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P. B. M. W. M. Timmermans · W. Hoefke · H. Stähle
and P. A. van Zwieten

Structure-Activity Relationships in Clonidine-Like Imidazolidines and Related Compounds

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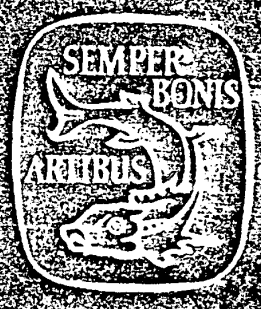

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62 figures and 39 tables

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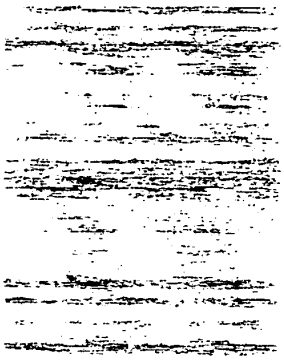

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I. General Introduction

Imidazoline derivatives have attracted the interest of pharmacologists and clinicians. They are mainly applied as sympathomimetic agents in practical medicine as decongestive drugs. Well-known examples are naphazoline, xylometazoline, tramazoline and other compounds, which are commercially available nasal decongestants. The imidazoline compounds applied in practical pharmacotherapy are predominantly α-sympathomimetic agents. Their therapeutic activity is modest: they can temporarily relieve the unpleasant symptoms of rhinitis or conjunctivitis, as a result of vasoconstriction within the congested tissues. The vasodilatation in inflamed tissues, brought about by histamine and probably other endogenous vasodilators is thus diminished by topically applied imidazoline derivatives and theoretically also by other vasoconstrictor agents, like adrenaline. The interest for imidazoli(di)ne derivatives has received a new impetus by the discovery of clonidine.

Clonidine (2-(2,6-dichlorophenylimino)imidazolidine) was developed in the sixties with the aim to obtain an additional imidazoline decongestant. Clonidine was initially known as *St-155*. The notation *St* is derived from the chemist Stähle, who was the first to synthesize this molecule and a considerable number of related imidazolidine derivatives with *St* and *STH* code numbers as well.

1.1. Influence of Clonidine on Arterial Pressure

In 1962 clonidine synthesized by Stähle, after being proven pharmacologically as nasal decongestant, was submitted as a 0.3 % solution to the medical department of C. H. Boehringer Sohn, Ingelheim in order to test its compatibility in man. Mrs. Schwandt, a member of the trial group, administered to herself 10 – 15 drops (about 1 to 2 mg), instead of 2, since she had a cold. «What could happen to harmless nose drops?» However, all sorts of things happened, for the dose amounted to about 10-fold of the therapeutic one applied at present. Strong sedation, dryness of the mouth, bradycardia and hypotension were observed. These effects continued for more than 24 hours. After this very first experiment on that lady Dr. Martin Wolf, a physician and member of the trial group, tested the compound systematically himself and recognized that the drug's decongestant properties were far less interesting than its potent hypotensive activity (Wolf, 1962, personal communication).

The influence of clonidine on arterial pressure is biphasic: both in animals and man clonidine causes an initial but transient rise in blood pressure, which is followed by a more prolonged and pronounced depressor phase (fig. 1). The initial pressor effect reflects vasoconstriction, brought about by the stimulation of peripheral α-adrenoceptors in the arterioles. This action is hardly unexpected in view of the imidazoline structure of the drug. The potent hypotensive and antihypertensive properties of clonidine, however, were rather surprising and prompted further investigations. A detailed analysis of its pharmacological effects has been presented by Hoefke and Kobinger (1966). Thereupon this compound has been introduced into clinical medicine for the treatment of arterial hyperten-

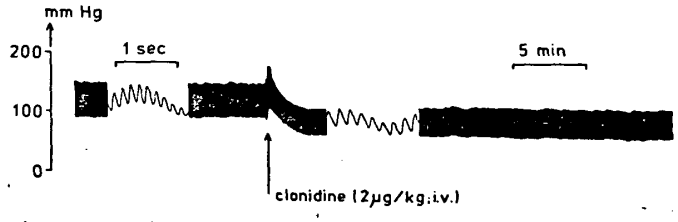


Fig. 1: Effect of intravenous clonidine (2µg/kg) on arterial pressure and heart rate of a normotensive rat anaesthetized with pentobarbitone (75 mg/kg, i.p.). From Timmermans and van Zwieten (unpublished result).

sion. In various countries the drug was registered under the commercial names Catapresan® or Catapres®. In humans its effective dose can be as low as 75-150 μg per os daily.

In the present review paper we shall not discuss the clinical advantages or disadvantages of clonidine compared with those of the various other antihypertensive agents, nor shall we deal with other applications of this drug. We only mention the possibility to apply clonidine in special cases of migraine without further discussing this possibility.

1.2. Mechanism of the Hypotensive/ Antihypertensive Activity of Clonidine

Soon after the discovery of the potent blood pressure lowering properties of clonidine, experimental arguments were presented in favour of a central hypotensive effect, at least in acute animal experiments. The mechanism of the acute, centrally-induced hypotensive effect of clonidine and related drugs has been described in full detail in review papers by the following authors: Schmitt, 1971, 1977; Kobinger, 1973, 1978; Stähle and Hoefke, 1975; van Zwieten, 1975a; Hoefke, 1976, 1980; Walland, 1977. The general opinion concerning the central hypotensive effect of clonidine may be summarized by the following theory, which has received ample experimental evidence from various groups: Clonidine is a relatively lipophilic drug, which easily penetrates into the central nervous system, where it achieves a high concentration in the brain and the cerebrospinal fluid. In the ponto-medullary area *α-adrenoceptors* have been demonstrated to occur. These receptors are excited by clonidine, an α-adrenoceptor stimulant drug. The α-receptor stimulation leads to an increased activity of hypothetical inhibitory neurons, probably the bulbospinal neurons, thus reducing the tone of the peripheral sympathetic nervous system. Consequently, arterial blood pressure and cardiac frequency will diminish. The α-adrenoceptor stimulant activity of clonidine is blocked by α-sympatholytic drugs which can penetrate into the central nervous system. Accordingly, piperoxan and yohimbine (Schmitt et al., 1971, 1973) and also prazosin (Cavero and Roach, 1978; Timmermans et al., 1979a) interfere with the acute hypotensive effect of clonidine, probably by a competitive mechanism of interaction. This mechanism of clonidine proves to be a general principle which can be extended to its structurally related derivatives. It may also hold true for the central hypotensive effect of α-methyl-DOPA (review by van Zwieten, 1976).

Most elegant studies in tetraplegic patients by Reid and coworkers (1977) have demonstrated unequivocally that the hypotensive mechanism of clonidine in man is also located within the central nervous system.

During the last few years the attention has been focused upon the considerable agonistic activity of clonidine at presynaptic α-adrenoceptors (for reviews on the basic principles see Langer, 1977; Starke et al., 1977, Westfall, 1977). Most of the studies which have led to this view were carried out on isolated organ preparations. Attempts have been made to extrapolate these findings towards a stimulation of central, presynaptic α-adrenoceptors by clonidine as an initiation of its central hypotensive effect. This hypothesis, however, is still subject to debate. A presynaptic nature of the receptive sites is favoured by the observations that clonidine diminishes the turnover of noradrenaline in the brain (Andén et al., 1970, 1976) and reduces the stimulation-induced release of neurotransmitter from brain slices (Farnebo and Hamberger, 1971; Starke and Montel, 1973). Moreover, the central hypotensive effect of clonidine can be readily reduced by piperoxan and yohimbine, α-sympatholytic drugs preferentially acting at presynaptic α-adrenoceptors (Starke et al., 1975a; Borowski et al., 1976; Drew 1976; Doxey et al., 1977). On the contrary, some reports strongly suggest that the cardiovascular depressant action of clonidine is due to activation of central, postsynaptic α-adrenoceptors, or at least of α-adrenoceptors on non-catecholaminergic neurons, since the drug acts independently of storage and synthesis of endogenous catecholamines in the central nervous system (Haeusler, 1974; Kobinger and Pichler, 1976; Warnke and Hoefke, 1977). In addition, prazosin which preferably blocks postsynaptic α-adrenoceptors (Cambridge et al., 1977; Doxey and Easingwood, 1978) has also been shown to

antagonize the central hypotensive effect of clonidine (Cavero and Roach, 1978; Timmermans et al., 1979a).

It has been postulated that the central hypotensive effect of clonidine might involve H_2-histamine receptors in the brain as demonstrated in rats (Karppanen et al., 1976, 1977) and rabbits (Hoefke, unpublished results). In fact, clonidine has been reported to activate histamine H_2-receptors (Karppanen and Westermann, 1973; Csongrady and Kobinger, 1974; Audisier et al., 1976). However, H_2-histamine receptive sites could not be shown to play a role in the hypotensive response to clonidine in conscious, renal hypertensive cats (Finch and Hicks, 1976) and dogs (Delbarre, Huchet and Schmitt, unpublished results).

1.3. Scope and Aims of the Present Survey

This review will treat the structure-activity relationships (SAR) of clonidine-like imidazolidines and related compounds with respect to various cardiovascular actions. Apart from the SAR concerning circulatory effects attention will be paid to other pharmacological and physiological properties of clonidine and its analogues, like sedation, local anaesthetic activity, inhibition of gastric secretion, action on H_2-histamine receptors etc. It is the aim of the present review to obtain insight into the relationship between molecular structure and biological activity of imidazolidines and related molecules. In addition, attempts will be made to differentiate between the various pharmacological actions within series of structurally related imidazolidines.

Several structural, physicochemical and quantum chemical parameters will be described as well. These molecular properties have been used to generate quantitative relationships. The emphasis of the present survey will be laid upon the interaction between clonidine and its structurally related imidazolidine compounds and the α-adrenoceptors within the central nervous system. From quantitative SAR studies a certain picture of the features of this central α-adrenoceptor has emerged. Finally, speculations about new antihypertensive drugs, based upon the present knowledge of the central α-adrenoceptor, will be submitted.

II. The Chemistry of Clonidine and Structurally related Derivatives; 2-(Arylimino)imidazolidines

2.1. History of Development

Structures possessing an imidazoline moiety (fig. 2), like naphazoline (Ar = α-naphthyl), oxymetazoline (Ar = 2,6-dimethyl-4-t-butyl-5-hydroxyphenyl), xylometazoline (Ar = 2,6-dimethyl-4-t-butylphenyl) and similar compounds are classical drugs and are used as nasal decongestants because of their local vasoconstrictor effect (also see Chapter I). On the other hand, tolazoline (Ar = phenyl) and phentolamine (Ar = N-4-methylphenyl, N-3-hydroxyphenylamino) are known as α-adrenolytic agents. These molecules are substituted amidines in which the amidine function is incorporated into an imidazoline ring. Additionally, this portion is connected with an aromatic nucleus via a methylene ($-CH_2-$) bridge.

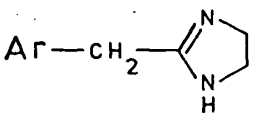


Fig. 2: General structure of the classical 2-(arylmethylene)imidazolines.

The first fundamental alteration of this basic structure was reported by Najer et al. (1959, 1960). They replaced the imidazoline ring by an oxazoline nucleus and the $-CH_2-$ ring junction

by an -NH- group. This gave rise to the general structure depicted in fig. 3.

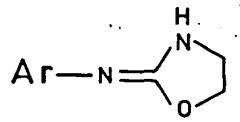


Fig. 3: General structure of 2-(arylimino)-oxazolidines.

The compounds also exhibited strong vasoconstrictor activity (Guidicelli et al., 1959).

The same authors prepared a number of substituted 2-(phenylimino)imidazolidines (Najer et al., 1961), which are cyclic guanidine derivatives (fig. 4).

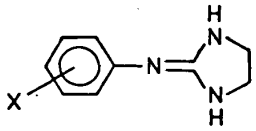


Fig. 4: General structure of 2-(arylimino)imidazolidines.

These compounds, however, appeared less active than similar derivatives possessing a methylene bridge with respect to vasoconstrictor activity. At approximately the same time a patent application was filed by C. H. Boehringer Sohn, Ingelheim (W. Germany) for an imidazolidine with the code designation St-155 (fig. 5).

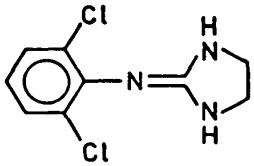


Fig. 5: Structural formula of clonidine (2-[2,6-dichlorophenylimino]imidazolidine).

St-155, later known as clonidine, was unique in having a double ortho-substitution by chlorine at the phenyl ring.

The more or less accidental discovery of clonidine as a potent antihypertensive drug (see Chapter I) has led to the synthesis of a large number of derivatives, mainly at the research laboratories of C. H. Boehringer Sohn, Ingelheim, by Stähle and his co-workers. In spite of this great number none of them has become a therapeutically useful antihypertensive drug more beneficial than clonidine itself (Graubner and Wolf, 1966).

St-600 (2-[2-methyl-5-fluorophenylimino]-imidazolidine) seemed an exception in this respect. This substance is less active than clonidine, but acts longer and probably possesses a more favourable ratio between antihypertensive efficacy and side effects (Stähle, 1974; Hoefke et al., 1975; Kho et al., 1975).

Compounds more or less related to the fundamental structure of clonidine have been developed more recently by several companies. The most important of them have been summarized in table 1. Some of these hypotensive agents have been subjected to detailed pharmacological studies (cfr. Stähle, 1974; van Zwieten, 1975a; Schlittler, 1977). In spite of a considerable research effort none of these compounds has as yet been introduced into clinical medicine. Many of these structures will be treated separately in the course of this review.

2.2. Synthetic Approach to Clonidine and Structurally Related Imidazolidines

2.2.1. Available Methods

A study of the literature concerning the available synthetic routes leading to imidazolidines of the clonidine-type reveals that, apart from some remarks in current journals (see section II.1.) one is completely at the mercy of industrial patents. After the discovery of clonidine it was solely industrial research that gave the impulse to the development of the synthetic methods and the optimization of the reaction conditions, so that many derivatives became available for pharmacological screening. The most important preparative pathways to the title compounds have been reviewed recently (Timmermans, 1976). Upon closer examination of these synthetic methods a division into two general classes can be made.

Table 1: Survey of teams. From Stäh

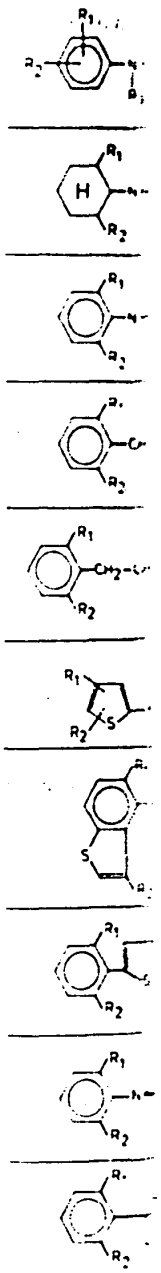
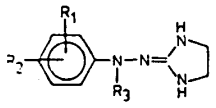
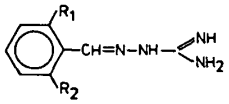
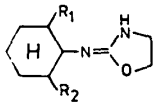
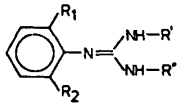
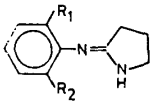
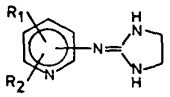
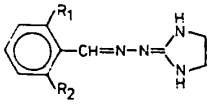
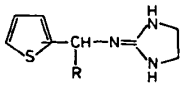
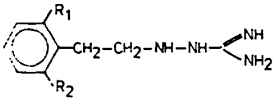
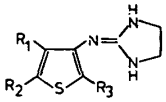
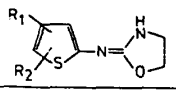
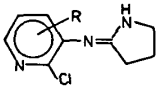
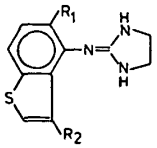
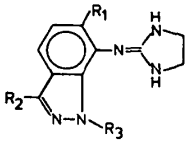
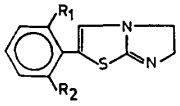
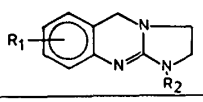
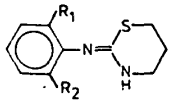
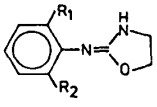
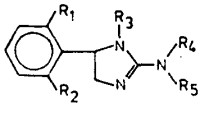
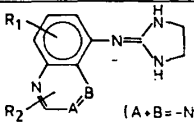


Table 1: Survey of hypotensive agents structurally more or less related to clonidine developed by several research teams. From Stähle (1974), with permission.

	Ciba 1966		Wyeth 1969
	Bayer 1966		Seperic 1971
	Bayer 1967 Searle 1971		Beecham 1969
	Sandoz 1967		Sumitomo 1969 Ciba - Geigy 1970
	Wander 1965		Hoechst 1969
	Du Pont 1970		Searle 1971
	Pfizer 1970		Wander 1971
	Sandoz 1969		Smith Kline 1969 and French
	Bayer 1962		Du Pont 1965 Bayer 1966 Lab. Daussee 1958
	Mead Johnson 1972 Bristol Myers 1971		Pfizer 1972 (A+B=-N=CH- or -CH=N-)

The first includes procedures in which the parent compounds are built up in one step by condensation of the separate ring systems (see Chart 1)

The nitrogen bridge originates from the aromatic portion through nucleophilic attack by the amine nitrogen. These methods, however, are only useful for obtaining a limited number of molecules and are by no means successful with respect to 2,6-di-substituted analogues, which is probably due to steric hindrance of such groups (Najer et al., 1961; C. H. Boehringer Sohn, Ingelheim, 1967). An exception in this respect is the procedure described by Chemie Linz AG (1974), which is reported to proceed in good yields (Chart 2).

In the second class of preparation the molecules are formed stepwise, starting from the aromatic moiety. All these methods clearly tend to create precursors with suitable leaving groups, which facilitate the cyclization of the imidazolidine part with ethylene diamine (see Chart 3).

The methods reported are very similar (Bloom, 1959; Najer et al., 1961; Boehringer Sohn, Ingelheim, 1965, 1966, 1967, 1969, 1971; Stähle et al., 1971, 1973; Pook et al., 1974; Lehmann et al., 1969a, 1969b, 1970; Toldy and Rados, 1968; Rouot et al., 1976; Shinuchi and Yoshikowa, 1974; Timmermans et al., 1978a). Consequently, the decision which synthetic pathway is to be followed in preparing imidazolidines of interest will depend on the versatility of the method concerned and also on the convenience in obtaining the precursors.

2.2.2. Readily Applicable Synthetic Procedures

Clonidine and its phenyl-substituted derivatives have generally been prepared in acceptable

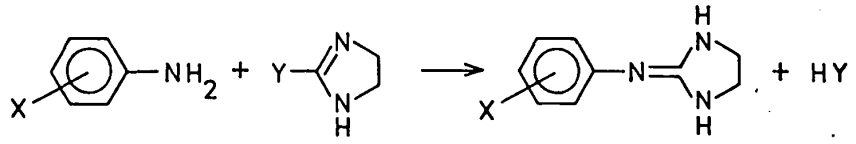


Chart 3: Preparation of 2-(arylimino)imidazolidines from suitable precursors and ethylene diamine.

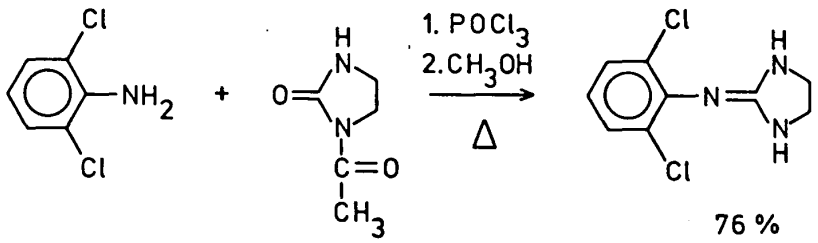


Chart 2: Preparation of clonidine in good yields from 2,6-dichloroaniline and N-acetyl-2-imidazolidinon (Chemie Linz AG, 1974).

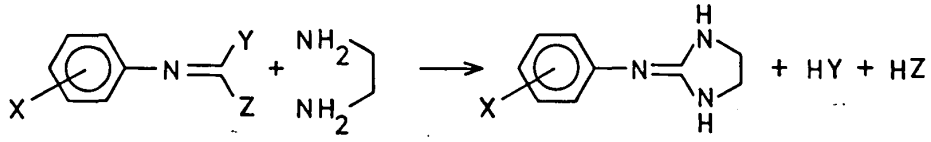
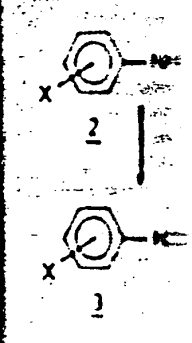


Chart 1: Preparation of 2-(arylimino)imidazolidines in one step.



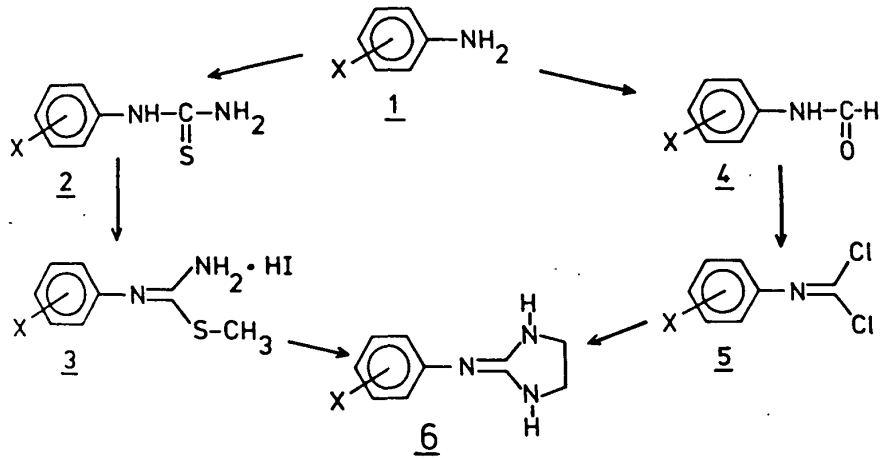


Chart 4: Synthetic routes most frequently used for the synthesis of clonidine and its structurally related derivatives.

yields via two methods. A schematic representation of these two routes is depicted in Chart 4.

Both pathways start from the substituted aniline *(1)*. N-aryl-S-methylisothiuronium iodides *(3)* are obtained by alkylation of N-arylthioureas *(2)*, which in their turn are available from the corresponding anilines *(1)* in good yields. Formylation of the anilines *(1)* results in the formation of formamides *(4)* , which are converted into the N-aryldichloroimines *(5)*. Both N-aryl-S-methylisothiuronium iodides *(3)* and N-aryldichloroimines *(5)* lead readily to 2-(arylimino)imidazolidines *(6)* in satisfactory yields. These two procedures are applicable to a variety of substituted molecules and the precursors *(3)* and *(5)* are rather easily accessible.

By heating N-aryl-S-methylisothiuronium iodides *(3)* with ethylene diamine in methanol or ethanol Bloom (1959) and Najer et al., (1961) prepared a number of 2-(arylimino)-imidazolidines *(6)*. This procedure was unsuccessful for 2,6-disubstituted compounds. The method could only be applied to the synthesis of a variety of imidazolidines after a modification. The reaction is then carried out preferably in the absence of a solvent by heating the components for about an hour at temperatures between 100 and 200°C (C. H. Boehringer Sohn, Ingelheim, 1966, 1971). Generally these conditions give satisfactory yields of the desired products with a relatively short reaction time and are also useful for the synthesis of 2,6-di-substituted derivatives. However, higher temperatures and longer reaction times are required and lower yields are obtained in comparison to unsubstituted or mono-ortho-subsituted congeners. Stähle et al. (1971, 1974), Pook et al. (1974), Rouôt et al. (1976) and Timmermans et al. (1978a) following this method prepared a great number of derivatives, including clonidine, in acceptable yields.

N-aryldichloroimines *(5)* proved excellent precursors for the preparation of 2-(arylimino)-imidazolidines *(6)*. Toldy and Rados (1968) synthesized a series of phenyl-substituted compounds, among which 2,6-di-substituted derivatives from the corresponding N-aryldichloroimines *(5)*. The reaction with ethylene diamine is effectuated in ethyl acetate in the presence of triethylamine. Clonidine and many structurally related imidazolidines were prepared in good yield according to this method (Lehmann et al., 1970; Rouot et al., 1976; Timmermans et al., 1978a). C. H. Boehringer Sohn, Ingelheim (1969) described the application of this reaction in obtaining nitro-substi-

tuted analogues, inaccessible via all the procedures reported so far.

In cases where both the N-aryl-S-methyl-isothiuronium iodide *(3)* and the N-aryldichloroimine *(5)* were applied to obtain the same derivative, the procedure which utilized the latter as the precursor, gave the best yields (Timmermans et al., 1978a).

The synthesis of [^{14}C]-clonidine has already been reported in 1969 by Rehbinder and Deckers. Clonidine with [^{14}C]-labelled 4 and 5 positions of the imidazolidine ring has been prepared by Ehrhardt (1972). Stiasni and Stähle (1978) have increased the radio-chemical yield of [^{14}C]-clonidine by more than 10-fold by modifying the earlier synthesis. The same authors were successful in obtaining [^{3}H]-labelled clonidine by catalytic debromination of 4-bromoclonidine with tritium gas. Deuteroclonidine became available through condensation with ethylene-[$^{2}H_4$]-diammonium dichloride.

III. Physicochemical and Quantum Chemical Properties of Clonidine and Structurally related Imidazolidines

3.1. Introduction

Clonidine and its analogues make up a series of congeneric drugs and have been the subject of a number of structure-activity relationship studies. These investigations have attempted to relate structural information with several biological actions. This Chapter compiles the experimental data on the molecular properties of clonidine and its derivatives. We will encounter most of these parameters in the treatment of the considerations on biological activity and molecular structure of imidazolidines (Chapter VI).

Section 2 of this Chapter deals with the dissociation constants which are related to the electronic properties of the imidazolidines and indispensable to acid/base equilibria. Lipophilicity as accounted for by partition coefficients is treated in section 3. This parameter is highly important when drug transport processes and hydrophobic interactions are concerned. Finally, the molecular structure and conformation of clonidine and related substances are considered in section 4. In this connection spectroscopic studies and quantum chemical calculations are reported.

3.2. Dissociation Constants of Clonidine and Structurally Related Imidazolidines

Imidazolidines of the clonidine-type are (weak) bases. In aqueous medium an equilibrium exists between these (weak) bases and their conjugated protonated forms. Dissociation constants reveal the proportions of the different ionic species of a substance at any chosen pH. The pK_a value together with the pH of the medium determines which fraction is undissociated and thus available for penetration through various lipid barriers. Furthermore, the pK_a value represents a parameter which reflects the over-all electronic properties of the substituents attached to the phenyl ring.

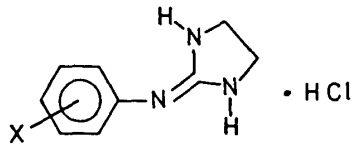
3.2.1. pK'_a Values

The pK'_a values of clonidine and a large number of imidazolidines determined by potentiometric titration of the hydrochlorides in water (20°C) are listed in table 2 (Timmermans and van Zwieten, 1978a). From the values enumerated in this table it can be concluded that considerable differences in pK'_a exist within this series of congeneric molecules. The pK'_a of clonidine (*1*; 2,6-di-Cl) is also reported to be 8.1 (20°C) by Stähle (1972) and 8.05 (25°C) by Struyker Boudier et al. (1974). The unsubstituted derivative *(15)* proved to have a value of 10.2 (20°C) (Pook et al., 1974). According to Struyker Boudier et al. (1974) the pK'_a value (25°C) of compound no. 2 (2,6-di-Br) amounts to 8.13, of no. *8* (2,6-di-Et) to 10.86 and of no. *6* (2-Cl,6-Me) to 9.76. Additionally, Rouot et al. (1976) determined the pK'_a values of a number of imidazolidines, including clonidine, in a 50 % aqueous ethanol mixture. These pK'_a values are

not directly comparable with the ones given in table 2 due to the depression of the pK'_a by ethanol as solvent.

In order to calculate the percentage of the imidazolidines, which is unionized in blood, pK'_a values were established which are comparable to those prevailing under physiological conditions (blood, 37°C). The influences of ionic strength and temperature on the dissociation reactions of clonidine and its derivatives were measured experimentally and agreed very well with the ones resulting from theoretical calculations (Timmermans and van Zwieten, 1978a). The corrections for ionic strength and tempera-

Table 2: Apparent dissociation constants (pK'_a) at 20°C and operational dissociation constants ($pK°_a$) of clonidine and structurally related imidazolidines. The $pK°_a$ represents the dissociation constant prevailing under physiological conditions (blood, 37°C). The percentages of protonated form and free base in blood (37°C, pH = 7.4) were calculated with the aid of this $pK°_a$. Data from Timmermans and van Zwieten (1978a).



Compd.	X	pK'_a	$pK°_a$	% protonated	% free base
1	2,6-di-Cl	8.05 ± 0.04	7.80	71.53	28.47
2	2,6-di-Br	7.80 ± 0.04	7.55	58.53	41.47
3	2,6-di-F	8.18 ± 0.04	7.90	75.97	24.03
4	2-Br,6-Cl	7.93 ± 0.02	7.65	63.96	36.04
5	2-Cl,6-F	8.01 ± 0.04	7.73	68.09	31.91
6	2-Cl,6-Me	9.40 ± 0.01	9.10	98.04	1.96
7	2,6-di-Me	10.53 ± 0.01	10.18	99.83	0.17
8	2,6-di-Et	10.61 ± 0.01	10.24	99.85	0.15
9	2,3-di-Cl	8.55 ± 0.02	8.27	87.96	12.04
10	2,4-di-Cl	8.73 ± 0.01	8.43	91.43	8.57
11	2,5-di-Cl	8.50 ± 0.03	8.22	86.80	13.20
12	2-Me,4-Cl	9.99 ± 0.03	9.74	99.46	0.54
13	2-Cl,4-Me	9.41 ± 0.01	9.11	98.08	1.92
14	2,4-di-Me	10.56 ± 0.01	10.21	99.84	0.16
15	H	10.05 ± 0.02	9.67	99.54	0.46
16	2-Cl	9.15 ± 0.04	8.82	96.32	3.68
17	2-Me	10.23 ± 0.01	9.90	99.68	0.32
18	2,4,6-tri-Cl	7.75 ± 0.02	7.50	55.73	44.27
19	2,4,6-tri-Br	7.46 *	7.21	39.24	60.76
20	2,4,6-tri-Me	10.78 ± 0.03	10.40	99.90	0.10
21	2,6-di-Cl,4-Br	7.72 *	7.46	53.44	46.56
22	2,6-di-Cl,4-Me	8.29 ± 0.03	8.01	80.27	19.73
23	2,6-di-Cl,4-OMe	8.57 ± 0.04	8.29	88.56	11.44
24	2,6-di-Cl,4-NO_2	6.86 *	6.65	15.17	84.83
25	2,4-di-Cl,6-Me	9.03 ± 0.03	8.70	95.23	4.77
26	2,4-di-Me,6-Cl	9.59 ± 0.03	9.29	98.73	1.27
27	2,6-di-Me,4-Cl	10.25 ± 0.02	9.92	99.69	0.31
28	2,6-di-Me,4-Br	10.21 ± 0.01	9.88	99.66	0.34

* Calculated

ture induce changes in such a way that under physiological conditions (blood, 37°C) the imidazolidine nitrogeneous bases are weaker than in distilled water (20°C) (see table 2.) With the aid of these so-called operational dissociation constants ($pK°_a$) the acid/base equilibria in blood (37°C, pH = 7.4) were calculated (see table 2). For example, under physiological conditions clonidine is present in its protonated (acid) form for about 70 %, leaving approximately 30 % for the free base. Compound no. *20* (2,4,6-tri-Me), which possesses a very high $pK°_a$ value (10.40) exists only for 0.1 % as an uncharged species.

3.2.2. Correlations of pK'_a with Electronic Substituent Constants

Hammett equation-like correlations (Hammett, 1940) have been reported for the dissociation reaction of clonidine and its phenyl substituted derivatives. For series of imidazolidines possessing similar ortho substituents linear relationships were found between electronic Hammett σ constants of meta and para substituents and $\Delta\ pK'_a$ ($= pK'_{a\,X} - pK'_{a\,H}$) (Timmermans and van Zwieten, 1978a). The reaction constants which measure the sensitivity to substitution in the phenyl ring were found to be positive and almost the same for each series (average value: + 1.48). The positive value of the reaction constant indicates that the dissociation is favoured by withdrawal of electrons from the reaction site. Moreover, by comparing this average value of + 1.48 with the one reported for the dissociation (25°C) of meta and para substituted anilinium ions (+ 2.767; Gould, 1959), it is obvious that the dissociation reaction of 2-(arylimino)imidazolidine ions is less sensitive to the influence of meta and para substituents than the dissociation of corresponding substituted anilinium ions. The observation that virtually indentical reaction constants resulted for mono-ortho and di-ortho-substituted derivatives (Timmermans and van Zwieten, 1978a) points to similar reaction sensitivities of these two classes of compounds to electrical effects excerted by meta and para substituents. A possible explanation may be that the dissociation is determined by one and the same conformation of these two groups of molecules, since only then the electronic influences of the substituents through inductive and resonance factors are equal.

A number of correlation equations have been derived relating the pK'_a of imidazolidines with various sets of electronic substituent constants in order to study the character of the electronic effects of the substitutents on this dissociation reaction. These regression equations have been summarized in table 3.*

An equation with excellent statistics was obtained by correlating pK'_a with the sum of the σ constants of the phenyl attached substituents, when for the ortho groups the values derived for

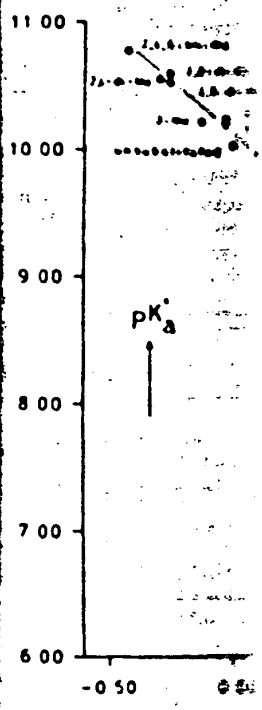


Fig. 6: Relationship between [illegible] related imidazolidines. The [illegible] and van Zwieten [illegible]

phenols [illegible] employed (table [illegible]) relationship is [illegible] cate that the effects of the [illegible] 2-(arylimino-[illegible] with those in [illegible]

Table 3: Linear regression equations generated by correlations between pK'_a and a number of electronic substituent constants for clonidine and structurally related imidazolines. All P values < 0.001

Equation		Number of Derivatives	Statistical Tests r	s	F	Eq. no.
$pK'_a = -1.567\ \Sigma\sigma_{o,m,p}$	+ 10.139	28	0.996	0.103	3196	1[a]
$pK'_a = -4.343\ \Sigma\delta$	+ 9.557	28	0.963	0.310	332	2[a]
$pK'_a = -1.188\ \Sigma F$	+ 10.421	28	0.965	0.302	350	3[a]
$pK'_a = -1.286\ \Sigma F$	$-$ 1.333 ΣR + 10.106	28	0.980	0.236	296	4[a]
$\Delta\ pK'_a = -1.84\ \Sigma\mathcal{F}$	$-$ 1.83 $\Sigma\mathcal{R}$ $-$ 0.40	22	0.98	0.24	202	5[b]

[a] From Timmermans and van Zwieten (1978 a).
[b] From Rouot et al. (1976).

* For all of the regressions [illegible] Chapter and throughout this [illegible] least squares was used [illegible] in order to determine the [illegible] theoretical means to the [illegible] correlation coefficient, r, the [illegible] and the significance of the [illegible] may consult Draper and [illegible] detailed statistical [illegible] made by using a rank [illegible] Spearman [illegible]

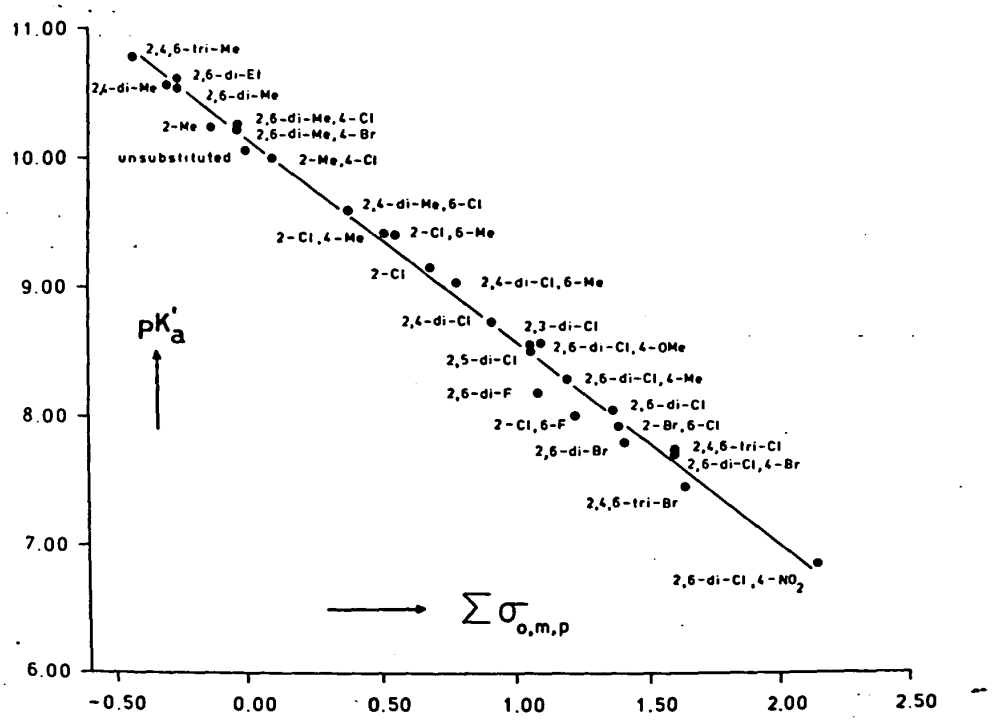


Fig. 6: Relationship between pK'_a and $\Sigma\sigma_{o,m,p}$ (phenyl-attached substituents) for clonidine and its structurally related imidazolidines. The relationship is described mathematically by equation 1 (table 3). From Timmermans and van Zwieten (1978a) with permission.

f electrons from the comparing this aver- the one reported for neta and para substi- '67; Gould, 1959), it ation reaction of 2- ns is less sensitive to ara substituents than ponding substituted vation that virtually ts resulted for mono- ted derivatives (Tim- n, 1978a) points to of these two classes l effects excerted by A possible explana- iation is determined rmation of these two only then the elec- ubstituents through tors are equal. equations have been imidazolidines with ubstituent constants cter of the electronic on this dissociation equations have been

llent statistics was , with the sum of the tached substituents, he values derived for

number of electronic 0.001

F	Eq. no.
3196	1[a]
332	2[a]
350	3[a]
296	4[a]
202	5[b]

phenols (Barlin and Perrin, 1966) were employed (table 3, eq.1.). This most significant relationship is visualized in fig. 6 and may indicate that the effects of the ortho substituents in 2-(arylimino)imidazolidines are comparable with those in phenols. The spectroscopic substituent constant, δ, of Seth-Paul and van Duyse (1972) also appeared a convenient parameter to predict the basicity of the member imidazolidines, compounds possessing ortho-situated fluorine and bromine excepted (eq. 2.). The very acceptable correlation between pK'_a and the inductive component (propagated through σ bonds) of the electronic effect of the substituents, Σ F (tabulated by Norrington et al., 1975) stresses a major importance of inductive contributions (eg. 3.) However, equation 4 shows that resonance contributions (conjugation between π electrons), as expressed by Σ R (Norrington et al., 1975), cannot be ignored. Equation 5 has been reported by Rouot et al. (1976). It comprises comparable inductive, $\Sigma\mathcal{F}$, and resonance, $\Sigma\mathcal{R}$, contributions and possesses indentical statistical quality as equation 4.

[a] For all of the regression equations, given in this Chapter and throughout this review the method of least squares was used. Statistical tests were performed in order to determine the «goodness of fit» of the theoretical model to the data. These involved the correlation coefficient, r, the standard deviation, s, and the significance of the regression, F. The reader may consult Draper and Smith (1966) for more detailed statistical information. Calculations were also made by using a rank correlation test according to Spearman (1904).

It should be expected that resonance interaction through delocalization of the positive charge developed in this protonation reaction is not very great since the delocalization of this positive charge rather occurs in the guanidine portion of the molecules. In the protonated 2-(arylimino)imidazolidines the positive charge is dispersed over the guanidine moiety and is not solely concentrated at the bridge nitrogen atom, where the protonation takes place (see section 3.4.3.). The rather small value found for the reaction constant (see above) is in agreement with this explanation.

3.3. Lipophilicity of Clonidine and Structurally Related Imidazolidines

The most widely and frequently used parameter for structure-activity studies in biological systems has been the lipophilicity. Overall lipophilic behaviour of drugs, as expressed by their octanol/water partition coefficients, is in many cases suitable to describe drug transport processes and also the hydrophobic interactions between drug and receptor are often adequately accounted for by this molecular parameter (for reviews see Hansch, 1971, 1973; Lien, 1974).

It is obvious that the lipid solubility will determine the hypotensive potency of clonidine-like drugs to a great extent, since this action is brought about via a central mechanism. The molecules have to penetrate from the blood into the brain, thereby passing the blood-brain barrier. Generally, penetration through lipid barriers is limited to the uncharged drug species, the charged molecules being more soluble in the aqueous medium. As a consequence the acid/base equilibrium under physiological conditions (37°, pH = 7.4) will greatly influence the penetration properties of the present imidazolidines. For this reason apparent partition coefficients at pH = 7.4 have appeared most useful describing the over-all lipophilic properties of these substances.

3.3.1. Partition Coefficients

The apparent partition coefficients (log P') of clonidine and a large series of congeneric substances have been measured in the octanol/buffer (pH = 7.4) system (Rouot et al., 1976; Timmermans et al., 1977a). The data have been gathered in table 4 and show that within this series of structurally similar molecules a wide range of lipophilicity is covered (> 4 log P' units; also see table 38). Therefore, distinct differences in lipophilic behaviour will be encountered. In addition, apparent partition coefficients (pH = 7.4) from the system chloroform/water have been reported for a limited number of imidazolidines (Struyker Boudier et al., 1974). However, this partition system is considered not as predictive as the octanol/water model.

For ionisable compounds, like the present imidazolidines, one distinguishes the apparent partition coefficient (P') and the true partition coefficient (P) of the neutral species. For bases the relation between P' and P is given by the following equation:

$$P = P' [1 + 10^{(pK_a - pH)}]$$

In this equation P' is corrected for ionisation, assuming that the ionized form does not partition. The apparent partition coefficients (P') of clonidine and its derivatives, which react with water to yield ions, were corrected for ionisation and expressed as the true partition coefficient (log P) of the free bases (table 4). This table shows that the great differences in lipophilic properties of the imidazolidines are mainly due to the involvement of pK_a. For instance the true partition coefficients (log P) of the free bases of compound no. 4 (2-Br, 6-Cl) and no. 7 (2,6-di-Me) are approximately the same (1.73 and 1.86 respectively). However, their apparent partition coefficients (log P') differ more than two log P units, which is caused by the great difference in pK_a (see table 2).

The lipophilic character of imidazolidine free bases has also been evaluated by their ΔR_M values obtained from thin-layer chromatography (Rouot et al., 1976). The R_f values of the imidazolidine free bases were determined by means of a reverse thin-layer chromatographic technique. The mobile phase was sufficiently alkaline to keep the molecules in their neutral form. R_M was calculated from R_f according to $R_M = \log (1/R_f - 1)$. The chromatographic ΔR_M ($= R_{M(X)} - R_{M(H)}$) values are additionally listed in table 4.

Table 4: Appar… true partition co… related deriva…

Compd.	X
1	2,6-di-Cl
2	2,6-di-Br
3	2,6-di-F
4	2-Br,6-Cl
5	2-Cl,6-F
6	2-Cl,6-Me
7	2,6-di-Me
8	2,6-di-Et
9	2,3-di-Cl
10	2,4-di-Cl
11	2,5-di-Cl
12	2-Me,4-Cl
13	2-Cl,6-Me
14	2,4-di-Me
15	H
16	2-Cl
17	2-Me
18	2,4-…
19	2,4-…
20	3,4-…
21	3,4-…
22	3,4-…
23	3,4-…
24	3,4-…
25	3,4-…
26	3,4-…
27	3,4-…
28	3,4-…
29	3,4-…
30	3,4-…
31	3,4-…
32	3-Me,…
33	3,4-…
34	4-Br
35	3,4-…
36	3,4-…
37	3,4-…

em (Rouot et al., 1976; 977a). The data have been nd show that within this similar molecules a wide is covered (> 4 log P' 8). Therefore, distinct dif- behaviour will be encoun- pparent partition coeffi- m the system chloroform/ rted for a limited number truyker Boudier et al., partition system is consi- ive as the octanol/water

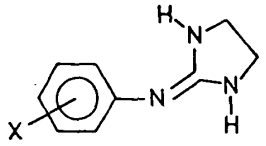
pounds, like the present listinguishes the apparent P') and the true partition neutral species. For bases P' and P is given by the

$= 10^{(pK_a - pH)}]$

s corrected for ionisation, ized form does not parti- rtition coefficients (P') of vatives, which react with re corrected for ionisation true partition coefficient ses (table 4). This table differences in lipophilic azolidines are mainly due pK_a. For instance the true log P) of the free bases of , 6-Cl) and no. 7 (2,6-di- the same (1.73 and 1.86, , their apparent partition fer more than two log P' by the great difference in

cter of imidazolidine free valuated by their ΔR_M thin-layer chromatogra- 6). The R_f values of the es were determined by n-layer chromatographic phase was sufficiently olecules in their neutral d from R_f according to: The chromatographic Δ) values are additionally

Table 4: Apparent partition coefficients (log P') determined in the octanol/aqueous buffer (pH = 7.4) system, true partition coefficients of the free bases (log P) and chromatographic ΔR_M values of clonidine and structurally related derivatives. Data from Rouot et al. (1976) and from Timmermans et al. (1977 a).



Compd.	X	log P'[a]	log P'[b]	log P[a]	ΔR_M[b]
1	2,6-di-Cl	0.62	0.83	1.59	0.38
2	2,6-di-Br	1.21	1.15	1.96	0.61
3	2,6-di-F	−0.16	0.02	0.93	−0.24
4	2-Br,6-Cl	0.87	–	1.73	–
5	2-Cl,6-F	0.52	–	1.45	–
6	2-Cl,6-Me	−0.57	−0.40	1.71	0.45
7	2,6-di-Me	−1.54	−1.65	1.86	0.42
8	2,6-di-Et	−0.88	−0.84	2.60	0.95
9	2,3-di-Cl	0.57	–	2.01	–
10	2,4-di-Cl	0.29	0.69	1.90	0.76
11	2,5-di-Cl	0.65	0.95	2.04	0.71
12	2-Me,4-Cl	−1.06	−0.58	1.80	0.75
13	2-Cl,4-Me	−0.48	−0.28	1.80	–
14	2,4-di-Me	−1.66	−1.46	1.78	0.43
15	H	−1.92	−1.80	1.01	0.00
16	2-Cl	−0.67	–	1.36	–
17	2-Me	−1.82	–	1.28	–
18	2,4,6-tri-Cl	1.47	1.38	2.18	1.00
19	2,4,6-tri-Br	2.24	–	2.74	–
20	2,4,6-tri-Me	−1.28	–	2.32	–
21	2,6-di-Cl,4-Br	1.97	–	2.66	–
22	2,6-di-Cl,4-Me	0.73	–	1.92	–
23	2,6-di-Cl,4-OMe	0.15	–	1.60	–
24	2,6-di-Cl,4-NO_2	1.92	–	2.10	–
25	2,4-di-Cl,6-Me	0.47	–	2.38	–
26	2,4-di-Me,6-Cl	−0.36	–	2.10	–
27	2,6-di-Me,4-Cl	−0.62	–	2.50	–
28	2,6-di-Me,4-Br	−0.28	–	2.80	–
29	2,4,5-tri-Cl	–	1.63	–	1.20
30	2,6-di-i-Pr	–	−0.21	–	1.32
31	2-OMe,4-Me	–	−1.56	–	−0.03
32	2-Me,5-Cl	–	−0.30	–	0.72
33	2,5-di-OMe	–	−1.78	–	−0.42
34	4-Br	–	−0.50	–	0.58
35	2,4-di-OMe,5-Cl	–	−1.37	–	−0.07
36	2-CF_3	–	0.00	–	–
37	2,6-di-Cl,4-OH	–	−0.40	–	–

[a] From Timmermans et al. (1977a).
[b] From Rouot et al. (1976).

3.3.2. Relationships Between log P, Δ R_M and Hydrophobicity Constants

One of the most useful features of the partition coefficient for structure-activity investigations is its additive character. The incremental change in hydrophobic (lipophilic) behaviour of a substance on introduction of the substituent X is represented by π (X). In the ideal case log P is the sum of π-constants over the entire molecule (Hansch, 1971; Hansch et al., 1963).

The additive-constitutive character of log P applied to phenyl-substituted imidazolidines was investigated by a correlation of log P (see table 4) with the Hansch hydrophobic substituent constant π (Timmermans et al., 1977a). The sum of π-constants (Σπ) over the substituents attached to the phenyl ring of the molecules was employed. For the correlation of log P with Σπ the π-values from the phenoxyacetic acid series (Fujita et al., 1964) proved superior over other sets of constants. The relationship found is expressed by equation 6 and is visualized in fig. 7.

$$\log P = 0.731\ \Sigma\pi + 0.898 \quad \text{(eq. 6)}$$
$$n = 28;\ r = 0.972;\ s = 0.177;$$
$$F = 444\ (P < 0.001)$$

Accordingly, a linear relationship (eq. 7) between ΔR_M (see table 4) and Σπ (phenoxyacetic acid series) has been established for 21 clonidine-like imidazolidines (Rouot et al., 1976):

$$\Delta R_M = 0.60\ \Sigma\pi - 0.21 \quad \text{(eq. 7)}$$
$$n = 21;\ r = 0.94;\ s = 0.16;$$
$$F = 149\ (P < 0.001)$$

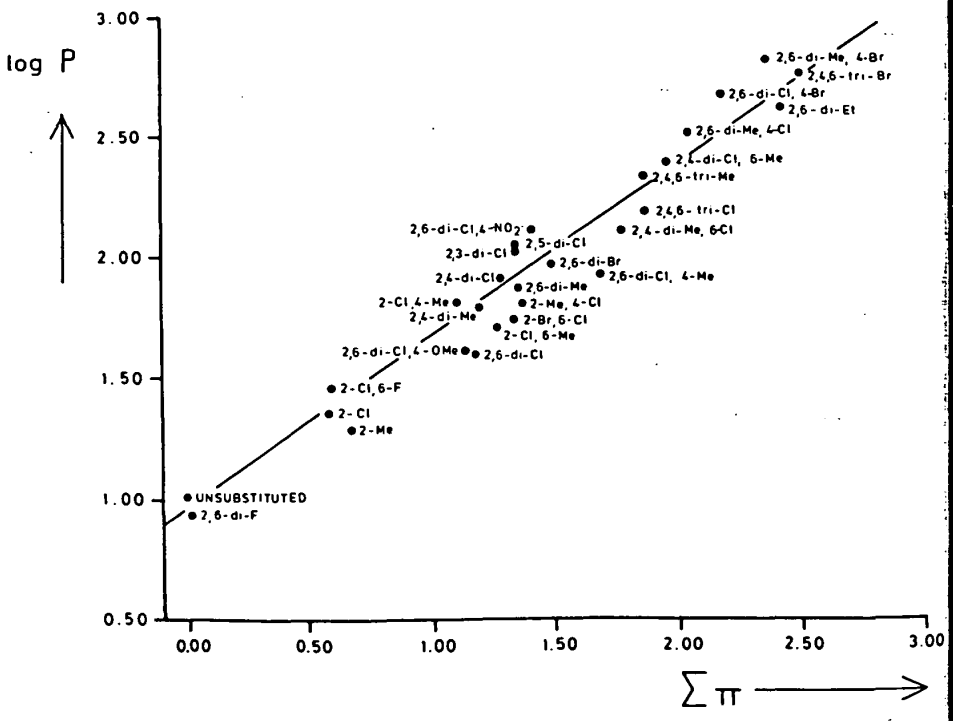


Fig. 7: Relationship between log P of phenyl-substituted imidazolidines (free bases, see table 4) and Σπ (phenyl-attached substituents) taken from the phenoxyacetic acid series (Fujita et al., 1964). The relationship is described mathematically by eq. 6. From Timmermans et al. (1977a), with permission.

These correlations sho
Apparently, the ortho sul
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they exert no more influen
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has not already been accou

3.3.3. Lipophilicity and

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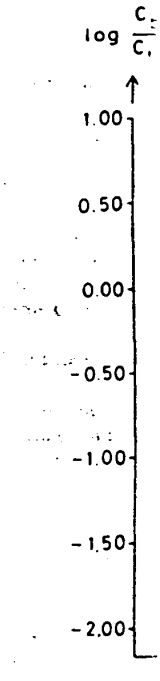

Fig. 8: Dependence of the abs
partition coefficient, log P', fo
mathematically by eq. 9. From

These correlations showed no anomalies. Apparently, the ortho substituents of 2-(arylimino)imidazolidines behave in such a way that they exert no more influence on the neighbouring imidazolidine moiety, as far as this influence has not already been accounted for by π .

3.3.3. Lipophilicity and Brain Disposition

The brain concentrations of clonidine and a number of analogues have been determined after intravenous administration to anaesthetized, normotensive rats (Timmermans et al., 1977a, 1977b). To connect these data directly to the biological (hypotensive) effect, they were established at the moment of maximal decrease in blood pressure. These data were used in order to investigate whether the lipophilicity of the compounds, expressed by log P', was the major contributing factor for the penetration ability into the central nervous system. The quotient of log brain concentration (ng/g w.w.)/dose (µg/kg) administered intravenously, log ($C_{brain}/C_{i.v.}$), was calculated to account for the tendency of the imidazolidines to penetrate into the brain. Upon correlating log ($C_{brain}/C_{i.v.}$) with log P' the following equations (8 and 9) have been derived:

$$\log (C_{brain}/C_{i.v.}) = 0.555 \log P' - 0.327 \quad \text{(eq. 8)}$$
$$n = 14;\ r = 0.947;\ s = 0.269;\ F = 104\ (P < 0.001)$$

$$\log (C_{brain}/C_{i.v.}) = -0.133\ (\log P')^2 + 0.574 \log P' - 0.094 \quad \text{(eq. 9)}$$
$$n = 14;\ r = 0.987;\ s = 0.139;\ F = 212\ (P < 0.001)$$

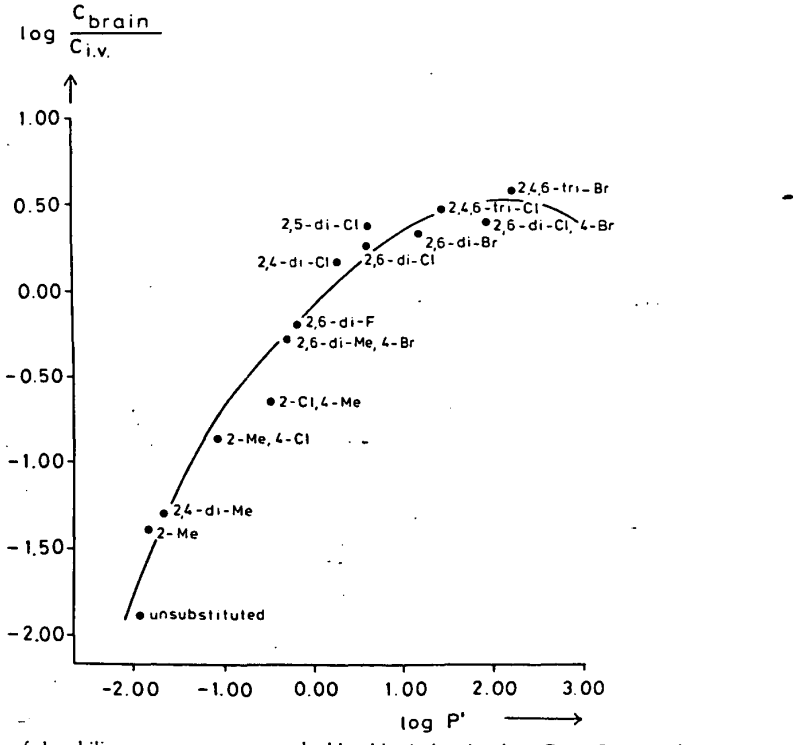

Fig. 8: Dependence of the ability to penetrate across the blood-brain barrier, log ($C_{brain}/C_{i.v.}$), on the apparent partition coefficient, log P', for clonidine and structurally related imidazolidines. The relationship is expressed mathematically by eq. 9. From Timmermans et al. (1977a), with permission.

Equation 9 is a highly significant improvement over equation 8 and is presented in fig. 8.

It is a parabolic relationship reflecting a very close correlation between the log ($C_{brain}/C_{i.v.}$) values and the lipophilicity of the imidazolidines. Therefore, after their intravenous administration the penetration ability of the compounds into the brain can be described by log P'. The ideal lipophilic character (log P') for maximal transport from the blood to the brain was calculated from equation 9 and amounted to 2.16. This value implies that a clonidine-like drug will possess the most favourable ratio between the dose applied intravenously and its brain concentration, when its log P' has a value of 2.16.

3.4. Molecular and Conformational Structure of Clonidine and Related Imidazolidines

Investigations have been performed to establish the molecular and conformational structure of clonidine free base and protonated form, since both species are present under physiological conditions.

3.4.1. Spectroscopic Studies

In older literature clonidine free base has been generally expressed in the amino form (fig. 9). However, a number of authors have shown that this compound exists in solution predominantly in the imino form by using ^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy (Rouot et al., 1973; Pook et al., 1974; Jackman and Jen, 1975).

An ^1H-NMR spectrum of clonidine base is visualized in fig. 10.

Their conclusions were based upon comparison of the NMR spectral data with those of model compounds in which the double bond was fixed within (amino form) or outside (imino form) the 5-membered ring. Therefore, clonidine base possesses a structure with an exocyclic double bond. Formally speaking, clonidine should be considered an imidazolidine derivative and not a compound derived from imidazoline.

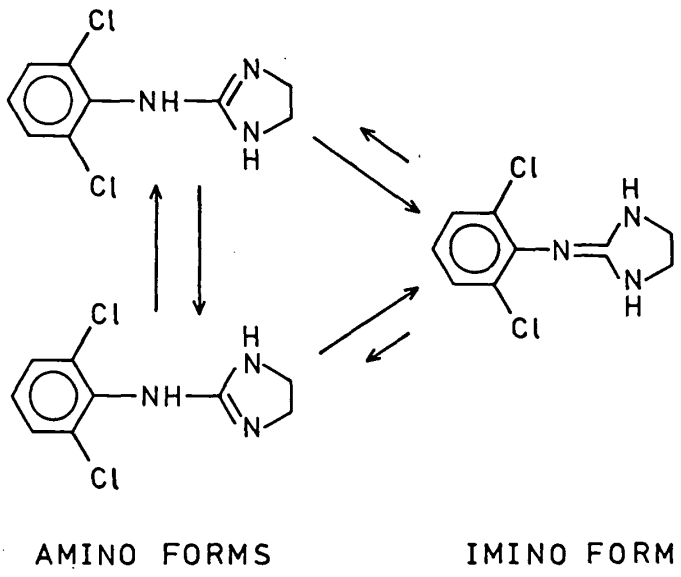

Fig. 9: Tautomeric equilibria between imino and amino forms of clonidine free base.

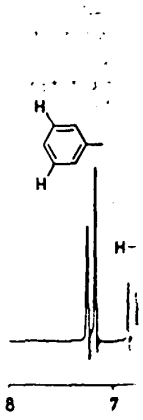

Fig. 10: ^1H-NM
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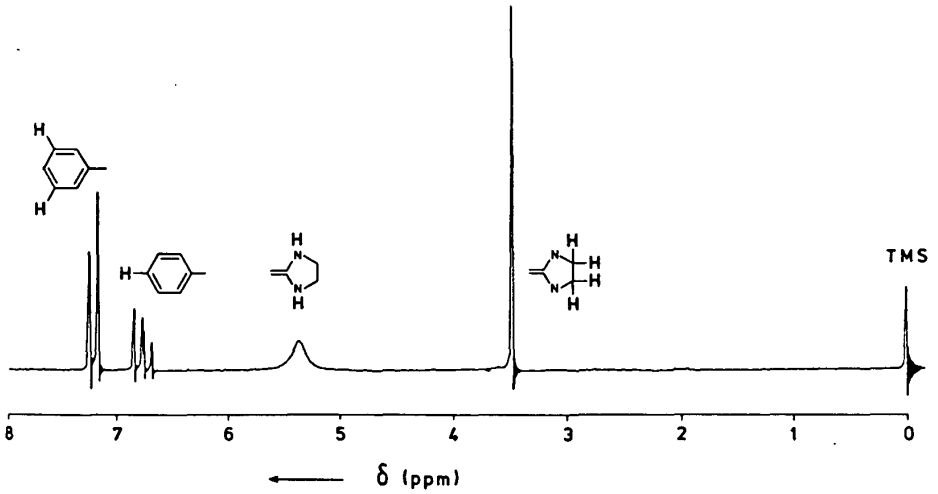

Fig. 10: ^{1}H-NMR spectrum of clonidine base (100 MHz; 1.0 M in $CDCl_3$ at 30°C). The chemical shifts (δ) are expressed in ppm relative to tetramethylsilane as internal standard. The methylene protons of the imidazolidine ring appear as a four-proton singlet at δ = 3.50 ppm and the NH-protons as a two-proton singlet at δ = 5.30 ppm. The phenyl protons are seen as multiplets at δ = 6.80/7.25 ppm (Timmermans, unpublished results).

Indications for the imino form of clonidine base have also been obtained from infrared spectroscopy (Rouot et al., 1973). The absorption frequency of the exocyclic C = N function in model imidazolidines is found at 1660 cm^{-1} (clonidine base: 1675 cm^{-1}). On the contrary, the C = N group in imidazolines with fixed amino forms absorbs near 1615–1630 cm^{-1}. In addition, data from ultraviolet spectroscopy (Rouot et al., 1973) support the view that in clonidine base the connection between the bridge nitrogen and the imidazolidine ring mainly occurs via an exocyclic double bond.

Upon protonation of clonidine base a proton will attach to a point where the electrostatic potential is strongly negative. Although the highest negative charge is found at both imidazolidine nitrogen atoms (Timmermans et al., 1977e), the protonation will take place at the bridge nitrogen atom, since only then stabilisation of the molecule can occur through delocalization of the positive charge in the guanidine portion. This leads to the structure as depicted in fig. 11.

Consequently, the protonation of clonidine base is an orbital-controlled reaction. Accordingly, alkylation reactions carried out on clonidine base resulted in the formation of products in which the alkyl residue was found at the bridge atom (Stähle and Pook, 1971). In the protonated form of clonidine the π-electrons of the double bond are delocalized and the positive charge is dispersed over the three nitrogen atoms. This situation corresponds with spectroscopic data (Rouot et al., 1973).

3.4.2. Crystallographic Studies

Two virtually identical crystal structures of clonidine hydrochloride have been reported (Byre et al., 1976; DeTitta, unpublised data, 1976). The crystals are monoclinic with space

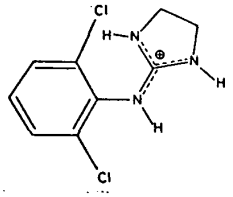

Fig. 11: Structure of protonated clonidine.

group C2/c. The 2,6-dichlorophenyl and the imidazolidine ring are found planar. The values of the structural elements of the ring junction in the crystal structure of clonidine hydrochloride (see fig. 12) are: r_s = 1.42 Å; r_d = 1.33 Å; α = 123°; β = 117° and θ = 75°. The arrangement of bonds about the bridging nitrogen atom is nearly planar.

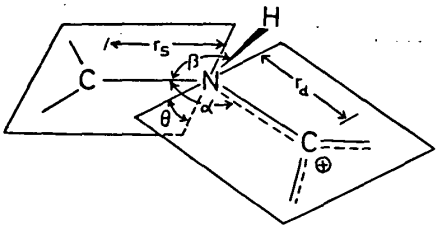

Fig. 12: Structural elements of the ring junction in clonidine hydrochloride (protonated form).

The short distance of r_d is indicative for a certain degree of double bond character and proves the proposed structure of protonated clonidine as given in fig. 10. In the crystal of clonidine hydrochloride the planes of the phenyl ring and the imidazolidine ring form an angle of 75°. The crystal structure is held together by hydrogen bond linking two-fold screw related molecules. The crystal structure packing diagram of clonidine hydrochloride is visualized in fig. 13.

The structure of crystalline clonidine base has not been reported yet.

3.4.3. Quantum Chemical Studies

Attempts have been made at establishing the equilibrium geometry/preferred conformation of clonidine base and protonated form by means of CNDO/2 calculations (Meerman-van Benthem et al., 1975; Timmermans et al., 1977e). By energy optimization of the ground state equilibrium geometry of clonidine base, the following values of the bridge parameters (see fig. 14) were found: r_s = 1.40 Å; r_d = 1.32 Å; α = 111.5° and θ = 34°.

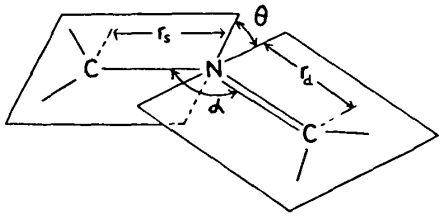

Fig. 14: Structural elements of the ring junction in clonidine free base.

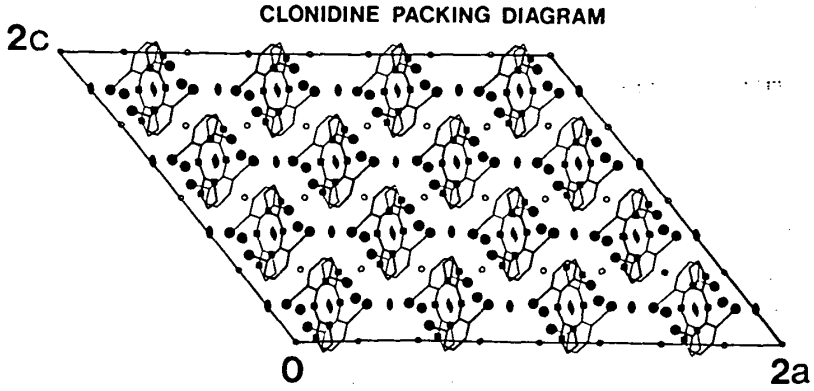

Fig. 13: Clonidine hydrochloride packing diagram. Code: Circles = chlorines; squares = nitrogen; lines = carbons. Unpublished data from DeTitta (1976), with permission.

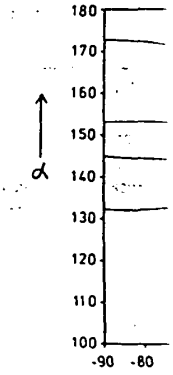

Fig. 15: Energy conto
given in atomic units
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Subsequently, a
r_s and r_d was calcu
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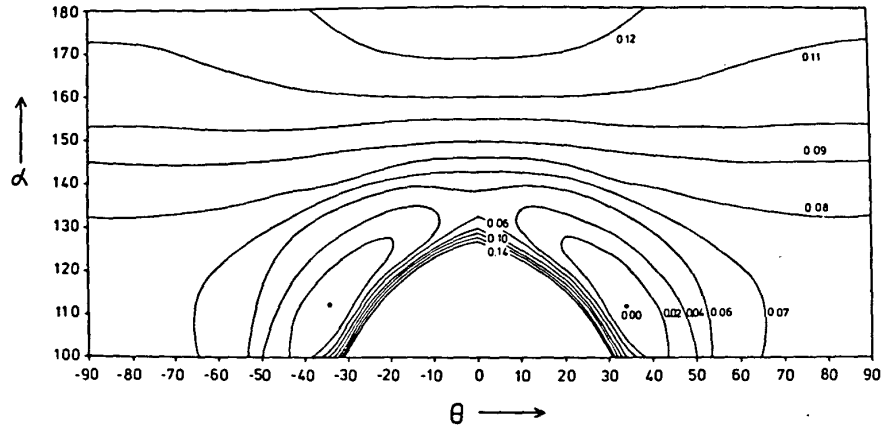

Fig. 15: Energy contours of clonidine base as a function of α and θ for r_s = 1.40 Å and r_d = 1.32 Å. Energies are given in atomic units relative to the energy of the equilibrium geometry. From Meerman-van Benthem et al. (1975), with permission.

Subsequently, a potential surface for optimal r_s and r_d was calculated by varying the angular parameters α and θ (fig. 15).

As can be seen from fig. 15, clonidine base proved to be stable in a non-perpendicular conformation. The CNDO/2 studies show an energy minimum for the base with the planes of the phenyl and imidazolidine rings forming a dihedral angle af 34°. This structure of the base permits the cyclic guanidine and the phenyl moieties to be conjugated. The NH . . . Cl repulsion only keeps this molecule from adopting a planar structure (θ =0°).

With the aid of the same CNDO/2 calculation procedure Timmermans et al. (1977c) have tried to find the equilibrium geometry of the protonated form of clonidine. Energy optimization resulted in the following values for the parameters (fig. 12) comprising the ring junction in protonated clonidine: r_s = 1.40 Å; r_d = 1.36 Å; α = 116°; β = 122° and θ = 40°. Upon variation of the interplanar angle θ the energy curve was obtained as given in fig. 16.

The shape of this energy curve can be interpreted as that conjugational effects are responsible for the energy lowering when θ is decreasing. For low values of θ the strong NH . . . Cl repulsion causes a net increase in energy. In the protonated form of clonidine θ = 40° is found to be the result of these two mutually conflicting effects. Compared to the free base of clonidine the angle between both ring systems (θ) of the protonated form is larger by 6°. The length of the double bond (r_d) proved to be 1.36 Å. This is larger than its value in the free base (r_d = 1.32) due to loss of double bond character. A planar

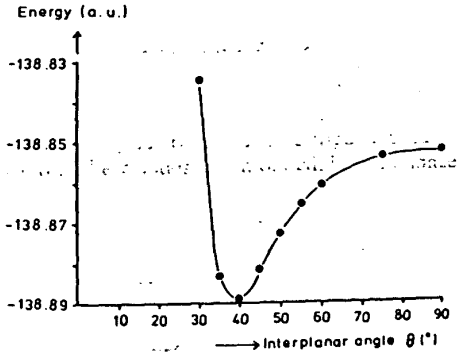

Fig. 16: Energy curve of protonated clonidine as a function of the interplanar angle θ. From Timmermans et al. (1977c), with permission.

structure was found for the bridge nitrogen and its three neighbouring atoms.

The preferred conformations of clonidine-like derivatives (free bases and protonated forms) have been calculated on the basis of the results of the CNDO/2 calculations on the free base and the protonated form of clonidine (Timmermans et al., 1977c). The equilibrium geometries of both clonidine species were taken as the starting points for these calculations, since the spectroscopic data of the congeners showed similar features as observed for clonidine itself (see above). To perform these calculations the bond lengths and the van der Waals radii of the substituents different from chlorine at the ortho positions were taken into account. Concomitantly, the preferred conformation of the free base of the unsubstituted compound was found nearly planar ($\theta = 15°$). This is also the case for all mono-ortho-substituted molecules. When the preferred conformation is determined by a fluorine atom, the interplanar angle is enlarged to 30° and if bromine or a methyl group is present, this angle amounts to 50°. In the preferred conformations of the protonated forms the dihedral angles are larger by 5° with respect to the ones in the corresponding free bases.

The preferred conformations of a considerable number of imidazolidine derivatives (free bases and protonated forms) were taken as inputs for PPP-MO calculations in order to obtain several quantum chemical indices of potential assistance in the structure-activity relationship studies (Timmermans et al., 1977c). The PPP-MO calculation method offers an opportunity to calculate π-electron properties of molecules, which are mostly encountered in applications to medicinal chemistry. Accordingly, a rather detailed list of quantum chemical parameters have been calculated which involved energy levels, bond orders an π-electron charge densities.

In order to check the validity of the employed PPP-MO calculation procedure, some of these calculated quantum chemical indices were correlated with the pK'_a values (see table 2) determined experimentally. For instance, a very acceptable linear correlation was derived between pK'_a and its associated ΔE_π value for 17 di-ortho-substituted imidazolidines (equation 10).

$$pK'_a = 11.21\ \Delta E_\pi - 29.41 \quad \text{(eq. 10)}$$
$$n = 17;\ r = 0.927;\ s = 0.489;\ F = 92 \quad (P < 0.005)$$

ΔE_π stands for the difference in π-electron energy between the protonated form and its corresponding free base. A meaningful relationship was also established between pK'_a and the calculated π-electron charge density at the bridge nitrogen atom of the protonated forms, qN_7 (equation 11).

$$pK'_a = -133.98\ qN_7 + 173.90 \quad \text{(eq. 11)}$$
$$n = 17;\ r = 0.964;\ s = 0.346;\ F = 200 \quad (P < 0.001)$$

The charge density at N_7 can be regarded most important in determining the ultimate course of the protonation reaction.

3.4.4. Concluding Remarks

A perpendicular position of both rings in clonidine base and protonated form has been suggested (Jen et al., 1972; Rouot et al., 1973; Wermuth et al., 1973; Jackman and Jen, 1975). However, the presence of a predominantly exocyclic double bond in clonidine base suggests the effort for conjugation in this molecule. This conjugation is only possible for a non-perpendicular structure. Both crystallographic (Byre et al., 1976; DeTitta, 1976) and quantum chemical (Meerman-van Benthem et al., 1975; Timmermans et al., 1977c) studies confirm a conformation which is not perpendicular. Still there is a great discrepancy between the structure found in the crystal and the one calculated on the lone gas phase molecule. A limitation of CNDO calculations is the underestimation of non-bonded repulsions. Accordingly, on optimization the distances between non-bonded atoms are too small. Due to the presence of such an interaction in clonidine (NH . . . Cl) the value for the dihedral angle will be higher than the calculated one.

It is always difficult to compare calculated conformations directly which the ones actually existing in the crystal. The theoretical calculations are on a single molecule in the gas phase and the crystal studies are on a molecule frozen into an array of its own kind. Thus, the calcula-

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IV. Structure-Activit
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4.1. Introduction

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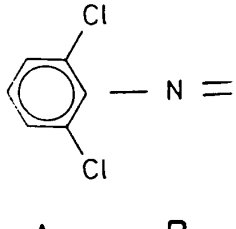

A B

Fig. 17. Subdivision of clonidine in
structural units.

tions do not account for the perturbation introduced by the neighbouring molecules. The same holds true for a comparison with conformations in solution in which the substance is surrounded by solvent molecules. Finally, the structure of a drug, whether determined in the crystal or derived from solution or from calculations in the gas phase, does not necessarily yield the appropriate information. It is not sure that the drug engages the receptor in its preferred conformation. It is possible that the substance has to distort its conformation and encounters the receptor in a non-preferred one. However, it can be imagined that in series of structurally similar derivatives these perturbations are about the same. The close similarity may be thought to warrant a comparison which in any case stresses relative values rather than absolute ones.

IV. Structure-Activity Relationships in Clonidine-Like Imidazolidines and Related Compounds

4.1. Introduction

The most important biological actions of clonidine and its related compounds are connected with the effects of these drugs on circulation. Intravenous administration of clonidine provokes a biphasic effect on arterial pressure. A short-lasting increase in pressure is followed by a prolonged decrease. Bradycardia accompanies both phases (also see Chapter I). It is obvious that the hypotension represents the therapeutically most useful action. Therefore, after the discovery of clonidine structural modifications have been made in this molecule mainly in order to obtain information about the molecular features underlying this hypotensive property.

In the molecule of clonidine at least three possibilities for a structural modification are present, viz. the aromatic portion (A), the bridge B and the imidazolidine moiety (C) (see fig. 17).

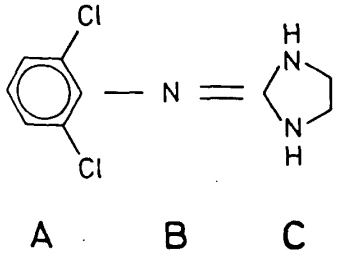

Fig. 17: Subdivision of clonidine in three fundamental, structural units.

The series of compounds used in the majority of studies aiming at relating the chemical structure with biological activity contain one or more of these three fundamental modifications.

The first part of this Chapter deals with the structure-activity relationships with respect to cardiovascular actions of clonidine-like imidazolidines and related compounds. The influence of structural alterations in the clonidine molecule on hypotensive activity (section 4.2.1.), central hypotensive activity (section 4.2.2.) and antihypertensive activity (section 4.2.3.) established with the aid of various different experimental animals will be described. In addition, the bradycardic (section 4.2.4.) and the hypertensive activities (section 4.2.5.) of clonidine-like imidazolidines and related compounds will be treated as well.

The second part of this Chapter is devoted to the structure-activity relationships obtained for side-effects and other pharmacological actions of these molecules. A number of them are related to the α-sympathomimetic property of the agents and others originate from studies to separate side-effects from hypotensive efficacy. We will report the available data on sedation (section 4.3.1.), α-adrenergic activity on rabbit intestine (section 4.3.2.), local anaesthetic activity (section 4.3.3.), antisecretory activity (section 4.3.4.), effects on histamine H_2-receptors (section 4.3.5.), presynaptic activity (section 4.3.6.) and, finally some miscellaneous actions (section 4.3.7.).

This Chapter only provides qualitative pictures of the structure-activity relationships. The tabulated data allow to a certain extent some general conclusions concerning the relationship between chemical structure and some biological actions out of this wide spectrum of pharmacological activities. Moreover, a few biological parameteres have been subjected to quantitative analyses. The attempts at a quantitative description of some of the structure-activity relationships will be reported in Chapter VI. Moreover, Chapter V puts various biological actions of these drugs against each other and is dealing with quantitative comparisons between them.

4.2. Structure-Activity Relationships with Respect to Cardiovascular Actions

4.2.1. Hypotensive Activity

4.2.1.1. Structural Changes in the Imidazolidine Ring

The structural modification involving the enlargement of the 5-membered (imidazolidine) ring of clonidine into a 6, 7 or 8-membered nucleus gave rise to a considerable diminution of hypotensive activity in the anaesthetized rabbit after intravenous administration (Stähle, 1974; Hoefke, 1976). Fig. 18 shows the activities of the compounds quantified as the logarithm of the reciprocal dose (mg/kg) required to cause a decrease in mean arterial pressure by 20 mm Hg (log 1/C).

Surprisingly, the molecules in which the imidazolidine moiety was expanded to a 7 or a 8-membered ring showed an increase in hypotensive activity compared to the 6-membered analogue *(St-404)*, although they are much less potent than clonidine itself.

The hypotensive effect of a number of derivatives possessing various different hetero aromatic ring systems has been quantified following intravenous administration to anaesthetized, normotensive rats (Timmermans and van Zwieten, 1978b; Timmermans and van Zwieten, submitted for publication). The compounds studied were: *St-404* (nitrate), compound *44–549* (fumarate) in which the imidazolidine portion together with the nitrogen bridge has been replaced by a bicyclic system, xylazine (2-[2,6-dimethylphenylimino]-perhydro-1,3-thiazine.HCl; *Bay 1470*) and 2-(2,6-dimethylphenylimino)oxazolidine.HCl (*Bay c 6014*; *LD 2855*). Their structures have been depicted in fig. 19.

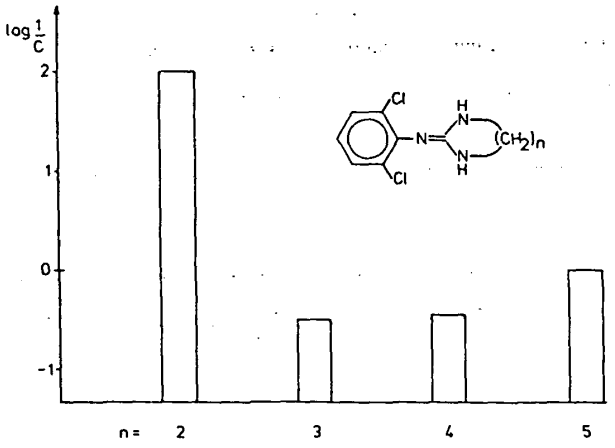

Fig. 18: Influence of expansion of the imidazolidine ring of clonidine on the hypotensive activity in anaesthetized rabbits after intravenous administration. C = ED_{20} = effective dose (mg/kg) lowering arterial pressure by 20 mm Hg. From Stähle (1974) and Hoefke (1976), with permission.

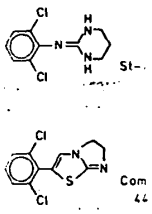

Fig. 19: Structures various different h

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Fig. 20: Influence hypotensive activity reciprocal ED_{10}, ex $ED_{\ }$, in µg/kg. Fro

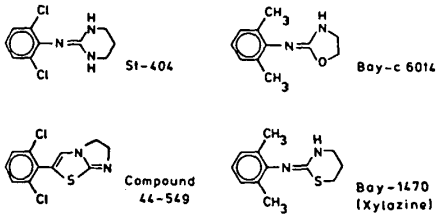


Fig. 19: Structures of some clonidine-like drugs with various different hetero aromatic ring systems.

Their activities have been compared with clonidine (.HCl) and its 2,6-dimethyl analogue (*St-95*.HCl) (see fig. 20). In fig. 20 the logarithm of the reciprocal ED_{30}, expressed in µmol/kg, is given on the ordinate. The line at the bottom of fig. 20 shows the corresponding ED_{30} in µg/kg (= dose required to invoke a 30 % decrease in mean arterial pressure).

Fig. 20 shows the particularly high hypotensive activity of compound *44–549* as quantified in the anaesthetized, normotensive rat. This drug was found approximately 5 times more potent than clonidine in reducing mean arterial pressure by 30 %. Compound *44–549* proved to possess potent hypotensive activity in experimental animals (Boyajy et al., 1972). Moreover, experiments by van Zwieten (1975b) revealed its pronounced central hypotensive action as studied through infusions of low doses into the left vertebral artery of anaesthetized cats. In this animal model compound *44–549* appeared a little less effective than clonidine. As in the anaesthetized rabbit (see fig. 18) a similar great loss of hypotensive potency of *St-404* compared to clonidine was found in the anaesthetized, normotensive rat. If the imidazolidine ring is replaced by an oxazolidine moiety, like in *Bay c 6014*, the hypotensive potency increases considerably compared to the parent compound in which an identically substituted phenyl nucleus is present (*St-95*; 2,6-di-Me). On the other hand, substitution of this part by a perhydro-1,3-thiazine structure, like in xylazine, led to a substance of which the hypotensive activity is

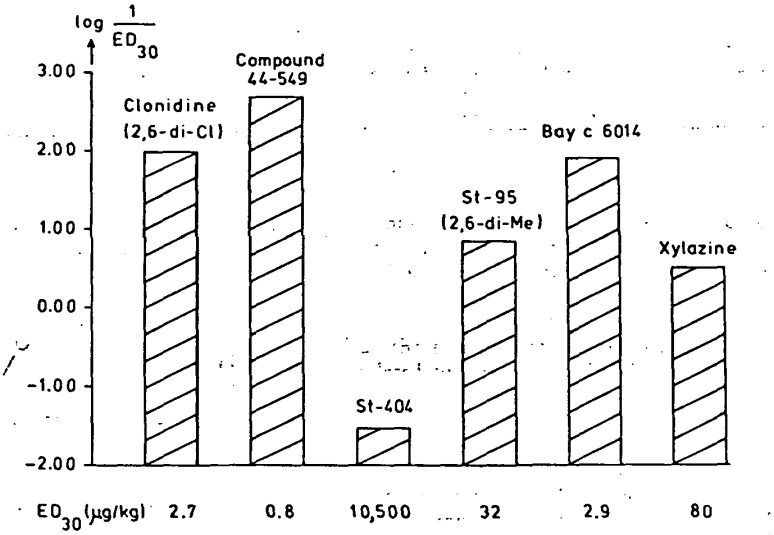


Fig. 20: Influence of replacement of the imidazolidine part by various hetero aromatic ring systems on the hypotensive activity in anaesthetized, normotensive rats after intravenous administration. The logarithm of the reciprocal ED_{30}, expressed as µmol/kg, is given on the ordinate. The line at the bottom shows the corresponding ED_{30} in µg/kg. From Timmermans and van Zwieten (submitted for publication).

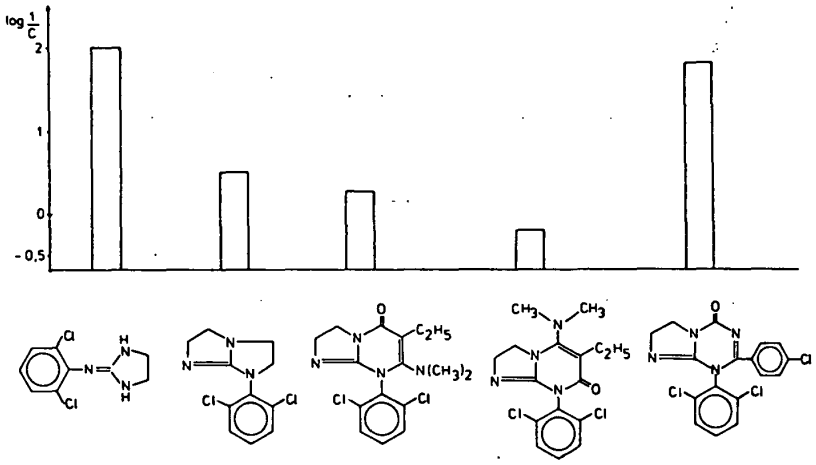


Fig. 21: Hypotensive activity of some annellated structures of clonidine following intravenous administration to anaesthetized rabbits. C = dose, mg/kg, decreasing mean arterial pressure by 20 mm Hg. From Stähle (1974) and Hoefke (1976), with permission.

reduced. The pharmacological properties of xylazine *(Bay 1470)* have been first described by Kroneberg et al., (1966, 1967). The mechanism of its hypotensive action has been further characterized as being closely related to that of clonidine (Schmitt et al., 1970; Heise and Kroneberg, 1971; Antonaccio et al., 1973; Finch, 1974). *Bay c 6014 (LD 2855)* was probably the first substance described with what at present can be called clonidine-like properties (Beauvallet et al., 1959). The pronounced hypotensive action of this agent has been reported by Giudicelli and Schmitt (1970). Its mechanism of action is comparable to that of clonidine.

Phenyl-substituted 2-(arylimino)pyrrolidines have been tested for hypotensive effects in conscious DOCA/saline hypertensive rats (Hershenson and Rozek, 1971). The data presented do not permit an exact comparison of potencies due to the variation in the pretreatment averages. However, 2,6-di-ortho-substitution appeared optimal, although the 2,6-di-ethyl analogue was ineffective. Furthermore, compounds with a 5-membered pyrrolidine ring proved more potent than correspondingly substituted 6-membered perhydropyridine derivatives. This result is in agreement with the findings on imidazolidines (see above).

The imidazolidine ring of clonidine has been structurally altered by ring closure reactions into annellated bicyclic structures (Stähle and Köppe, 1973). The hypotensive activity of these annellated clonidine molecules was distinctly reduced in comparison with clonidine upon intravenous administration to anaesthetized rabbits, some compounds within the imidazo [1,2-a] s-triazine series excepted (Stähle, 1974; Hoefke, 1976) (see fig. 21).

In a search for new centrally acting antihypertensive model compounds, substances with the general structure, as shown in fig. 22, were developed (Clough et al., 1978).

The compound with X = 2,6-di-Cl was as potent as clonidine in lowering blood pressure and heart rate of anaesthetized rats. The struc-

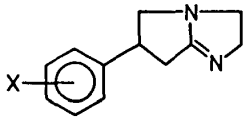


Fig. 22: Substituted 6-phenyl-2,3,6,7-tetrahydro-5H-pyrrolo-[1,2-a]-imidazoles.

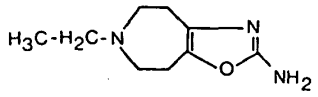
tures with X = 2-F and X = 2-Cl,6-F compounds behave following intraveno administration to c dogs. In addition, cats sympathetic ef after intravenous ar

The hypotensive 2,3; azepexole) ha characterized to be use of 6 test proce 1977). Like cloni biphasic effect on l by bradycardia af tion. A central n demonstrated by in 9,3,3 decreased pe activity, facilitated ally-mediated baro potency (R) of *B-1* 0.01–0.03, taking = 1). These resu molecular structur hitherto known st

Fig. 24: Hypotensive ... to anaesthetized, ... ordinate. The lin ... Zwieten 1980).

tures with X = 2-Br,6-Cl, X = 2,6-di-Cl,3-Me and X = 2-Cl,6-F were found less active. All compounds behave as active antihypertensives following intravenous, oral and intraventricular administration to conscious, renal hypertensive dogs. In addition, in chloralose-anaesthetized cats sympathetic efferent activity was reduced after intravenous and central application.

The hypotensive action of *B-HT 933* (fig. 23; azepexole) has been pharmacologically characterized to be of the clonidine-type by the use of 6 test procedures (Kobinger and Pichler, 1977). Like clonidine, the drug showed a biphasic effect on blood pressure accompanied by bradycardia after intravenous administration. A central nervous site of action was demonstrated by intracisternal injections. *B-HT 933* decreased peripheral sympathetic nerve activity, facilitated the flexor reflex and the vagally-mediated baroreceptor reflex. The relative potency (R) of *B-HT 933* was approximately 0.01–0.03, taking clonidine as the standard (R = 1). These results are unexpected as its molecular structure differs considerably from hitherto known substances of the clonidine-type. It is interesting that the structure of *B-HT 933* apparently fits into the α-adrenoceptor pattern.



B-HT 933

Fig. 23: Structural formula of *B-HT 933* (azepexole; 2-amino-6-ethyl-4,5,7,8-tetrahydro-6H-oxazolo-[5,4-d]-azepin).

4.2.1.2. *Structural Changes in the Ring Junction*

Replacement of the bridge nitrogen atom of clonidine by a methylene (– CH_2 –) group, a sulphur or an oxygen atom yields bridge analogues of clonidine. The hypotensive activities of these derivatives have been studied following intravenous administration to anaesthetized, normotensive rats (Timmermans and van Zwieten, 1980). The results have been presented in fig. 24.

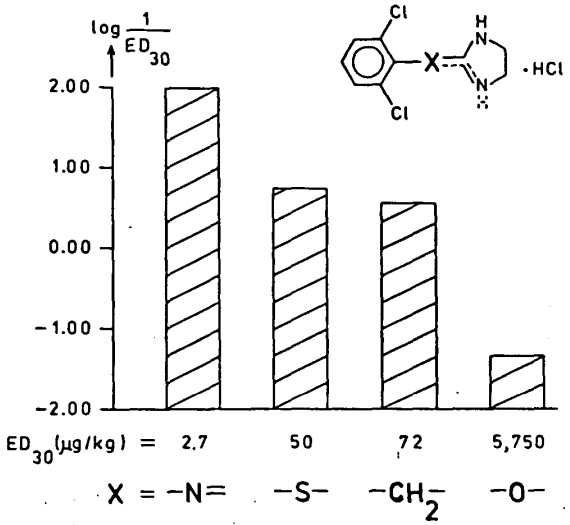


Fig. 24: Hypotensive activity of mono-atomic bridge analogues of clonidine following intravenous administration to anaesthetized, normotensive rats. The logarithm of the reciprocal ED_{30}, expressed in µmol/kg, is given on the ordinate. The line at the bottom shows the corresponding ED_{30} in µg/kg. From Timmermans and van Zwieten (1980).

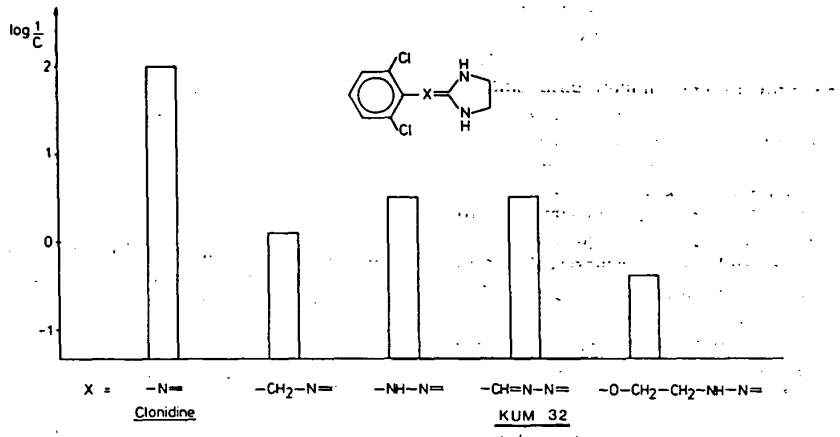


Fig. 25: The influence of extension of the ring junction in clonidine upon hypotensive activity following intravenous administration to anaesthetized rabbits. C = dose, mg/kg, for a 20 mm Hg decrease in mean arterial pressure. From Stähle (1974) and Hoefke (1976), with permission.

It follows from fig. 24 that the presence of a nitrogen atom between the two ring systems, like in clonidine, is essential for high hypotensive activity. The compounds possessing a ring junction occupied by methylene or sulphur are much less active. Upon including an oxygen the hypotensive activity is dramatically reduced. It should be remarked that these bridge analogues of clonidine are all centrally acting hypotensive drugs. Central α-adrenoceptors are involved in the mechanism of action, the oxygen derivative excepted for which a different mode of action underlies its central hypotensive effect (Timmermans et al., 1979b).

The influence of extension of the bridge between the phenyl and the imidazolidine ring upon hypotensive activity has been investigated. Fig. 25 shows the biological data obtained for a series of clonidine derivatives with two or more atoms composing the bridge (Stähle, 1974; Hoefke, 1976). The substances were injected into anaesthetized rabbits.

As can be concluded from this figure, bridge extension is accompanied by a loss of hypotensive activity which is particularly great when the ring junction exceeds three atoms. This conclusion only seems valuable for compounds possessing an imino (C = N) function in the ring junction. Lofexidine (X = – O-CH-CH_3) showed the same hypotensive activity as clonidine in anaesthetized rabbits.

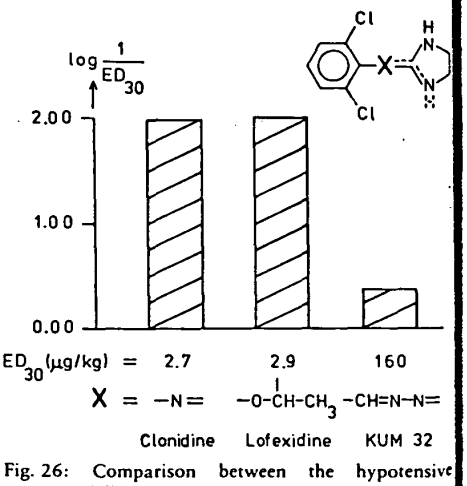


Fig. 26: Comparison between the hypotensive activities following intravenous injection into anaesthetized, normotensive rats of clonidine, lofexidine and *KUM 32*. The logarithm of the reciprocal ED_{30}, expressed in µmol/kg, is given on the ordinate. The line at the bottom shows the corresponding ED_{30} in µg/kg. From Timmermans and van Zwieten (1980).

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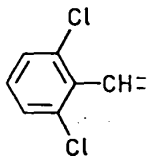

Fig. 27: 4-Amino-
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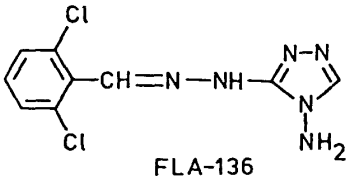
Investigations by means of intravenous administrations of lofexidine. HCl to anaesthetized, normotensive rats also showed that this structural modification of the bridge has yielded a molecule for which a high hypotensive activity is retained comparable to that of clonidine (Timmermanns and van Zwieten, 1980) (see fig. 26).

In accordance with the findings in the anaesthetized rabbit (fig. 25) the derivative KUM 32, studied as hydroiodide in which three atoms (−CH=N-N=) connect both cyclic parts proved also much less effective than clonidine in the anaesthetized, normotensive rat (fig. 26).

FLA-136 which resembles clonidine in structure (fig. 27) has been reported to possess antihypertensive activity at high doses in conscious, hypertensive rats following oral application (Eriksson and Florvall, 1976).



FLA-136

Fig. 27: 4-Amino-3-(2,6-dichlorobenzylidenehydrazino)-1,2,4-triazol *(FLA-136)*.

Similarly to clonidine, this compound was recently described to decelerate the synthesis and the turnover of noradrenaline in rat brain (Andén and Grabowska, 1977). However, in contrast to clonidine the hypotensive effect of intravenous *FLA-136* in anaesthetized rats is not preceded by an initial increase in blood pressure and its mechanism of action seems to be different (Timmermans et al., 1979c).

Substances displaying hypotensive and antihypertensive activities have been discovered in a chemical class which may be called «open-ring imidazolidines». The most promising molecules possess a structure consisting of a 2,6-dichlorophenyl moiety connected to a guanidine portion via a two-atomic bridge of varying composition. Guanabenz *(Wy 8678;* fig. 28) is one of the first examples out of this class. It induces cardiovascular effects similar to those of

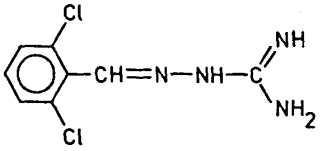


Fig. 28: 2,6-Dichlorobenzylidene aminoguanidine (*Wy 8678*; guanabenz).

clonidine, although it is much less potent (Natoff et al., 1968; Baum et al., 1969, 1970; Natoff and Stanton, 1969). The drug diminishes sympathetic nerve activity in the cat (Baum and Shropshire, 1970, 1976; Baum et al., 1970). Like clonidine, guanabenz reduces the turnover rate of brain noradrenaline in rats (Bolme et al., 1973). In view of the pharmacological similarity between clonidine and guanabenz a common mechanism of action may be suggested.

A number of hydroxylated derivatives of guanabenz of the type visualized in fig. 29 (R = H or CH_3) have also been found to possess antihypertensive properties. *SAH 43-663* (R = H) does not differ from clonidine in its pharmacological effects, although higher doses are needed (Sandoz Patent, 1968).

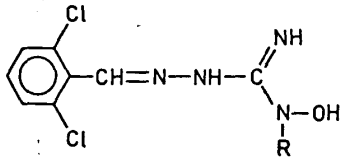


Fig. 29: N-(2,6-Dichlorobenzylidene amino), N-hydroxyguanidines.

Earlier work on arylalkylaminoguanidines, which were found to have antihypertensive activity in animals and man (Bream et al., 1970), has been continued by varying the chain linking aromatic moiety and guanidine group (Bream et al., 1975). High antihypertensive activity in DOCA/salt hypertensive rats was found to be associated with a series of phenylacetylguanidines. Optimal antihypertensive potency was observed for the 2,6-dichlorophenyl derivative (*BS 100–141;* fig. 30).

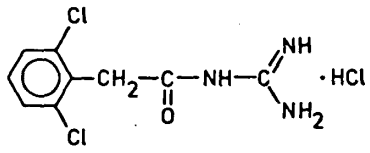


Fig: 30: 2,6-Dichlorophenylacetylguanidine hydrochloride (*BS 100-141;* guanfacine).

Pharmacological studies have demonstrated that *BS 100–141* induces cardiovascular actions similar to those of clonidine. Evidence for a central site of action has been demonstrated in anaesthetized dogs and cats and it causes dose-dependent reductions in the splanchic (sympathetic) nerve activity in the latter species (Scholtysik et al., 1975). The compound was found 20 to 40 times less potent than clonidine (Hoefke, unpublished results; Saameli et al., 1975). *BS 100–141* stimulates presynaptic cardiac α-adrenoceptors in cats and rabbits (Scholtysik, 1974; Pacha et al., 1975). Like clonidine, *BS 100–141* has been reported to reduce sympathetic activity in man, but it possesses a longer duration of action (Zamboulis et al., 1978). The sedative effects of *BS 100–141* have been described slightly compared to clonidine (Kleinlogel et al., 1975; Saameli et al., 1975). The threshold dose for inducing sedation in dogs was found about 100 times greater for *BS 100–141* than for clonidine (Scholtysik et al., 1975). Some clinical studies (Jäättelä, 1976a, 1976b); Dubach et al., 1977) have shown the usefulness of this drug (guanfacine) in antihypertensive therapy.

Recently, «open ring» derivatives of clonidine (arylguanidines) have been studied with respect to their acute haemodynamic effects in anaesthetized rats (Rouot et al., 1978). It could be shown that by opening the imidazolidine portion and/or omitting one or both of the resulting N-methyl groups, molecules are obtained possessing some hypotensive and bradycardic activities. They are found 100–1000 times less potent than their corresponding «closed» analogues.

4.2.1.3. *Structural Changes in the Aromatic Part; Substitution at the Phenyl Ring*

A few compounds related to clonidine in which the major structural difference is found in the aromatic (phenyl) nucleus have been subjected to pharmacological studies in order to test their hypotensive activities. Their formulae have been visualised in fig. 31.

The 2,6-dichlorophenyl moiety of clonidine has been replaced by a substituted thiophene ring in tiamenidine *(Hoe 440)*. It has been found that this compund exhibits haemodynamic effects which are similar to those of clonidine in their mechanism. Its blood pressure-lowering

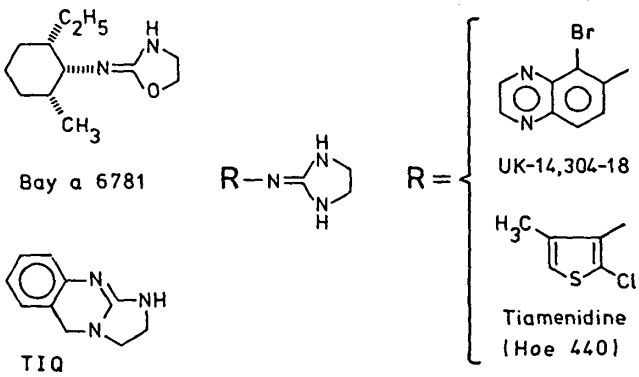


Fig. 31: Collection of formulae of some drugs distantly related to clonidine possessing structural changes in the aromatic portion.

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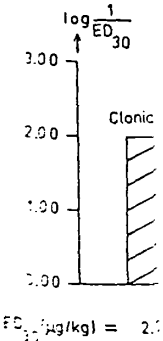

Fig. 32: Comparis
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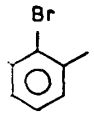

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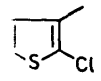

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effect at about a 3–10 times higher dose is usually as strong as that of clonidine and the toxicity and many of the side-effects are reported to be considerably diminished (Kersting, 1973; Lindner and Kaiser, 1974; Simon et al., 1976).

In *Bay a 6781* it draws the attention that this molecule contains a cyclohexyl moiety instead of an aromatic nucleus. Its effect in inhibiting noradrenaline release has been described (Werner et al., 1970, 1972). This oxazolidine derivative is a centrally acting hypotensive drug as potent as clonidine, at least in animal experiments. The mechanism of action is probably the same as that of clonidine (Schümann and Werner, 1971; Jacobs et al., 1972).

A quinoxaline system is found in the experimental compund *UK-14,304–18* (Pfizer). In animal experiments this substance is somewhat less active than clonidine and it seems likely that the mechanism which underlies the hypotensive effect is identical to that of clonidine (Pfizer, personal communication).

A comparison has been made between the hypotensive activities of clonidine (.HCl), *Bay a 6781* (.HCl) and *UK-14,304–18* (tartrate) following intravenous administration to anaes-

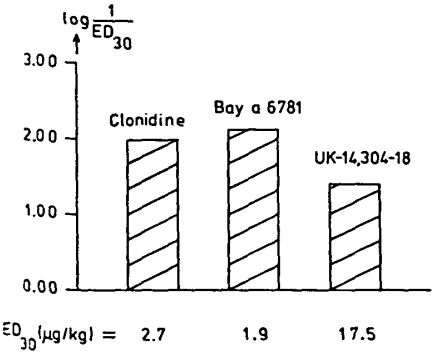

Fig. 32: Comparison between the hypotensive activities of clonidine, *Bay a 6781* and *UK-14, 304-18* upon intravenous administration to anaesthetized, normotensive rats. The logarithm of the reciprocal ED_{30}, expressed in µmol/kg, is given on the ordinate. The line at the bottom shows the corresponding ED_{30} in µg/kg. From Timmermans et al. (submitted for publication).

thetized, normotensive rats (Timmermans et al., submitted for publication). Dose-response curves were used to quantify their potencies as the dose required to invoke a 30 % decrease in mean arterial pressure (ED_{30}) (see fig. 32).

It strikes that the hypotensive activity of *Bay a 6781* exceeds that of clonidine following intravenous application to anaesthetized rats, in spite of the profound structural modification made in this molecule. *UK-14,304–18* exhibited a somewhat less hypotensive potency, although it can be considered an active derivative. *UK-14,304–18* also appeared less active than clonidine in normal human subjects (Ashton and Rawlins, 1978).

Imidazoquinazolines, like 1,2,3,5-tetrahydroimidazo [2,1-b] quinazoline *(TIQ)* were reported by Jen et al. (1972) as a new class of potential antihypertensive agents. TIQ was found to be the most effective compound in this series in lowering blood pressure in the metacorticoid hypertensive rat and the conscious neurogenic hypertensive dog by oral administration. α-Adrenergic blockade would explain the mechanism of action (Jen et al., 1972). The molecule of *TIQ* can be regarded a rigid 2-(arylimino)imidazolidine due to the presence of a methylene bridge, which interconnects the two ring systems. As a consequence thereof the molecule is forced into a nearly planar structure.

The acute effects of *TIQ* on blood pressure have been evaluated following application via the blood stream to anaesthetized, normotensive rats and cats (Timmermans and van Zwieten, 1978b) (see fig. 33).

TIQ caused an acute fall of a short duration which was followed by a recovery of the blood pressure to normal preinjection values, and thereupon a decrease gradually started. The α-sympatholytic properties of *TIQ* as suggested by Jen et al. (1972) could not be confirmed.

In spite of the pronounced peripheral vasopressive action (also see section 4.2.5.) of the classical α-sympathomimetic drugs naphazoline, oxymetazoline, tetryzoline, tramazoline and xylometazoline (for structural formulae see fig. 34) a dose-dependent reduction in arterial pressure could be established for these agents, oxymetazoline excepted, following intravenous administration to pentobarbitone (75 mg/kg, i. p.) anaesthetized, normotensive rats (Timmer-

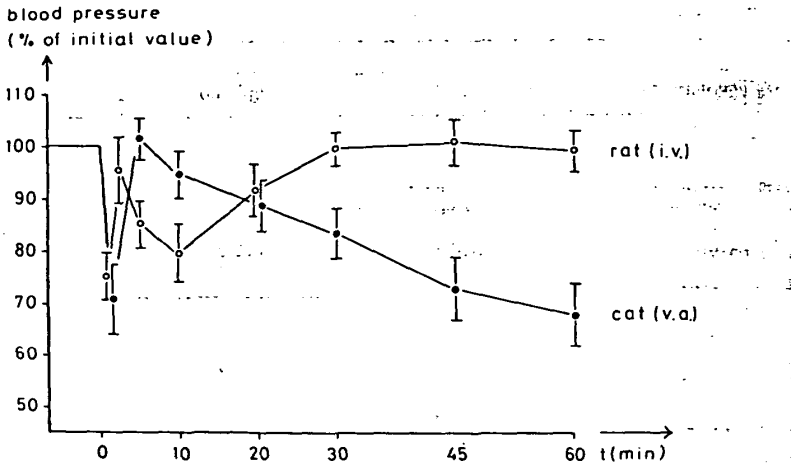

Fig. 33: Effect of *TIQ* on mean arterial blood pressure (means ± S.E.M.) following intravenous application o 10 mg/kg to anaesthetized, normotensive rats (o — o; n = 5) and after infusion of 3 mg/kg into the left vertebra artery of chloralose-anaesthetized cats (● — ●; n = 4). From Timmermans and van Zwieten (1978b) with permission.

mans et al. (1978 b). Fig. 35 shows the dose-response curves of the maximal effects after intravenous injections.

Within this series of 4 drugs naphazoline appeared most active in decreasing arterial pressure. These results agree with earlier observations made for intravenous tetryzoline (Hutcheon et al., 1958) and tramazoline (Struyker Boudier et al., 1974), but are at variance with

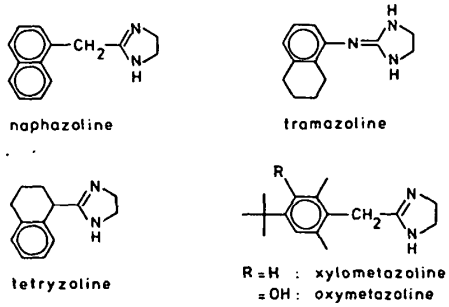

Fig. 34: Structural formulae of classical α-sympathomimetic drugs.

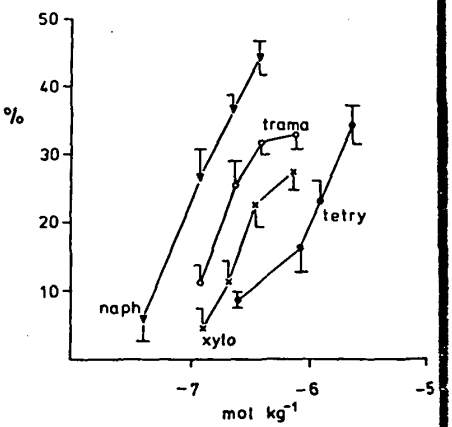

Fig. 35: Dose-response characteristics of the maxima decrease in mean arterial pressure after intravenou administration of naphazoline (naph), tramazolin (trama), xylometazoline (xylo) and tetryzoline (tetry to pentobarbitone-anaesthetized, normotensive rat (means ± S.E.M.; n = 4–5). Ordinate: Decrease i mean arterial pressure (% of pre-injection value). Abs cissa: Log_{10} dose (mol/kg). From Timmermans et al (1978b), with permission.

reported data on syste
zoline and tramazoline
1971); Kobinger and
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Table 5: Hypotensive activ
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anaesthetized rabbits. The
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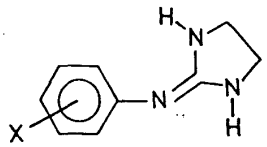
Compound	X
Unsubstituted	
St- 465	H
Mono-substituted (ortho)	
St- 371	2-CF_3
97	2-Et
96	2-Cl
391	2-Br
Mono-substituted (meta)	
St- 622	3-Cl
Mono-substituted (para)	
Hpt-1198	4-OH
St-2019	4-cyclo-Pr

reported data on systemic naphazoline, tetryzoline and tramazoline (Schmitt and Fénard, 1971); Kobinger and Pichler, 1975; Struyker Boudier, 1975).

A very great number of phenyl-substituted clonidine-like imidazolidines have been synthesized and tested for hypotensive activity. These compounds differ in the substituents attached at the phenyl ring and/or in the substitution pattern at this moiety. Of all chemical classes of molecules ever studied for potential hypotensive activity after the discovery of clonidine, this type of derivatives is far in the majority and gives the opportunity of systematically exploring the structure-activity relationship. Table 5 comprises the results for 156 imidazolidines which have been investigated with the aid of anaesthetized rabbits following intravenous administration. The figures listed are the doses (mg/kg) which lower arterial pressure by 20 mm Hg in this animal. The data summarized have been composed from the ones reported by Stähle (1974), Hoefke (1976), Hoefke et al. (in preparation and unpublished findings at C. H. Boehringer Sohn, Ingelheim (Hoefke and coworkers).

Table 5: Hypotensive activity, characterized as ED_{20} (effective dose, mg/kg, lowering arterial pressure by 20 mm Hg of phenyl-substituted imidazolidines obtained from dose-response curves after intravenous administration to anaesthetized rabbits. The compounds have been ranked in order of hypotensive potency within each set with identical substitution pattern. > : Weak or no activity at the amount indicated; higher doses not studied. The figures summarized have been composed from data reported by Stähle (1974), Hoefke (1976), Hoefke et al. (in preparation) and Hoefke (unpublished).



Compound	X	ED_{20} (mg/kg)	Compound	X	ED_{20} (mg/kg)
Unsubstituted					
St- 465	H	> 3.0			
Mono-substituted (ortho)					
St- 371	2-CF_3	0.3	*St- 90*	2-Me	1.2
97	2-Et	0.4	*681*	2-F	1.7
96	2-Cl	1.0	*690*	2-NO_2	> 1.0
391	2-Br	1.0	*2013*	2-SMe	> 1.0
			693	2-I	> 3.0
Mono-substituted (meta)					
St- 622	3-Cl	1.0	*St- 621*	3-Br	> 3.0
Mono-substituted (para)					
Hpt-1198	4-OH	2.7	*St- 624*	4-Cl	> 3.0
St-2019	4-cyclo-Pr	> 1.0	*625*	4-Br	> 3.0

Table 5 (continued)

Compound	X	ED_{20} (mg/kg)	Compound	X	ED_{20} (mg/kg)
Di-substituted (2,3)					
STH-2224	2,3-di-Br	0.006	*STH-2167*	2-Cl,3-F	0.1
2100	2-Cl,3-Br	0.01	*2169*	2-Br,3-F	0.1
St- 606	2-Me,3-Br	0.01	*St- 450*	2-Me,3-Cl	0.1
STH-2165	2-Br,3-Cl	0.015	*608*	2-Cl,3-Me	0.45
St- 476	2,3-di-Cl	0.03	*615*	2-Br,3-Me	> 3.0
Di-substituted (2,4)					
St- 375	2-Cl,4-Me	0.014			
691	2-Cl,4-NO_2	0.03	*St- 459*	2-Cl,4-F	0.9
612	2-Br,4-Me	0.05	*604*	2-F,4-Me	1.0
Hpt- 829	2,4-di-Me	0.10	*444*	2-Cl,4-Et	1.0
St- 692	2-Cl,4-NH_2	0.10	*486*	2-F,4-Cl	1.5
341	2-Me,4-Cl	0.10	*613*	2-CF_3,4-Br	3.0
455	2-Et,4-Cl	0.10	*Hpt-1203*	2-Cl,4-OMe	3.0
678	2-Br,4-Cl	0.12	*St-1948*	2,4-di-CF_3	> 1.0
Kö-1164	2-Cl,4-Br	0.15	*STH-2168*	2-CF_3,4-F	> 1.0
St- 363	2,4-di-Cl	0.17	*St- 611*	2,4-Di-F	> 3.0
592	2,4-di-Br	0.17	*607*	2-Me,4-F	> 3.0
453	2-Cl,4-t-Bu	0.25	*Hpt-1204*	2-Cl,4-OH	> 3.0
680	2-Me,4-Br	0.3	*St- 684*	2-Cl,4-CN	> 3.0
893	2-F,4-Br	0.45	*670*	2-OMe,4-Cl	> 3.0
STH-2173	2-Br,4-F	0.50	*601*	2-OH,4-Cl	> 3.0
Di-substituted (2,5)					
St- 600	2-Me,5-F	0.03	*St- 591*	2-Cl,5-Me	0.7
590	2-Me,5-Br	0.04	*610*	2,5-di-F	2.0
STH-2160	2-Br,5-F	0.06	*589*	2-Br,5-Me	0.3
St- 585	2-Me,5-Cl	0.065	*599*	2-OMe,5-Cl	> 1.0
STH-2162	2-Cl,5-F	0.1	*1949*	2-F,5-Me	> 1.0
St- 549	2-Br,5-Cl	0.1	*665*	2-Cl,5-OH	> 1.0
609	2-Cl,5-OMe	0.2	*587*	2-Cl,5-CF_3	> 3.0
475	2,5-di-Cl	0.25	*614*	2-Br,5-CF_3	> 3.0
603	2-Cl,5-Br	0.3	*672*	2-OH,5-Cl	> 3.0
602	2,5-di-Br	0.3	*2047*	2,5-di-OH	> 3.0
Di-substituted (2,6)					
St- 155	2,6-di-Cl	0.01	*St- 95*	2,6-di-Me	0.1
STH-2130	2-Br,6-F	0.01	*1697*	2-Me,6-Et	0.3
St-1974	2-CF_3,6-F	0.04	*1959*	2-Br,6-Me	0.4
1923	2-Cl,6-F	0.04	*1946*	2,6-di-OH	0.7
464	2,6-di-Br	0.045	*1945*	2,6-di-OMe	1.0
93	2-Cl,6-Me	0.05	*2038*	2-NH_2,6-NO_2	> 1.0
1964	2-Cl,6-CF_3	0.06	*1912*	2,6-di-F	> 3.0
1957	2,6-di-CF_3	0.06	*91*	2,6-di-Et	> 3.0
1962	2-Br,6-Cl	0.1	*1973*	2,6-di-i-Pr	> 3.0

Table 5 (continued)

Compound	X
Di-substituted (3,4)	
St-1943	3,4-di-OH
STH-2163	3-Br,4-F
2207	3,4-di-Br
2131	3-Cl,4-F
2120	3-CF_3,4-Cl
2147	3-CF_3,4-Br
Di-substituted (3,5)	
St- 474	3,5-di-Cl
593	3,5-di-CF_3
Tri-Substituted (2,4,5)	
St-1915	2,5-di-Cl,4-M
899	2,5-di-Cl,4-Br
737	2,4,5-tri-Cl
1824	2-Cl,4-Me,5-N
Tri-substituted (2,4,6)	
St-1942	2,6-di-Cl,4-M
888	2-Br,4-Cl,6-F
89	2,4,6-tri-Me
92	2,4,6-tri-Et
871	2,6-di-Cl,4-Br
894	2,4-di-Br,6-F
884	2-Cl,4-Br,6-M
921	2-Br,4-F,6-Mc
878	2,4-di-Cl,6-Br
732	2,4,6-tri-Cl
881	2,6-di-Et,4-Br
876	2-Br,4-Me,6-(
922	2,4-di-Br,6-M
906	2-Br,4-Cl,6-O
886	2-Br,4-t-Bu,6-
887	2,4-di-F,6-Br
Tri-substituted (3,4,5)	
STH-2084	3,4,5-tri-OH
Tetra-substituted	
St-1961	2,3,4,5-tetra-
1987	2,3,5,6-tetra-
Penta-substituted	
St-1956	2,3,4,5,6-pen-

Table 5 (continued)

Compound	X	ED_{20} (mg/kg)	Compound	X	ED_{20} (mg/kg)
Di-substituted (3,4)					
St-1943	3,4-di-OH	0.25	*STH-2108*	3-F,4-Me	> 1.0
STH-2163	3-Br,4-F	0.5	*2121*	3-Me,4-F	> 1.0
2207	3,4-di-Br	> 1.0	*2073*	3-Cl,4-OH	> 1.0
2131	3-Cl,4-F	> 1.0	*2133*	3-NH_2,4-F	> 1.0
2120	3-CF_3,4-Cl	> 1.0	*2125*	3-NO_2,4-F	> 1.0
2147	3-CF_3,4-Br	> 1.0	*2114*	3-OH,4-Me	> 1.0
			St- 473	3,4-di-Cl	> 3.0
Di-substituted (3,5)					
St- 474	3,5-di-Cl	3.0	*St-2055*	3,5-di-OH	> 1.0
593	3,5-di-CF_3	> 1.0			
Tri-Substituted (2,4,5)					
St-1915	2,5-di-Cl,4-Me	0.01	*St-1827*	2-Cl,4-Me,5-NH_2	1.0
899	2,5-di-Cl,4-Br	0.015	*STH-2088*	2,4-di-OH,5-Cl	> 1.0
737	2,4,5-tri-Cl	0.020	*2082*	2-Br,4,5-di-OH	> 1.0
1824	2-Cl,4-Me,5-NO_2	0.25	*2070*	2,5-di-OH,4-Br	> 1.0
Tri-substituted (2,4,6)					
St-1942	2,6-di-Cl,4-Me	0.01	*St-1952*	2,6-di-Cl,4-CH_2OH	0.3
888	2-Br,4-Cl,6-F	0.02	*895*	2,6-di-Br,4-Me	0.34
89	2,4,6-tri-Me	0.03	*908*	2-Br,4-F,6-Cl	0.35
92	2,4,6-tri-Et	0.03	*Hpt-1128*	2,4-di-Me,6-Cl	0.45
871	2,6-di-Cl,4-Br	0.035	*1129*	2,6-di-Me,4-Cl	0.55
894	2,4-di-Br,6-F	0.06	*St-1988*	2,4,6-tri-F	1.0
884	2-Cl,4-Br,6-Me	0.06	*889*	2,4-di-Br,6-CF_3	1.0
921	2-Br,4-F,6-Me	0.06	*Hpt-1127*	2,4-di-Cl,6-Me	1.0
878	2,4-di-Cl,6-Br	0.085	*St- 739*	2,4,6-tri-Br	1.2
732	2,4,6-tri-Cl	0.09	*896*	2-Br,4-Cl,6-Me	1.3
881	2,6-di-Et,4-Br	0.10	*Hpt-1210*	2,6-di-Cl,4-OMe	1.8
876	2-Br,4-Me,6-Cl	0.11	*St- 904*	2,6-di-Br,4-Cl	3.0
922	2,4-di-Br,6-Me	0.16	*666*	2,6-di-Cl,4-OH	> 1.0
906	2-Br,4-Cl,6-OMe	0.2	*2040*	2,6-di-Br,4-CH_2OH	> 1.0
886	2-Br,4-t-Bu,6-Cl	0.2	*1984*	2,6-di-Cl,4-COOH	> 1.0
887	2,4-di-F,6-Br	0.28	*101*	2,6-di-Me,4-t-Bu	> 1.0
Tri-substituted (3,4,5)					
STH-2084	3,4,5-tri-OH	0.5	*STH-2090*	3,5-di-Br,4-NH_2	> 1.0
Tetra-substituted					
St-1961	2,3,4,5-tetra-F	> 1.0	*St- 123*	2,3,5,6-tetra-Me	> 1.0
1987	2,3,5,6-tetra-F	> 1.0			
Penta-substituted					
St-1956	2,3,4,5,6-penta-F	> 1.0	*St-1924*	2,3,4,5,6-penta-Cl	> 1.0

Upon closer examination of this table 5 a few generalizations regarding the structure-activity relationship in this class of molecules can be made. The following qualitative considerations may give insight into the influence of substitution at the phenyl ring on hypotensive activity. However, in doing so, one should always keep in mind that the overall lipophilic properties of the drugs are a prerequisite for high hypotensive activity. The penetration of the compounds from the blood into the brain is superimposed upon the demands which are made upon the structural properties for a most favourable drug-receptor complex formation.

If the phenyl ring contains no substituent or is substituted with more than three groups, clonidine-like drugs result with very diminished hypotensive properties. Mono-substitution is most beneficial at the ortho-position. No effective substances are obtained within series with mono-substitution at the meta or the para-position. Among mono-ortho-substituted compounds those possessing large groups, which favour lipophilicity, are most potent.

Di-substitution increases hypotensive activity over mono-substitution, provided that it is performed at the correct position at the phenyl ring. An illustrative example is given in fig. 36 (Stähle, 1974; Hoefke, 1976).

The alteration of the 2,6-substitution of the chlorine atoms in clonidine into other substitution patterns is accompanied by a loss of hypotensive activity. The relatively high effectiveness of the 2,3-dichloro derivative is worth mentioning, when compared to the corresponding 2,5-isomer. The 2,4-compound occupies an intermediate position. Additionally, the hypotensive activity drops further for the 3,5 and 3,4-analogues.

Within 2,3-di-substituted imidazolidines the order of hypotensive activity for the substituents at the 3-position is: Br > Cl > F > Me. This order is found in 2-Cl as well as in 2-Br derivatives (fig. 37).

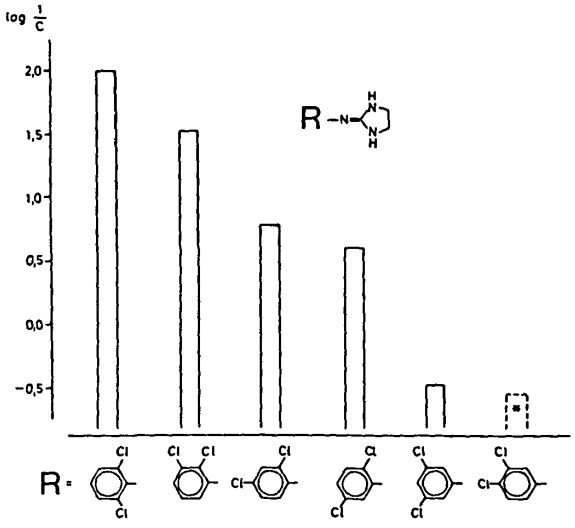
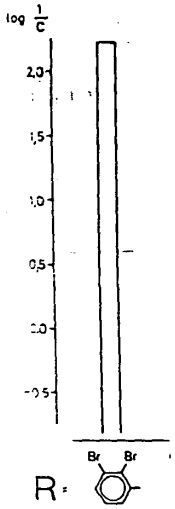


Fig. 36: Influence of the substitution pattern in dichlorophenyl-imidazolidines on hypotensive activity in anaesthetized rabbits following intravenous administration. The logarithm of the reciprocal dose (C, mg/kg) required to decrease arterial pressure by 20 mm Hg (ED_{20}) is given on the ordinate. The corresponding numerical values of the ED_{20} are listed in table. 5. *St*-473 (3,4-di-Cl) showed weak activity up to 3 mg/kg. By analogy with Stähle (1974) and Hoefke (1976), with permission.



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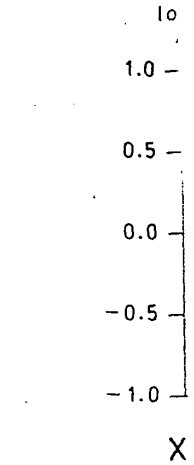

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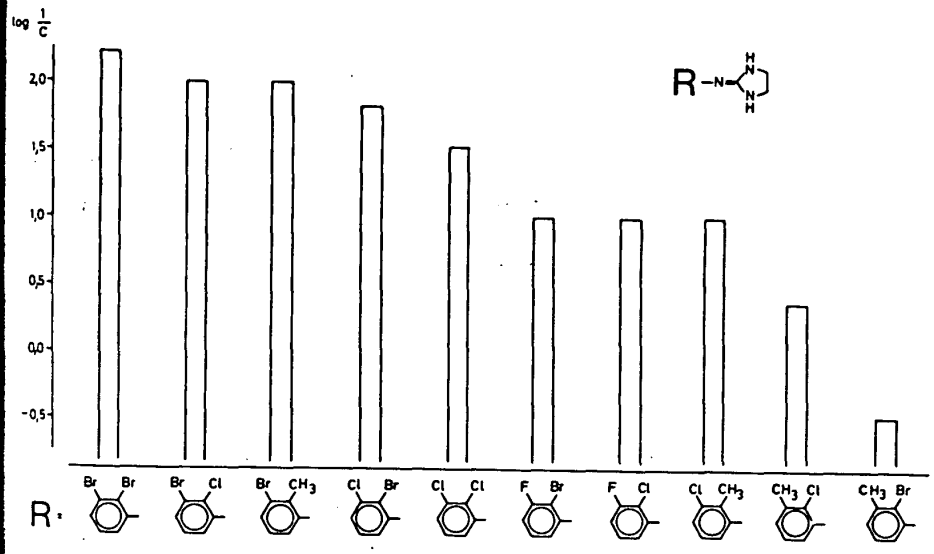

Fig. 35: Influence of various substituents located at the 3-position in 2-Br, 2-Cl and 2-Me-substituted (phenylimino)imidazolidines on hypotensive activity following intravenous administration to anaesthetized rabbits. The logarithm of the reciprocal dose (mg/kg) invoking a decrease in mean arterial pressure by 20 mm Hg (ED_{20}) is given on the ordinate. The corresponding numerical values of the ED_{20} are listed in table 5. From Hoefke et al. in preparation).

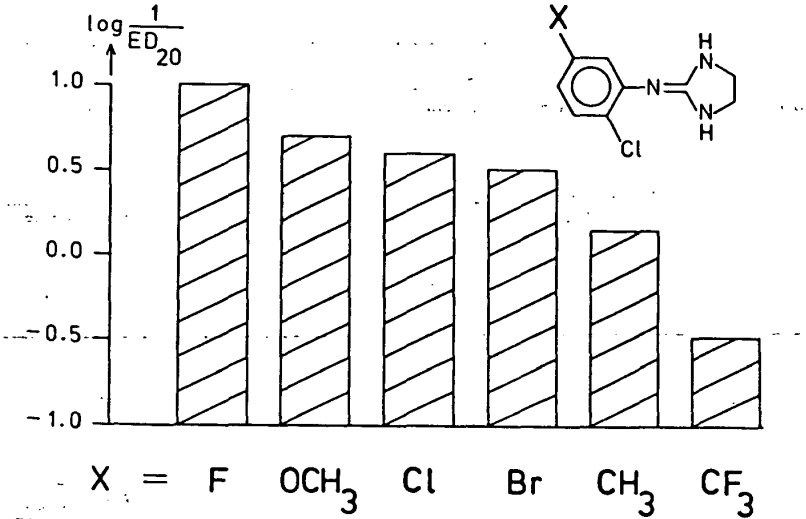

Fig. 36: The influence of various different substituents located at the 5-position in (2-chlorophenylimino)imidazolidines on hypotensive activity following intravenous administration to anaesthetized rabbits. The logarithm of the reciprocal dose (mg/kg) invoking a decrease in mean arterial pressure by 20 mm Hg (ED_{20}) is given on the ordinate. The corresponding numerical values of the ED_{20} are listed in table 5.

Molecules possessing a 2,4-di-substitution pattern preferably select a methyl group at their 4-position for high hypotensive activity. The ranking order is: Me > Br > Cl > F. This order of hypotensive activity can be deduced from 2-Cl, 2-Br, 2-F and 2-Me congeners. The 5-position in 2,5-di-substituted substances displays a surprisingly different preference for certain substituents to yield active hypotensive drugs. An example is given in fig. 38.

A fluorine at the 5-position in 2-Cl, 2-Br and 2-Me-substituted imidazolidines is the best candidate for high hypotensive activity followed by a methoxy group. The ranking order is further: Cl > Br > Me > CF_3. It is striking that this selection of substituents parallels the bulkness of the group almost perfectly. Small substituents, like fluorine and methoxy, favour hypotensive potency over larger ones such as bromine and CF_3.

Generally, derivatives with a 2,6-di-substitution pattern are the most active ones within the whole series of phenyl-substituted imidazolidines. Halogen atoms or a lipophilic group, like CF_3, should be placed at the ortho-positions. Every combination of chlorine, bromine, CF_3 and fluorine yields highly active molecules, an ortho, ortho' double fluorine excepted. Alkyl-substitution (methyl, ethyl, i-propyl) diminishes hypotensive activity (fig. 39).

Generally, but not in all cases a substituent at the 4-position reduces blood pressure-lowering activity compared to corresponding 2,6-di-substituted compounds in which this place at the phenyl ring is left unoccupied. The ranking order for the para-position in these substances is: Me > Br > Cl > OMe, which is in agreement with the order found for the 4-position in 2-4-analogues. As holds true for any position at the phenyl ring the incorporation of an hydroxy function abolishes the effectiveness. This is also encountered for polar groups, like CH_2OH and COOH. On the other hand, the introduction of an additional substituent at the 6-position of 2,4-di-substituted analogues increases hypotensive activity in many cases.

The acute effects of clonidine and 26 of its phenyl-substituted analogues on arterial pressure have been quantified in anaesthetized, normotensive rats after intravenous administra-

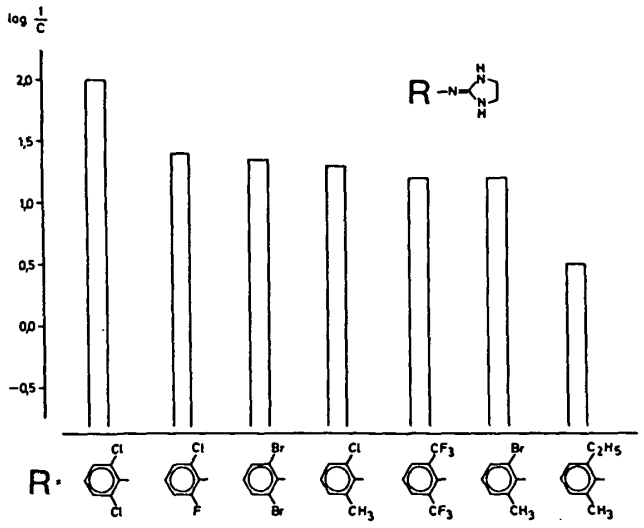

Fig. 39: Hypotensive activity in anaesthetized rabbits after intravenous administration of clonidine and some 2,6-di-substituted analogues. The logarithm of the reciprocal ED_{20}, expressed in mg/kg, is given on the ordinate. The corresponding numerical values of the ED_{20} are listed in table 5. From Hoefke et al. (in preparation).

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Zwieten, 1977a).
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Table 6: Hypotensiv
ED_{25}, of clonidine a
tion to anaesthetize

Compd. no.	X
1	2,6-di-Cl
2	2,6-di-Br
3	2,6-di-F
4	2-Br,6-Cl
5	2-Cl,6-F
6	2-Cl,6-Me
7	2,6-di-Me
9	2,3-di-Cl
10	2,4-di-Cl
11	2,5-di-Cl
12	2-Me,4-Cl
13	2-Cl,4-Me
14	2,4-di-Me
15	H
16	2-Cl
17	2-Me
18	2,4,6-tri-Cl
19	2,4,6-tri-Br
20	2,4,6-tri-Me
21	2,6-di-Cl,4-Br
22	2,6-di-Cl,4-M
23	2,6-di-Cl,4-O
24	2,6-di-Cl,4-N
25	2,4-di-Cl,6-M
26	2,4-di-Me,6-(
27	2,6-di-Me,4-(
28	2,6-di-Me,4-I

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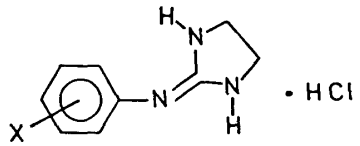
translated from the

tion (Timmermans 1976; Timmermans and van Zwieten, 1977a). The quantification of the hypotensive activity of the substances was achieved by calculating the logarithm of the reciprocal dose, expressed as μmol/kg, connected with a standard response of 30% decrease in arterial pressure (log $1/ED_{30}$). Dose-response curves were used to evaluate this

Table 6: Hypotensive activity, characterized as ED_{30}, and potency in decreasing cardiac frequency, quantified as ED_{25}, of clonidine and related imidazolidines calculated from dose-response curves after intravenous administration to anaesthetized, normotensive rats. Data from Timmermans and van Zwieten (1977a).



Compd. no.[a]	X	Hypotensive activity ED_{30} (μg/kg)	log ED_{30} (μg/kg)	log $1/ED_{30}$ (μmol/kg)	Potency in decreasing cardiac frequency ED_{25} (μg/kg)	log ED_{25} (μg/kg)
1	2,6-di-Cl	2.7	0.43	1.99	11[b]	1.04
2	2,6-di-Br	5.5	0.74	1.81	13	1.11
3	2,6-di-F	600	2.78	−0.41	235	2.37
4	2-Br,6-Cl	2.9	0.46	2.03	5.6	0.75
5	2-Cl,6-F	12.5	1.10	1.30	16	1.20
6	2-Cl,6-Me	19	1.28	1.11	28	1.45
7	2,6-di-Me	32	1.50	0.85	60	1.78
9	2,3-di-Cl	13	1.11	1.31	36	1.56
10	2,4-di-Cl	61	1.78	0.64	71	1.85
11	2,5-di-Cl	150	2.18	0.25	190	2.28
12	2-Me,4-Cl	275	2.44	−0.05	150	2.18
13	2-Cl,4-Me	53	1.72	0.67	64	1.81
14	2,4-di-Me	810	2.91	−0.56	560	2.75
15	H	25000	4.40	−2.10	–	–
16	2-Cl	170	2.23	0.13	280	2.45
17	2-Me	990	2.99	−0.67	510	2.71
18	2,4,6-tri-Cl	21	1.32	1.16	110[b]	2.04
19	2,4,6-tri-Br	580	2.76	−0.13	850	2.93
20	2,4,6-tri-Me	280	2.45	−0.07	160	2.20
21	2,6-di-Cl,4-Br	60	1.78	0.76	80	1.90
22	2,6-di-Cl,4-Me	21	1.32	1.12	23	1.36
23	2,6-di-Cl,4-OMe	2000	3.30	−0.83	1350	3.13
24	2,6-di-Cl,4-NO_2	4500	3.65	−1.16	4000	3.60
25	2,4-di-Cl,6-Me	79	1.90	0.55	82	1.91
26	2,4-di-Me,6-Cl	80	1.90	0.51	47	1.67
27	2,6-di-Me,4-Cl	240	2.38	0.03	200	2.30
28	2,6-di-Me,4-Br	700	2.84	−0.36	375	2.57

[a] Numbering refers to table 4.
[b] Extrapolated from the dose-response curve.

biological parameter. For this series of structurally very similar molecules this concentration was found in the middle of the linear part of nearly all the dose-response characteristics and resembled a 50% maximal activity in most cases. The results have been summarized in table 6.

The data listed in table 6, connected with the hypotensive activity, will be examined more closely with the aid of some selections of compounds, so that the influences of the substituents on this cardiovascular phenomenon observed in the anaesthetized, normotensive rat, will be more clear. For instance, fig. 40 shows how the hypotensive activity varied when the chlorine atoms of clonidine were substituted by other halogen atoms or methyl groups. Its strikes immediately that the derivative in which one chlorine atom was replaced by bromine (no. *4*) possessed high hypotensive activity. The potency of this particular molecule was slightly higher than that of clonidine, although not significantly. Substitution of one chlorine atom by fluorine (no. *5*) was accompanied by a loss of blood pressure-lowering activity. Compared to clonidine its bromine analogue (no. *2*) was about twice less active. Moreover, substitution by fluorine atoms (no. *3*) resulted in a dramatic reduction in potency. Replacing one chlorine atom by methyl (no. *6*) also caused a decrease in hypotensive activity. It decreased further when both chlorine atoms were deputized for methyl groups (no. *7*). The variation in hypotensive activity within this series of 2,6-di-substituted molecules found in the anaesthetized, normotensive rat corresponds in a general sense with the one established in the anaesthetized rabbit described above.

From the selection of compounds which is presented in fig. 41 it can be concluded that any substitution at the 4-position of the phenyl ring of clonidine leads to a derivative of which the hypotensive activity declined compared to that of the parent compound with an unoccupied para-position. Substitution by chlorine (no. *18*) or methyl (no. *22*) resulted in approximately equipotent substances. But going from bromine (no. *21*) via methoxy (no. *23*) to nitro (no. *24*) a rapid fall in hypotensive activity was observed. In anaesthetized, normotensive rats the same

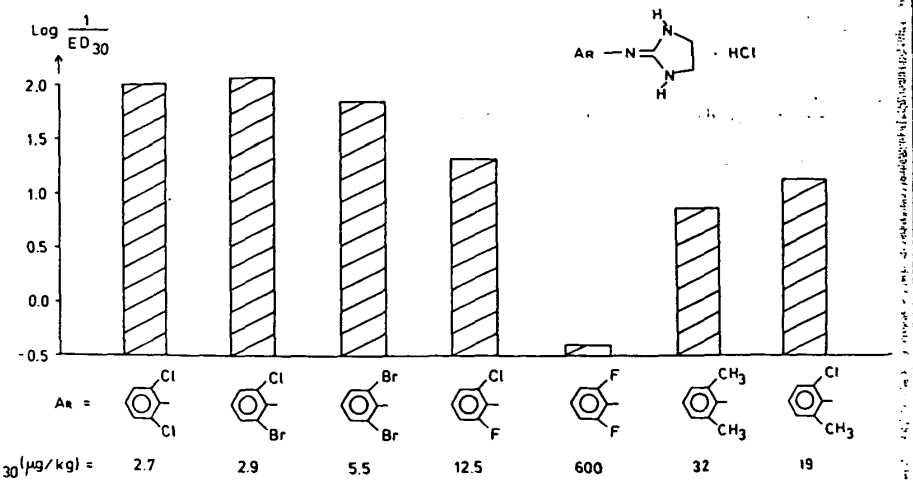


Fig. 40: Hypotensive activity in anaesthetized, normotensive rats after intravenous administration of clonidine and some 2,6-di-substituted analogues in which one or two chlorine atoms were replaced by other halogen atoms or methyl groups. The logarithm of the reciprocal ED_{20}, expressed in µmol/kg, is given on the ordinate. The line at the bottom lists the corresponding ED_{30} in µg/kg (also see table 5). From Timmermans and van Zwieten (1977a), with permission.

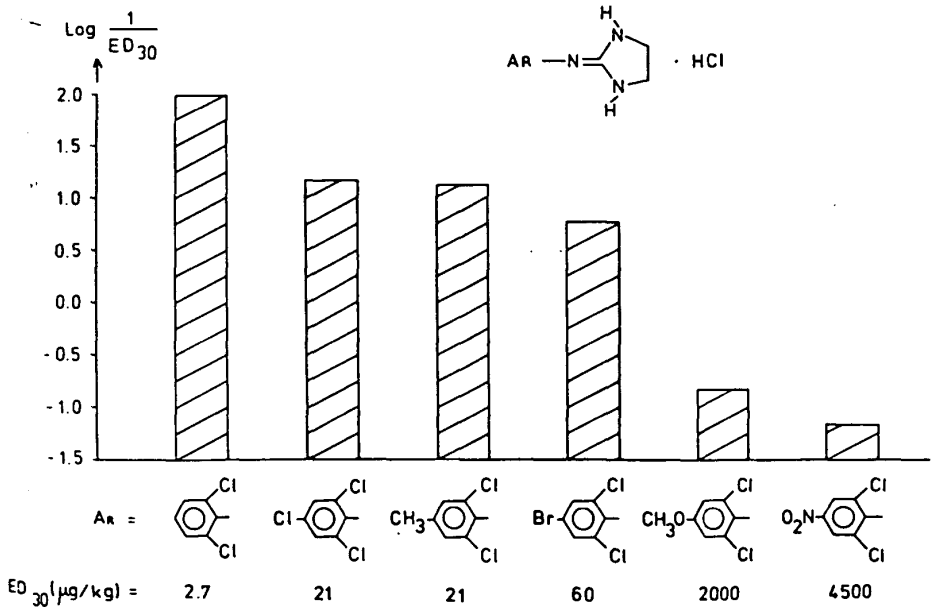


Fig. 41: Influence of para-substitution of clonidine on hypotensive activity in anaesthetized, normotensive rats after intravenous administration. The logarithm of the reciprocal ED_{30}, expressed in µmol/kg, is given on the ordinate. The line at the bottom lists the corresponding ED_{30} in µg/kg (also see table 6). From Timmermans and van Zwieten (1977a), with permission.

influences of 4-substituents on hypotensive activity (Cl ≈ Me > Br) were seen for 2,6-dimethyl-substituted imidazolidines.

In addition, upon changing the substitution pattern from 2,6 into 2,4 the hypotensive activity greatly diminished. A further loss of potency was observed for mono-ortho-substituted congeners. The unsubstituted analogue (no. *15*) was almost devoid of blood pressure-lowering activity in the anaesthetized, normotensive rat, resulting in an ED_{30} of 25 mg/kg. Comparable observations were made in the anaesthetized rabbit (see above).

In the normotensive, anaesthetized rat the alteration of the substitution pattern of the chlorine atoms of clonidine follows a similar relative sequence of activities as reported above for the anaesthetized rabbit (compare fig. 42 with fig. 36).

A few other studies have been made in which the hypotensive activities of a limited number of clonidine-analogues have been compared. Clonidine and 5 of its phenyl-substituted derivatives were injected intravenously into anaesthetized cats. The doses were chosen so that comparable hypotensive actions were achieved (Hoefke et al., 1975). The relative efficacy versus clonidine (= 1.0) was estimated from the relationship of approximately equipotent doses (see table 7).

All substances, *St-91* (2,6-di-Et) excepted, exerted a pattern of response typical for clonidine, i.e. an initial short-lasting blood pressure increase followed by a prolonged hypotension (and bradycardia). From table 7 it can be seen that these «clonidine positive» substances were all less active than clonidine. Compound *St-91* (2,6-di-Et) exhibited a distinct

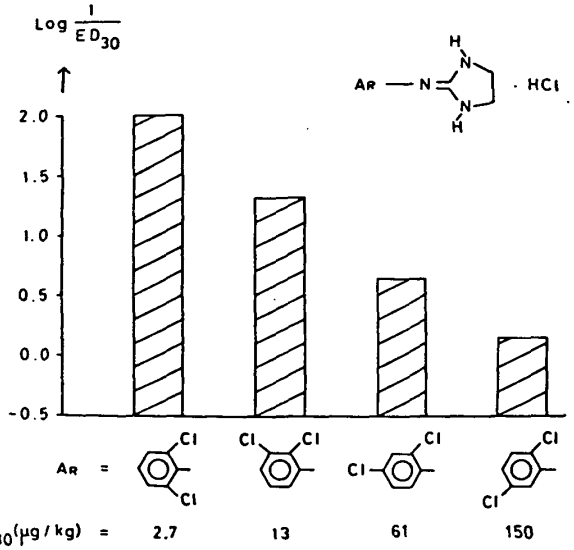


Fig. 42: Influence of alteration of the substitution pattern of the chlorine atoms of clonidine on the hypotensive activity in anaesthetized, normotensive rats after intravenous administration. The logarithm of the reciprocal ED_{30}, expressed in µmol/kg, is given on the ordinate. The line at the bottom lists the corresponding ED_{30} in µg/kg (also see table 6). From Timmermans and van Zwieten (1977a), with permission.

hypertensive effect, but only in 3 out of 14 experiments hypotension developed thereafter. Intravenous administration of these compounds to conscious and anaesthetized dogs yielded similar results (Hoefke et al., 1975). They evoked a decrease in arterial pressure preceded by an initial hypertensive phase. Compound *St-91* (2,6-di-Et) only produced a short-lasting rise in arterial pressure.

Table 7: Relative hypotensive potencies of some phenyl-substituted imidazolidines with respect to clonidine (= 1.0). The compounds were administered intravenously to anaesthetized cats. Data from Hoefke et al. (1975).

Compd. no.[a]	(X)	Dose range (µg/kg)	Relative hypotensive potency
St- 155	(2,6-di-Cl)	1 – 10	1.0
93	(2-Cl,6-Me)	10 – 30	0.3
375	(2-Cl,4-Me)	10 – 30	0.1 – 0.3
600	(2-Me,5-F)	30 – 100	0.03 – 0.1
608	(2-Cl,3-Me)	100 – 300	0.03 – 0.1
91	(2,6-di-Et)	3 – 300	[b]

[a] Numbering refers to table 5.
[b] Hypotension in 3 out of 14 experiments.

In a comparative study Laverty (1969) injected clonidine (0.2 mg/kg) and 6 analogues (1.0 mg/kg) subcutaneously into conscious, normotensive rats. The effects of the compounds on blood pressure were followed during 24 hours by using a tail-cuff technique. Falls in arterial pressure were noticed in these animals for all drugs and they had similar durations of action.

A screening of 23 phenyl-substituted imidazolidines including clonidine, for hypotensive activity by means of intravenous injections into anaesthetized, normotensive rats revealed a hypotensive action for 15 congeners only (Rouot et al., 1977). These 15 derivatives were studied in more detail. The dose was quantified for a 15 % decrease in arterial pressure 15 minutes after intravenous injection. The results are given in table 8.

Table 8: Hypotensive activities of 15 phenyl-substituted imidazolidines quantified following intravenous administration to anaesthetized, normotensive rats. Hypotensive activity is given as the logarithm of the reciprocal dose (mol/kg) causing a 15% decrease in arterial pressure 15 minutes after application. Data from Rouot et al. (1977).

Compd. no.[a]	(X)	Hypotensive activity	Compd. no.[a]	(X)	Hypotensive activity
2	(2,6-di-Br)	8	11	(2,5-di-Cl)	[b]
1	(2,6-di-Cl)	7.66	30	(2,6-di-i-Pr)	[b]
29	(2,4,5-tri-Cl)	7.26	32	(2-Me,5-Cl)	[b]
9	(2,3-di-Cl)	7.22	3	(2,6-di-F)	[b]
6	(2-Cl,6-Me)	7.12	36	(2-CF_3)	[b]
10	(2,4-di-Cl)	6.96	37	(2,6-di-Cl,4-OH)	[b]
13	(2-Cl,4-Me)	6.82	34	(4−Br)	[b]
18	(2,4,6-tri-Cl)	6.52			

[a] Numbering refers to table 4.
[b] Hypotension less than 15%.

Rouot et al. (1977) succeeded in the quantification of the hypotensive activities for only 8 compounds out of the 15 studied. It should be noted, however, that many of these derivatives found weakly or not effective by them, displayed readily measurable hypotensive properties according to other authors (see tables 5 and 6).

4.2.2. Central Hypotensive Activity

Among the various techniques, employed in order to obtain evidence for a central mechanism of action of hypotensive drugs, infusion via the cat's vertebral artery has been used frequently. This method has contributed to the discovery of the central hypotensive action of clonidine (see Chapter I). If it is observed that a drug administered in low doses via a vertebral artery is more effective than after systemic (usually intravenous) application, it seems very likely that the compound in question possesses substantial central hypotensive activity.

The central hypotensive activity of clonidine and 8 of its structurally related derivatives has been determined by infusions into the left vertebral artery of chloralose-anaesthetized cats (Timmermans and van Zwieten, 1977b). The effects were compared with those obtained after intravenous injections of identical doses in order to test possible central hypotensive activity. The compounds applied to the left vertebral artery immediately reduced arterial pressure to a larger degree than after systemic application. In addition, the α-sympatholytic agent piperoxan, administered via the same, central route significantly antagonized these centrally induced, hypotensive responses, indicating the involvement of central α-adrenoceptors (also see Schmitt et al., 1971, 1973).

In order to allow mutual comparison the maximal hypotensive effects of the imidazolidines after infusion via the cat's left vertebral artery are summarized in fig. 43.

Clonidine (2,6-di-Cl), compound no. *4* (2-Br, 6-Cl) and no. *2* (2,6-di-Br) appeared very active in lowering arterial pressure after infusion of low doses into the cat's left vertebral artery. The central hypotensive effects of the compounds proved dose-dependent in accordance to the one of clonidine reported earlier (Sattler and van Zwieten, 1967). The efficacy of no. *4* (2-Br, 6-Cl) was found comparable to clonidine. The high potency of this analogue is accentuated by its strong effect at a dose as low as 0.3 μg/kg. Compound no. *2* (2,6-di-Br) was a little less potent than clonidine. A somewhat higher dose of no. *5* (2-Cl, 6-F) was required to bring about comparable hypotensive effects as needed for the derivatives mentioned above. The compounds no. *11* (2,5-di-Cl), no. *16* (2-Cl) and no. *8* (2,6-di-Et) also showed central hypotensive activities, but they were considerably less active

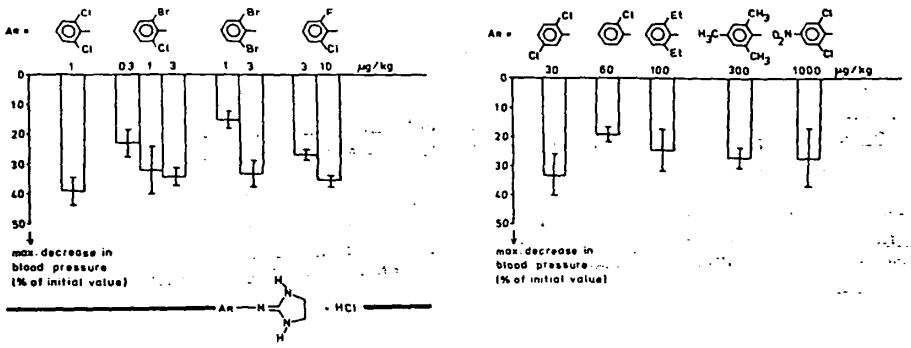


Fig. 43: Maximal decrease in mean arterial pressure of chloralose-anaesthetized cats (means ± S.E.M.) brought about by clonidine and a number of its structurally related imidazolidines after infusion into the left vertebral artery. From Timmermans and van Zwieten (1977b), with permission.

than clonidine. Rather high doses of no. *20* (2,4,6-tri-Me) and no. *24* (2,6-di-Cl, 4-NO_2) were necessary in order to diminish arterial pressure by approximately 30 % of the pre-injection value. For all compounds studied the doses (µg/kg) associated with a 25 % decrease in arterial pressure (ED_{25}) were estimated. The results are enumerated in table 9.

Table 9: Central hypotensive activities, quantified as estimated ED_{25}, of clonidine and a number of structurally related imidazolidines, infused into the left vertebral artery of chloralose-anaesthetized cats. Data from Timmermans and van Zwieten (1977b).

Compd. no.[a]	(X)	Central hypotensive activity ED_{25} (µg/kg)	log ED_{25} (µg/kg)
4	(2-Br,6-Cl)	0.44	−0.36
1	(2,6-di-Cl)	0.52	−0.28
2	(2,6-di-Br)	1.85	0.27
5	(2-Cl,6-F)	2.35	0.37
11	(2,5-di-Cl)	18.6	1.27
16	(2-Cl)	82	1.91
8	(2,6-di-Et)	100	2.00
20	(2,4,6-tri-Me)	270	2.43
24	(2,6-di-Cl,4-NO_2)	880	2.94

[a] Numbering refers to table 4.

Evidence for a central hypotensive action can also be obtained by direct administration of the drug to the brain. Injections into the cisterna cerebellomedullaris or into the cerebral ventricles are relatively simple experimental procedures in which the blood-brain barrier does not play a substantial part.

The central hypotensive activities of 5 imidazolidines have been compared with the parent compound clonidine by means of injections into the cisterna of anaesthetized cats (Hoefke et al., 1975). Each animal received the test substance first and after sufficient recovery clonidine as the reference drug. The doses were chosen in such a manner that similar hypotensive responses were obtained. The relative activity towards clonidine (= 1.0) was estimated (see table 10).

Intracisternal administration of the compounds elicited hypotensive responses similar as clonidine. All the substances, including *St-91* (2,6-di-Et), were effective via this application technique, although less active than clonidine itself. In rabbits *St-91* (0.3 µg/kg) was as active as 1.0 µg/kg of clonidine in reducing arterial pressure after intracisternal injection (Hoefke, 1976; Warnke and Hoefke, 1977).

Clonidine (1 µg/kg), naphazoline, oxymetazoline, *St-91* (2,6-di-Et), *St-1697* (2-Me, 6-Et), *St-363* (2,4-di-Cl) and xylazine, all in 10 µg/kg, were administered intracisternally to

Table 10: Rel
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The compound
thetized cats. D

Compd. no.[a]	(
St-155	(2,6-d
93	(2-Cl,
375	(2-Cl,
91	(2,6-di
608	(2-Cl,
600	(2-Me,

[a] Numbering refers t

atropine-treate
1975). A signif
observed for cl
nificant change
and *St-1697* (2-
in arterial press

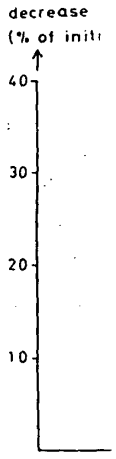


Fig. 44: Dose-respi
of clonidine (2,6-d
(means ± S.E.M.)

Table 10: Relative central hypotensive activities of some imidazolidines with respect to clonidine (= 1.0). The compounds were given intracisternally to anaesthetized cats. Data from Hoefke et al. (1975).

Compd. no.[a]	(X)	Dose range (µg/kg)	Relative central hypotensive activity
St-155	(2,6-di-Cl)	0.3 – 1.0	1.0
93	(2-Cl,6-Me)	3 – 30	0.1 – 0.3
375	(2-Cl,4-Me)	1 – 10	0.1 – 0.3
91	(2,6-di-Et)	3 – 30	0.1 – 0.3
608	(2-Cl,3-Me)	10 – 30	0.03
600	(2-Me,5-F)	30 – 100	0.01 – 0.03

[a] Numbering refers to table 5.

atropine-treated dogs (Kobinger and Pichler, 1975). A significant fall in blood pressure was observed for clonidine and xylazine and no significant changes for naphazoline, oxymetazoline and *St-1697* (2-Me, 6-Et). A significant increase in arterial pressure was noticed for *St-91* (2,6-di-Et) and *St-363* (2,4-di-Cl). On the other hand, naphazoline, oxymetazoline and *St-363* (2,4-di-Cl) injected intracisterally to vagotomized cats in a dose of 30 µg/kg significantly decreased blood pressure (Kobinger and Pichler, 1975).

The central hypotensive activity of a number of clonidine-like imidazolidines has also been demonstrated through intracerebroventricular injections into anaesthetized rats (Rouot et al., 1977). Most of the compounds already referred to in table 8 with respect to their hypotensive activity following intravenous application were employed, including no. *8* (*St-91*, 2,6-di-Et). All compounds which showed hypotensive activity following systemic application (see table 8) were also effective after central administration. Additionally, compounds no. *8* (2,6-di-Et), no. *37* (2,6-di-Cl, 4-OH) and no. *11* (2,5-di-Cl) provoked a hypotensive response, although this action was apparent only 45 minutes after administration of the latter.

4.2.3. Antihypertensive Activity

Clonidine and a number of its structurally related derivatives have been studied with

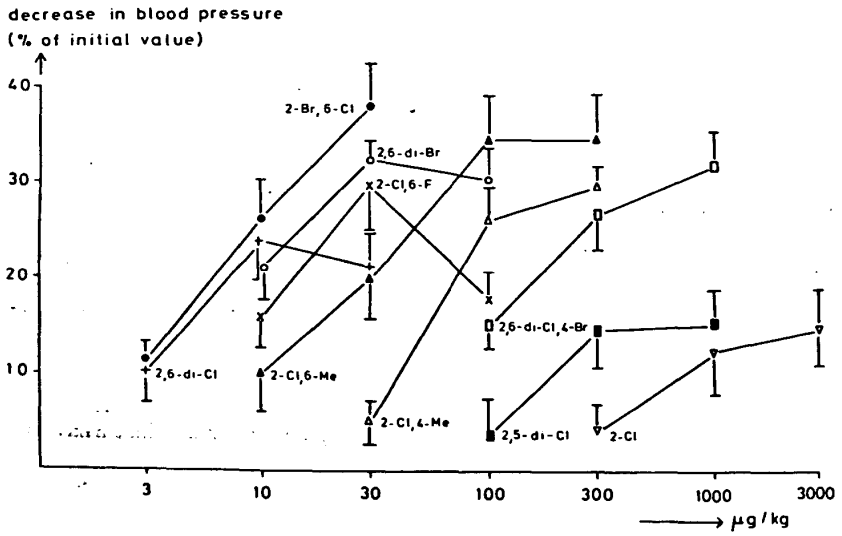


Fig. 44: Dose-response curves of the maximal decrease in mean arterial pressure after intravenous administration of clonidine (2,6-di-Cl) and some structurally related imidazolidines to conscious, genetically hypertensive rats (means ± S.E.M.). From Timmermans and van Zwieten (1977b), with permission.

respect to their antihypertensive properties in the conscious, genetically hypertensive rat after intravenous application (Timmermans and van Zwieten, 1977b). The antihypertensive effect of the drugs was established by determining the maximal decrease in mean arterial pressure after administration of various doses. The dose-response characteristics are given in fig. 44.

Compound no. *4* (2-Br, 6-Cl) appeared to be the most potent in this animal model. Its antihypertensive activity was very pronounced and exceeded that of clonidine at higher doses. For clonidine, compounds no. *2* (2,6-di-Br) and no. *5* (2-Cl, 6-F) it was observed that the effect on blood pressure was less at a higher dose than at a lower one, at which these drugs had apparently reached their maximal plateau of activity. Compound no. *11* (2,5-di-Cl) and no. *16* (2-Cl) affected blood pressure to a smaller extent. Their maximal responses amounted to a 15 % decrease. When the antihypertensive responses of the derivatives were compared one hour after injection the prolonged depressor effect of compound no. *13* (2-Cl, 4-Me) and especially of no. *6* (2-Cl, 6-Me) were remarkable.

The antihypertensive activity of the drugs was quantified by means of the logarithm of the dose (μg/kg), required to induce a 20 % decrease in mean arterial pressure (log ED_{20}), calculated from the dose-response curves. Compounds no. *11* (2,5-di-Cl) and no. *16* (2-Cl) were not able to reach this response and consequently no ED_{20} could be obtained. The data are reported in table 11.

By means of subcutaneous injections to conscious, genetically hypertensive rats Laverty (1969) investigated the antihypertensive properties of clonidine and 6 analogues by using a tail-cuff technique. A comparison was made beween approximately equipotent doses (see table 12).

Table 11: Antihypertensive activities, characterized as ED_{20}, of clonidine and a number of structurally related imidazolidines obtained from dose-response curves after intravenous administration to conscious, genetically hypertensive rats. Data from Timmermans and van Zwieten (1977b).

Compd. no.[a]	(X)	Antihypertensive activity ED_{20} (μg/kg)	log ED_{20} (μg/kg)
4	(2-Br,6-Cl)	6.0	0.78
1	(2,6-di-Cl)	7.2	0.86
2	(2,6-di-Br)	9.0	0.95
5	(2-Cl,6-F)	14	1.15
6	(2-Cl,6-Me)	30	1.48
13	(2-Cl,4-Me)	72	1.86
21	(2,6-di-Cl,4-Br)	112	2.05

[a] Numbering refers to table 4.

Table 12: A comparison between approximately equipotent doses (mg/kg; subcutaneously) of some phenyl-substituted imidazolidines on arterial pressure of conscious, genetically hypertensive rats. Data from Laverty (1969).

Compd. no.[a]	(X)	Antihypertensive property
St-155	(2,6-di-Cl)	0.1
464	(2,6-di-Br)	0.4
600	(2-Me,5-F)	0.4
608	(2-Cl,3-Me)	0.4
375	(2-Cl,4-Me)	0.7
612	(2-Br,4-Me)	1.5
678	(2-Br,4-Cl)	1.5

[a] Numbering refers to table 5.

A number of chemical classes more or less related to the fundamental phenyliminoimidazolidine structure of clonidine has been explored for antihypertensive activity in metacorticoid hypertensive rats and conscious, neurogenic hypertensive dogs following oral administration (Jen et al., 1975). Of all structures investigated clonidine was found most potent in these experimental animals. Replacement of one of the imidazolidine nitrogen atoms by a methylene group and/or attaching a methyl or an acetyl function at one or at both of them gave compounds retaining much of the antihypertensive potency. Compounds with other hetero atoms (oxygen or sulphur) had greatly diminished activities. Expanding or opening the imidazolidine ring reduced the potency. Aromatization of this nucleus (imidazoles)

afforded less potent compounds and fusion with a benzene ring nearly abolished activity. Many of these generalizations with respect to the structure-activity relatioship found by Jen et al. (1975) agree well with the features already dealt with above.

4.2.4. Bradycardic Activity

The fall in heart rate induced by clonidine is generally attributed to a central nervous inhibition of sympathetic tone. In addition, an increase in vagal tone has been shown to contribute to the reduction in cardiac frequency. Clonidine facilitates the vagally mediated part of the baroreceptor reflex. This facilitatory effect of clonidine upon this vagal reflex could be located within the medulla oblongata, the same region in which it has been shown that clonidine decreases the sympathetic activity. Finally, an action on presynaptic α-adrenoceptors in the heart, which impairs adrenergic transmitter release, may also contribute peripherally to the bradycardia (Kobinger, 1978; also see Chapter I).

The bradycardic properties of clonidine and 26 of its phenyl-substituted analogues have been evaluated following intravenous application to anaesthetized, normotensive rats (Timmermans and van Zwieten, 1977a). Dose-response curves were constructed from the effects measured at the moment of maximal decrease in blood pressure.

The potency of the clonidine-like drugs in causing bradycardia was quantified by means of the logarithm of the dose (μg/kg), required to obtain a 25 % decrease in heart rate (log ED_{25}), calculated from the dose-response characteristics. The data are reported in table 6.

As can be seen from table 6 (page 137), bradycardia was provoked by all the molecules. The unsubstituted derivative no. *15* was an exception in that it brought about only minor effects on cardiac frequency. Upon comparison of the bradycardic data with the hypotensive ones listed in the same table, it appears that, in general, these compounds were somewhat less active in decreasing heart rate by 25 % than in lowering arterial pressure by 30 % or about equally potent with respect to these two cardiovascular phenomena.

A dose-dependent reduction in cardiac frequency has also been obtained following intravenous injection of the classical α-sympathomimetic drugs naphazoline, oxymetazoline, tetryzoline, tramazoline and xylometazoline into anaesthetized, normotensive rats (Timmermans et al., 1978b). The curves are visualized in fig. 45. The sequence of bradycardic activity parallels the one of the hypotensive potency (see fig. 35), i. e. naphazoline > tramazoline > xylometazoline > tetryzoline.

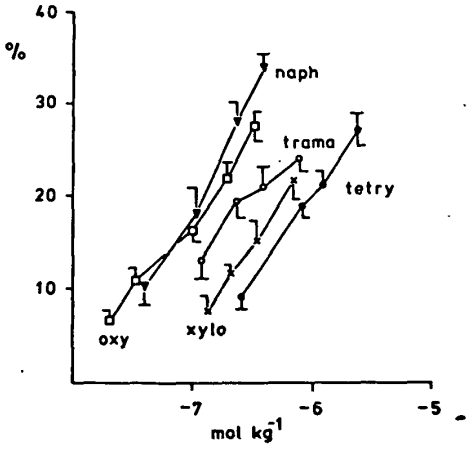


Fig. 45: Dose-response characteristics of the maximal decrease in heart rate after intravenous administration of oxymetazoline (oxy), naphazoline (naph), tramazoline (trama), xylometazoline (xylo) and tetryzoline (tetry) to pentobarbitone-anaesthetized, normotensive rats (means ± S.E. M.). Ordinate: Decrease in heart rate (% of pre-injection value). Abscissa: $\log_{10}$ dose (mol/kg). From Timmermans et al. (1978b), with permission.

The influence of clonidine and a number of structurally related imidazolidines on cardiac frequency has been determined following intravenous administration to conscious, genetically hypertensive rats (Timmermans and van Zwieten, 1977b). The decrease in heart rate was measured at the moment of maximal depressor effect and plotted against the logarithm of the dose applied (see fig. 46).

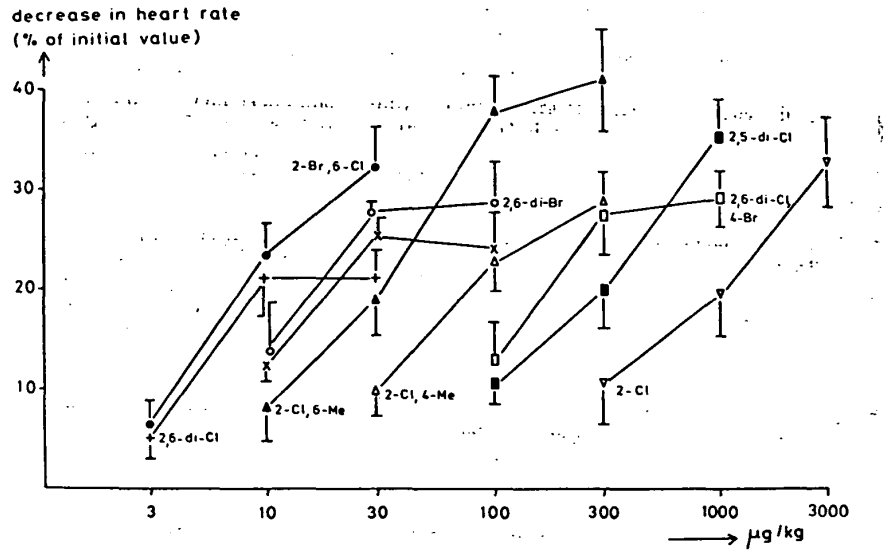


Fig. 46: Dose-response characteristics of the decrease in heart rate at the moment of maximal depressor effect after intravenous administration of clonidine (2,6-di-Cl) and some structurally related imidazolidines to conscious, genetically hypertensive rats (means ± S.E.M.). From Timmermans and van Zwieten (1977b), with permission.

Compound no. *4* (2-Br, 6-Cl) lowered cardiac frequeny to a comparable degree as clonidine at lower doses, but reached a higher plateau of maximal activity. Compound no. 6 (2-Cl, 6-Me) attained the greatest effect on heart rate. The derivatives no. *11* (2,5-di-Cl) and no. *16* (2-Cl) affected heart rate to a greater extent than blood pressure (cf. with fig. 44). The potency of the molecules in causing bradycardia was put on a comparable scale by the logarithm of the dose (µg/kg) which invoked a 20 % reduction in cardiac frequency (log ED_{20}), calculated from the dose-response curves. The data are reported in table 13.

Upon comparing the data listed in table 11 (antihypertensive activity; page 144) with those reported in table 13 (bradycardic potency) a comparable order of potency can be observed.

Bradycardia was measured in anaesthetized rats after stereotactic application of a number of imidazolidines and imidazolines in the anterior hypothalamic area (Struyker Boudier et al., 1975). The bradycardic activity of the drugs was

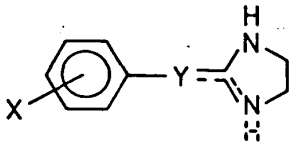
Table 13: Potency in decreasing cardiac frequency, quantified as ED_{20}, of clonidine and a number of structurally related imidazolidines. ED_{20} (dose, µg/kg, for a 20% reduction) was calculated from dose-response curves after intravenous administration to conscious, genetically hypertensive rats. Data from Timmermans and van Zwieten (1977b).

Compd. no.[a]	(X)	Potency in decreasing cardiac frequency ED_{20} (µg/kg)	log ED_{20} (µg/kg)
4	(2-Br,6-Cl)	7.9	0.90
1	(2,6-di-Cl)	9.3	0.97
2	(2,6-di-Br)	16	1.20
5	(2-Cl,6-F)	20	1.30
6	(2-Cl,6-Me)	33	1.52
13	(2-Cl,4-Me)	79	1.90
21	(2,6-di-Cl,4-Br)	170	2.23
11	(2,5-di-Cl)	310	2.49
16	(2-Cl)	1100	3.04

[a] Numbering refers to table 4.

expressed as the minimal dose causing a decrease in heart rate of at least 40 beats/minute lasting for at least 20 minutes (see table 14).

Table 14: Bradycardic activities of some imidazolidines and imidazolines. Minimal dose (nmol) required to induce a decrease in heart rate of more than 40 beats/minute after direct injections into the anterior hypothalamic area of anaesthetized rats. Data from Struyker Boudier et al. (1975).



Compd. no.[a]	(X)	Y	Minimal dose (nmol) inducing bradycardia of 40 beats/minute
St-1943	(3,4-di-OH)	N	3
155	(2,6-di-Cl)	N	7
464	(2,6-di-Br)	N	12
666	(2,6-di-Cl,4-OH)	N	20
Tramazoline			40
St- 600	(2-Me,5-F)	N	50
Tetryzoline			60
St- 91	(2,6-di-Et)	N	> 50
1913	(2,6-di-Cl)	CH_2	> 50
71	(2,4,6-tri-Me)	CH_2	>100
Naphazoline			>100
Oxymetazoline			>100
Xylometazoline			>100

[a] Numbering refers to table 5.

After intrahypothalamic injection only a limited number of the drugs tested induced bradycardia. Compound *St-1943* (3,4-di-OH) was the most potent one. Clonidine *(St-155), St-464* (2,6-di-Br), *St-666* (2,6-di-Cl,4-OH), tramazoline, *St-600* (2-Me, 5-F) and tetryzoline were also found effective in decreasing order of potency. Naphazoline, xylometazoline, *St-71* and also oxymetazoline were without effect at doses up to 100 nmol. *St-91* (2,6-di-Et) and *St-1913* lacked any effect up to doses of 50 nmol.

Bradycardia in vagotomized, atropine- pretreated rats has been used as a measure of the decrease in sympathetic activity of the central nervous system (Hoefke et al., 1975). The effect of clonidine and 5 of its congeneric substances (for structures see table 15) on cardiae frequency was measured 30 minutes after intravenous injection. All the drugs decreased heart rate dose-dependently with the exception of *St-91* (2,6-di-Et). The effective dose (μg/kg), reducing heart rate by 50 beats/minute was evaluated (see table 15). In contrast to the other derivatives, *St-91* (2,6-di-Et) revealed an inverse relationship between dose and degree of bradycardia. All the agents, including *St-91* (2,6-di-Et) lowered heart rate when injected intracisternally into anaesthetized cats. They were, however, less active than clonidine in this respect.

The same compounds were employed by Hoefke et al. (1975) to test a facilitation of the vagally mediated cardiodepressive reflex in anaesthetized rats with blocked β-receptors. The reflex bradycardia elicited by angiotensin was significantly increased by these substances, *St-91* (2,6-di-Et) excepted. On the other hand this compound *St-91* given intracisternally to anaesthetized dogs (9 μg/kg) substantially increased the bradycardia following angiotensin.

A number of α-adrenoceptor-stimulating substances have been studied for bradycardia in vagotomized, anaesthetized, normotensive rats pretreated with atropine (Kobinger and Pichler, 1975). Intravenous naphazoline, oxymetazoline and compound *St-91* (2,6-di-Et) did not exert dose-dependent changes in heart rate up to the amounts as indicated in table 16. However,

Table 15: Dose (μg/kg) reducing heart rate by 50 beats/minute (ED_{50}) for clonidine and some congeners 30 minutes after intravenous administration to vagotomized, anaesthetized, normotensive rats pretreated with atropine. Data from Hoefke et al. (1975).

Compd. no.[a]	X	ED_{50}(μg/kg)
St-155	(2,6-di-Cl)	5
93	(2-Cl,6-Me)	9.5
375	(2-Cl,4-Me)	41
608	(2-Cl,3-Me)	62
600	(2-Me,5-F)	300
91	(2,6-di-Et)	–

[a] Numbering refers to table 5.

clonidine, *St-1697* (2-Me, 6-Et), *St-363* (2,4-di-Cl) and xylazine *(Bay 1470)* showed bradycardia dose-dependently. The amount (μg/kg) diminishing cardiac frequency by 50 beats/minute (ED_{50}) was determined (see table 16). Xylazine (Bay 1470) and the two imidazolidine derivatives St-1697 (2-Me, 6-Et) and St-363 (2,4-di-Cl) were markedly less potent than clonidine.

Table 16: Sympathoinhibitory activity in vagotomized rats of some α-adrenergic agonists. ED_{50}: dose (μg/kg) decreasing heart rate by 50 beats/minute 30 minutes after intravenous application. Data from Kobinger and Pichler (1975).

Compd. no.[a]	X	ED_{50}(μg/kg)
St- 155	(2,6-di-Cl)	7.3
1697	(2-Me,6-Et)	60
Xylazine (Bay 1470)		115
St- 363	(2,4-di-Cl)	233
Oxymetazoline		> 30
St -91	(2,6-di-Et)	> 100
Naphazoline		> 1000

[a] Numbering refers to table 5.

A significant facilitation of the cardiodepressor reflex triggered by angiotensin was found for intracisternal injections of naphazoline (10 μg/kg) and oxymetazoline (20 μg/kg) into anaesthetized dogs with blocked β-receptors. In other experiments the same treatment had no facilitatory effect (Kobinger and Pichler, 1975). In these negative experiments, however, a significant increase in basal blood pressure was observed, due to leakage of the substances to the periphery, which counteracted the cardiodepressor reflex. Intracisternal administration of 10 μg/kg of compound *St-91* (2,6-di-Et) also significantly facilitated this reflex, but 20 μg/kg did not, whereas blood pressure was increased. An unequivocal and significant reflex facilitation without rise in blood pressure was observed for intracisternal injections of compound *St-1697* (2-Me, 6-Et), *St-363* (2,4-di-Cl) and xylazine *(Bay 1470)* with 10 μg/kg of each.

The central site of the reflex facilitation of all drugs tested was demonstrated by the absence of effectiveness following subcutaneous or intravenous administration of amounts effective after intracisternal application. In addition, clonidine (1 μg/kg) and the drugs mentioned above (10 μg/kg) lowered heart rate significantly after intracisternal injections into atropine-treated dogs. Moreover, naphazoline, oxymetazoline and *St-363* (2,4-di-Cl) given intracisternally to vagotomized cats in a dose of 30 μg/kg induced bradycardia as well. These results with naphazoline and oxymetazoline are in contrast to those of Schmitt and Fénard (1971) who reported no cardiovascular inhibition in dogs after intracisternal injections of 3–50 μg/kg.

4.2.5. Hypertensive Activity

The initial hypertensive phase observed following systemic administration of clonidine and other α-adrenergic agents reflects the excitation of peripheral, vascular α-adrenoceptors. This peripheral α-sympathomimetic activity has been quantified mainly with the aid of two animal models, viz. the pithed and the spinal rat.

Pithed rats were used in order to evaluate the hypertensive activities of 27 phenyl-substituted imidazolidines, including clonidine, upon intravenous administration (Rouot, 1974; Rouot et al., 1976, 1977). The α-sympathomimetic activity of the drugs was expressed by their pD_2 and their pC_{100} values, calculated from dose-response-curves (pC_{100}: negative logarithm of the dose, mol/kg, for 100% increase in blood pressure). Table 17 summarizes the results of these experiments. The activities of the substances varied considerably (more than 2 logarithmic units). In this experimental model the pD_2 value of noradrenaline amounted to 8.12, which shows the particularly high activity of some clonidine-like imidazolidines. Within this series of molecules studied clonidine itself was moderately active. A number of congeneric imidazolidines was more effective.

The hypertensive activity of clonidine and 5 analogues has been compared by intravenous injections into spinal rats (Hoefke et al., 1975). For all substances linear dose-response curves were drawn. From these the pC_{30} values were calculated (pC_{30}: negative logarithm of the dose,

Table 17: Peripheral hy
substituted imidazolidin
tained from dose-respo
application to pithed r.
piled from those reporte
et al. (1976, 1977).

Compd. no.[a]	(X)
9	(2,3-di-Cl)
29	(2,4,5-tri-Cl)
20	(2,4,6-tri-Me)
7	(2,6-di-Me)
14	(2,4-di-Me)
6	(2-Cl,6-Me)
32	(2-Me,5-Cl)
8	(2,6-di-Et)
1	(2.,6-di-Cl)
11	(2,5-di-Cl)
38	(2-Et)
39	(2,6-di-Me,4-O
40	(2-Cl,3-Me)
37	(2,6-di-Cl,4-OI
3	(2,6-di-F)
2	(2,6-di-Br)
12	(2-Me,4-Cl)
13	(2-Cl,4-Me)
33	(2,5-di-OMe)
18	(2,4,6-tri-Cl)
10	(2,4-di-Cl)
35	(2,4-di-OMe,5-
31	(2-OMe,4-Me)
36	(2-CF_3)
34	(4-Br)
15	(H)
30	(2,6-di-i-Pr)

[a] Numbering refers to table 4, n

mol/kg, increasing me
mm Hg, see table 18)
efficacy of compoun
found to be more ma
other agents were less

Spinal rats were also
the effects of some c
compounds after int
(Kobinger and Pichler
response curves, the
evaluated, elevating m
30 mm Hg and the pC
(see table 19).

Table 17: Peripheral hypertensive activities of phenyl-substituted imidazolidines (pD_2 and pC_{100} values) obtained from dose-response curves upon intravenous application to pithed rats. The data have been compiled from those reported by Rouot (1974) and Rouot et al. (1976, 1977).

Compd. no.[a]	(X)	pD_2	pC_{100}
9	(2,3-di-Cl)	7.89	8.00
29	(2,4,5-tri-Cl)	7.86	7.95
20	(2,4,6-tri-Me)	7.80	7.39
7	(2,6-di-Me)	7.74	7.85
14	(2,4-di-Me)	7.74	7.52
6	(2-Cl,6-Me)	7.74	7.82
32	(2-Me,5-Cl)	7.63	7.81
8	(2,6-di-Et)	7.61	7.85
1	(2.,6-di-Cl)	7.58	7.52
11	(2,5-di-Cl)	7.55	7.70
38	(2-Et)	7.55	7.15
39	(2,6-di-Me,4-OH)	7.51	7.42
40	(2-Cl,3-Me)	7.44	7.08
37	(2,6-di-Cl,4-OH)	7.26	7.22
3	(2,6-di-F)	7.19	7.17
2	(2,6-di-Br)	7.18	7.30
12	(2-Me,4-Cl)	7.11	7.21
13	(2-Cl,4-Me)	7.08	7.04
33	(2,5-di-OMe)	6.98	7.22
18	(2,4,6-tri-Cl)	6.92	6.92
10	(2,4-di-Cl)	6.69	6.75
35	(2,4-di-OMe,5-Cl)	6.68	6.38
31	(2-OMe,4-Me)	6.58	6.60
36	(2-CF_3)	6.11	6.23
34	(4-Br)	6.09	5.26
15	(H)	5.84	5.17
30	(2,6-di-i-Pr)	5.79	5.89

[a] Numbering refers to table 4, no. *38*, *39* and *40* excepted.

mol/kg, increasing mean arterial pressure by 30 mm Hg, see table 18). It can be seen that the efficacy of compound *St-91* (2,6-di-Et) was found to be more marked than clonidine. The other agents were less effective.

Spinal rats were also used in order to quantify the effects of some α-adrenoceptor-activating compounds after intravenous administration (Kobinger and Pichler, 1975). From the dose-response curves, the doses were graphically evaluated, elevating mean arterial pressure by 30 mm Hg and the pC_{30} values were calculated (see table 19).

Naphazoline, oxymetazoline, *St-91* (2,6-di-Et) and *St-1697* (2-Me, 6-Et) were approximately 3–5 times more active than clonidine, whereas *St-363* (2,4-di-Cl) and xylazine *(Bay-1470)* exerted only about 1/10–1/20 of the potency of clonidine. Table 19 also shows that (±) noradrenaline was 40 times more potent than clonidine in these investigations.

Dose-response curves for nine α-sympathomimetic drugs were determined in pithed rats after intravenous administration (Autret et al., 1971). The relative potencies calculated from the doses producing half the maximal response (65 mm Hg) by taking the hypertensive activity of (±) noradrenaline as 1 were: oxy-

Table 18: Peripheral hypertensive activities of clonidine and some analogues, expressed as pC_{30}. The compounds were administered intravenously to spinal rats. Data from Hoefke et al. (1975).

Compd. no.[a]	X	pC_{30}
St- 91	(2,6-di-Et)	7.66
155	(2,6-di-Cl)	7.28
93	(2-Cl,6-Me)	7.09
375	(2-Cl,4-Me)	6.59
608	(2-Cl,3-Me)	6.14
600	(2-Me,5-F)	6.07

[a] Numbering refers to table 5.

Table 19: Peripheral hypertensive activities of a number of α-adrenoceptor-stimulating drugs upon intravenous application to spinal rats. The potency is expressed by the pC_{30} value. Data from Kobinger and Pichler (1975).

Compd. no.[a] (X)	pC_{30}
(±) Noradrenaline	9.12
Oxymetazoline	8.24
St-1967 (2-Me,6-Et)	8.04
Naphazoline	7.94
St- 91 (2,6-di-Et)	7.71
155 (2,6-di-Cl)	7.47
363 (2,4-di-Cl)	6.52
Xylazine *(Bay-1470)*	6.29

[a] Numbering refers to table 5.

metazoline = 0.1, phenylephrine = 0.1, naphazoline = 0.087, clonidine = 0.029, *Bay c 6014 (LD 2855)* = 0.028, tramazoline = 0.030, tetryzoline = 0.01 and xylazine *(Bay 1470)* = 0.0003.

The vasopressor activity of a number of structurally dissimilar α-adrenergic drugs has been established in the pithed rat following intravenous administration (Timmermans et al., submitted for publication). Dose-response curves were employed in order to deduce the pC_{60} values of the agents as indices of hypertensive activity (pC_{60}: negative logarithm of the dose, mol/kg, for an increase in mean arterial pressure by 60 mm Hg). The results are reported in table 20.

Table 20: Peripheral hypertensive activities of some structurally dissimilar α-sympathomimetic drugs, expressed by pC_{60} values. The compounds were given intravenously to pithed rats. Data from Timmermans et al., submitted for publication. For structural formulae see previous sections.

Compd.	pC_{60}
Compound *44-549*	8.40
Oxymetazoline	8.24
Bay a 6781	8.11
Lofexidine	7.99
Naphazoline	7.83
Tramazoline	7.80
Clonidine	7.78
UK-14,304-18	7.56
Bay c 6014 (LD 2855)	7.51
St-1967	7.24
St-1913	7.17
Xylometazoline	7.12
Tetryzoline	6.90
KUM 32	6.23
Xylazine *(Bay-1470)*	5.98
St-404	5.21

The very high hypertensive activity of compound *44-549* comes to the fore. This molecule was found even more effective than oxymetazoline. Moreover, the potencies of *Bay a 6781* and lofexidine are also worth mentioning. The bridge analogues *St-1967* (–S–) and *St-1913* ($-CH_2-$) were less active than clonidine. *St-404* (expanded imidazolidine ring) exhibited an activity more than 300 times less than clonidine.

Upon comparing the results of the quantitative evaluations of the hypertensive effects of various α-sympathomimetic drugs, summarized above, a good agreement exists between the relative potencies found in these studies. This action is due to a direct stimulation of peripheral, vascular α-adrenoceptors. Pretreatment with reserpine does not alter the responses (Autret et al., 1971 and refs. quoted therein) and a competitive antagonism has been demonstrated for the α-adrenolytic drug phentolamine (Autret et al., 1971 and refs. quoted therein; Kobinger and Pichler, 1975).

Quantitative correlations between the hypertensive activity of clonidine-like imidazolidines and physicochemical parameters have been reported (Rouot, 1974; Rouot et al., 1976, 1977). This quantitative structure-activity relationship will be described in a separate section (see 6.5.).

4.3. Structure-Activity Relationships with Respect to Various Pharmacological Actions and Side-Effects

4.3.1. Sedation

The main side-effect of clonidine is sedation which is probably the most important but not too serious side-effect in the clinical use of this drug (Dollery et al., 1976). The central nervous action of clonidine is not limited to the brain stem (hypotension), but also involves sites at the level of the cerebral cortex. The sedative effects of clonidine were already observed in the very first animal experiments (Hoefke and Kobinger, 1966). Symptoms of sedation are also obvious upon administration of therapeutic doses to dogs, cats, rabbits, rats and mice.

Chicks have widely been used for the investigation of the sedative properties of α-sympathomimetic drugs, since during their first weeks of life the blood-brain barrier is still incomplete. Noradrenaline and α-methylnoradrenaline cause sleep, but are ineffective in older chicks as well as mammals. Clonidine as well provokes sleep in these animals (Zaimis, 1970;

Fügner and Hoefke, 1971; Delbarre and Schmitt, 1971), but does not show a decrease in activity in older animals. Sedation is believed to be mediated by central α-adrenoceptors. Like the hypotensive effect, it can be antagonized by a number of α-sympatholytic durgs (Delbarre and Schmitt, 1971; Fügner, 1971).

Fügner and Hoefke (1971) reported a screening method by which the sedative effects of a number of imidazolidines and imidazolines were determined quantitatively in chicks only a few days old. The results have been summarized in table 21. Within the four imidazolidine derivatives tested clonidine itself appeared most potent in causing sleep in 50% of young chicks. Surprisingly, the 2,6-di-ethyl derivative *(St-91)* was devoid of sedative properties. Moreover, the imidazolines naphazoline, tetryzoline and xylometazoline, therapeutically used as nasal decongestants, exhibited a strong sleep-inducing effect in these animals.

Table 21: Dose (mg/kg) producing sleep in 50% of young chicks (ED_{50}). Substances were given subcutaneously (s. c.) or intraperitoneally (i. p.). Data from Fügner and Hoefke (1971).

Compd. no.[a]	(X)	Administration	ED_{50} (mg/kg)	Age of chicks (days)
St-155	(2,6-di-Cl)	s. c.	0.066	7
375	(2-Cl,4-Me)	s. c.	0.6	7
600	(2-Me,5-F)	s. c.	1.9	6
Xylometazoline		i. p.	2.3	4
Tetryzoline		i. p.	3.1	1–2
Naphazoline		s. c.	3.2	2
St-91	(2,6-di-Et)	s. c./i. p.	–[b]	3–9

[a] Numbering refers to table 5.
[b] Not effective in the dose range 0.3–3.0 mg/kg.

Six clonidine analogues and the parent compound itself have been studied in rats for their effects on measures of rat behaviour including duration of chloral hydrate hypnosis, Y-runway activity, spontaneous cage activity and conditioned avoidance response (Laverty, 1969). The approximately equi-effective doses of these substances on the duration of hypnosis following chloral hydrate (300 mg/kg; i. p.) are shown in table 22. After chloral hydrate administration only compounds *St-464* (2,6-di-Br) and to a lesser degree *St-612* (2-Br, 4-Me) and *St-678* (2-Br, 4-Cl) behaved like clonidine in causing a significant prolongation of the sleeping time. Clonidine was by far the most active in this test.

Table 22: Comparison between approximately equi-effective doses (mg/kg; s. c.) of clonidine and some analogues on the duration of chloral hydrate (300 mg/kg; i. p.) induced hypnosis in rats. Data from Laverty (1969).

Compd. no.[a]	(X)	Sedative properties
St-155	(2,6-di-Cl)	0.05
464	(2,6-di-Br)	0.3
612	(2-Br,4-Cl)	>10
678	(2-Br,4-Cl)	>10
600	(2-Me,5-F)	25
608	(2-Cl,3-Me)	>50
375	(2-Cl,4-Me)	>50

[a] Numbering refers to table 5.

Delbarre and Schmitt (1971) studied the depressing action of a number of α-sympathomimetic agents administered intramuscularly in chickens (24–72 hours old) and mice. Clonidine, *Bay c 6014 (LD 2855)*, tetryzoline, naphazoline, tramazoline, xylazine *(Bay-1470)* and oxymetazoline induced a loss of the righting reflex in chickens and prolonged the sleeping time caused by chloral hydrate (250 mg/kg; i. p.) in mice. The effects were dose-dependent and the drugs investigated in chickens showed the following potency: clonidine = 1, *LD 2855* = 0.81, xylazine = 0.1, tetryzoline = 0.11, tramazoline = 0.11, naphazoline = 0.047 and oxymetazoline = 0.044. Clonidine (0.15–0.30 mg/kg) caused subhypnotic doses of chloral hydrate to induce sleep in mice. Naphazoline, tetryzoline and tramazoline were much less potent.

Clonidine and a number of its phenyl-substituted derivatives were studied with respect to a prolongation of the sleeping time in mice induced by hexobarbitone (75 mg/kg; i. p.)

(Hoefke et al., 1975). The compounds were given subcutaneously 30 minutes before hexobarbitone. The ED_{45}, which is the dose prolonging the sleeping time by 45 % of the control group, is listed in table 23. Table 23 shows that all the test substances prolonged the sleeping period. The dose, however, required to prolong the sleeping time by 45 % varied considerably.

Table 23: Prolongation of hexobarbitone sleeping time in mice (ED_{45}) by clonidine and 5 of its derivatives given s. c. 30 minutes earlier. Data from Hoefke et al. (1975).

Compd. no.[a] (X)	ED_{45} (mg/kg)
St-155 (2,6-di-Cl)	0.04
93 (2-Cl,6-Me)	0.10
375 (2-Cl,4-Me)	0.15
608 (2-Cl,3-Me)	0.16
600 (2-Me,5-F)	1.85
91 (2,6-di-Et)	3.00

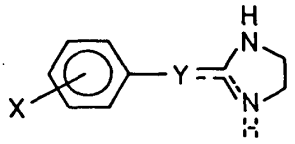
[a] Numbering refers to table 5.

It has been suggested that activation of α-adrenoceptors in the central nervous system induces sedation (see above). However, the biological data on the sedative effects of imidazolidines summarized above do not allow definite conclusions about the structure-activity relationship with respect to this action due to the insufficient number of compounds studied. In general, 2,6-di-ortho-substituted imidazolidines are more effective than corresponding compounds possessing a different substitution pattern. In intact animals the lipophilic properties of the drugs will certainly be involved in determining the sedative potency. On the other hand this molecular feature will be of less importance in young chickens in which the blood-brain barrier is not fully developed, although it cannot be ignored totally.

4.3.2. Adrenergic Activity at Rabbit Intestine

Peripheral α-adrenergic activity of imidazolidines and imidazolines has been measured on the isolated intestinal smooth muscle of the rabbit (Struyker Boudier et al., 1975). The pendular movements of isolated pieces of rabbit jejunum were registered upon increasing doses of the drugs. (–) Noradrenaline was employed as a reference (intrinsic activity = 1). The affinity of the drugs was expressed by means of the pD_2 value. The results of the experiments on rabbit intestine are summarized in table 24.

Table 24: Intrinsic activity constants (α) and pD_2 values for drug-iduced inhibition of the pendular movements of rabbit intestine. Data from Struyker Boudier et al. (1975).



Compd. no.[a] (X)	Y	α	pD_2
St-464 (2,6-di-Br)	N	0.2	4.9
Clonidine (2,6-di-Cl)	N	0.4	5.2
St- 600 (2-Me,5-F)	N	0.5	5.4
666 (2,6-di-Cl,4-OH)	N	0.3	5.4
93 (2-Cl,6-Me)	N	0.3	5.6
1913 (2,6-di-Cl)	CH_2	0.3	5.6
Tetryzoline		1	5.6
St- 71 (2,4,6-tri-Me)	CH_2	0.6	5.7
1943 (3,4-di-OH)	N	1	5.8
Xylometazoline		1	6.0
Naphazoline		1	6.1
St-91 (2,6-di-Et)	N	0.9	6.2
Tramazoline		1	6.2
(–) Noradrenaline		1	6.8
Oxymetazoline		1	8.8

[a] Numbering refers to table 5.

Within this group of compounds only a limited number possessed an intrinsic activity equal to 1 (tetryzoline, *St-1943*, xylometazoline, naphazoline, tramazoline and oxymetazoline). A few compounds displayed low intrinsic activity of 0.2–0.4 (*St-464*, clonidine, *St-666*, *St-93* and *St-1913*).Two imidazolidines (*St-600* and *St-71*) had an intermediate intrinsic activity of 0.5–0.6, whereas *St-91* possessed a value of 0.9.

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4.3.3. Local

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results are listed

Table 25: Local
rabbit cornea for
imidazolidines. D

Compd. no.[a] (X
29 (2,4,5-tri-Cl)
9 (2,3-di-Cl)
1 (2,6-di-Cl)
18 (2,4,6-tri-Cl)
2 (2,6-di-Br)
10 (2,4-di-Cl)
13 (2-Cl,4-Me)
32 (2-Me,5-Cl)
11 (2,5-di-Cl)
30 (2,6-di-i-Pr)
6 (2-Cl,6-Me)
3 (2,6-di-F)
36 (2-CF_3)
37 (2,6-di-Cl,4-(
34 (4-Br)

[a] Numbering refers to t
[b] Inactive; highest conc
[c] Highest concentration

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substances. As

With respect to the pD_2. values an activity range of about 4 logarithmic units was covered. Oxymetazoline having the highest pD_2 of 8.8 and *St-464* having the lowest value of 4.9.

The quantitative structure-activity relationship with respect to this biological action on rabbit intestine in which the pD_2 values have been correlated with a number of physicochemical parameters of the drugs will be treated in a separate section (ChapterVI, section 4).

4.3.3. Local Anaesthetic Activity

The local anaesthetic activity on the rabbit cornea has been established for clonidine and 14 analogues (Rouot et al., 1977) according to the method described by Bartsch and Knopf (1970). Log dose-response curves were constructed and the pD_2 values were determined graphically. The results are listed in table 25.

Table 25: Local anaesthetic activity (pD_2) on the rabbit cornea for clonidine and structurally related imidazolidines. Data from Rouot et al. (1977).

Compd. no.[a] (X)	Local Anaesthetic Activity (pD_2)
29 (2,4,5-tri-Cl)	2.15
9 (2,3-di-Cl)	1.74
1 (2,6-di-Cl)	1.48
18 (2,4,6-tri-Cl)	1.40
2 (2,6-di-Br)	1.33
10 (2,4-di-Cl)	0.92
13 (2-Cl,4-Me)	0.92
32 (2-Me,5-Cl)	0.92
11 (2,5-di-Cl)	0.87
30 (2,6-di-i-Pr)	0.87
6 (2-Cl,6-Me)	0.66
3 (2,6-di-F)	—[b]
36 (2-CF_3)	—[b]
37 (2,6-di-Cl,4-OH)	< 1[c]
34 (4-Br)	—[b]

[a] Numbering refers to table 4.
[b] Inactive; highest concentration studied: 3.10^{-1} mol/l.
[c] Highest concentration studied: 10^{-1} mol/l.

The local anaesthetic activity could only be quantified by means of a pD_2 value for 11 substances. As can be concluded from table 25 imidazolidines of the clonidine-type are weak local anaesthetics in this test. A significant linear relationship resulted upon correlating the pD_2 values (table 25) with the corresponding apparent partition coefficients from the octanol/buffer (pH = 7.4) system (table 5):

$$pD_2 = 0.48 \log P' + 0.93 \quad \text{(eq. 12)}$$
$$n = 11;\ r = 0.78;\ s = 0.30;$$
$$F = 14\ (P < 0.005)$$

Accordingly, a great deal of the local anaesthetic activity of the imidazolidines can be explained by their over-all lipophilic behaviour. Therefore, this action is probably mainly caused by a nonspecific mechanism.

4.3.4. Antisecretory Activity (Reduction of Gastric Acidity)

Another side-effect of clonidine is its antisecretory action, which manifests itself, for instance, by dryness of the mouth (inhibition of salivary secretion). In the clinical treatment this side-effect of clonidine is, however, not as important as sedation. For screening purposes it is easier to test the effects of potential antisecretory drugs on gastric secretion in rats according to the method of Shay et al. (1954), because in this species the alkaline saliva is a strong stimulus for acid production. Two signs of antisecretory activity are usually been studied in these experimental animals: a decrease in acidity (i. e. an increase in pH) of the gastric secretion and a reduction in volume of this secretion.

According to this method Hoefke et al. (1975) determined the influence of clonidine and 5 derivatives on the total gastric acidity in fasted rats. The compounds were injected subcutaneously into pylorus-ligated, starved rats. The volume of the gastric secretion was measured and the total acidity determined by titration 4 hours later. Dose-response curves were drawn by plotting acidity and volume versus log dose. With the aid of these dose-response characteristics drug-induced changes in gastric acidity and volume of secretion were quantified as the dose (mg/kg) which reduced the titratable acidity to 50 % ($ED_{50\ acid.}$) and produced a decrease in secretion volume of 50 % ($ED_{50\ vol.}$), respectively. The dose reducing acidity to 50 % (ED_{50}) of the controls was estimated (table 26).

Table 26: Dose (mg/kg) reducing total gastric acidity/4 hours to 50% (ED_{50}) in pylorus-ligated rats. Compounds were given subcutaneously immediately after surgical intervention. Data from Hoefke et al. (1975).

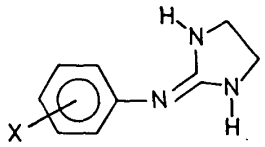
Compd. no.[a]	(X)	ED_{50} (mg/kg)
St-155	(2,6-di-Cl)	0.036
93	(2-Cl,6-Me)	0.057
375	(2-Cl,4-Me)	0.170
91	(2,6-di-Et)	1.15
608	(2-Cl,3-Me)	4.0
600	(2-Me,5-F)	5.0

[a] Numbering refers to table 5.

All substances decreased the secretion of gastric acid. Within this series of compounds clonidine was most active. *St-91* (2,6-di-Et) proved more effective than *St-608* (2-Cl, 3-Me) and *St-600* (2-Me, 5-F) in this test.

By using the same method a great number of clonidine derivatives has been tested for antisecretory activity (Hoefke and coworkers, unpublished results). The data have been collected in table 27. Concerning the structure-activity relationship, focused upon the potency of these imidazolidines to decrease gastric acidity, the most active compounds are found within the group of 2,3- and 2,6-disubstituted analogues. This is in accordance with their activity in decreasing blood pressure in anaesthetized rabbits (see table 5). A remarkable parallelism exists in the group of di-substituted imidazolidine between the influence on gastric acidity in rats and the blood pressure lowering activity in rabbits. This parallelism between hypotensive and antisecretory activities was quantified by using Spearman's rank correlation test (see Chapter V, section 2).

Table 27: Antisecretory activity of phenyl-substituted imidazolidines determined 4 hours after subcutaneous application to pylorus-ligated, fasted rats. $ED_{50\ acid.}$ = dose (mg/kg) diminishing gastric acidity by 50% of the controls. $ED_{50\ vol.}$ = dose (mg/kg) reducing total gastric secretion by 50% of the controls. >: Weak or no activity at the amount indicated; higher doses not tested. Unpublished data from Hoefke.



Compound no.[a]	X	$ED_{50\ acid.}$	$ED_{50\ vol.}$
Mono-substituted (ortho)			
St- 96	2-Cl	1.05	1.80
693	2-J	1.2	1.45
391	2-Br	1.55	2.85
371	2-CF_3	2.07	4.45
97	2-Et	5.1	20.0[b]
90	2-Me	6.2	9.2
681	2-F	>10.0	>10.0
1963	2-Phenyl	>10.0	>10.0

Table 27 (co

Compound n
Di-substituted
STH-2165
2224
2100
St- 606
2169
476
STH-2167
St- 450
608
Di-substituted
St- 678
375
453
680
592
363
692
Kö- 1164
St- 341
612
893
STH-2173
St- 459
691
604
486
455
1948
467
STH-2168
Di-substituted
STH-2160
2162
St- 585
600
603
475
594
1949
Di-substituted
St- 155
93
1962
STH-2130
St- 464
1964

Table 27 (continued)

Compound no.[a]	X	ED_{50} acid.	ED_{50} vol.
Di-substituted (2,3)			
STH-2165	2-Br,3-Cl	0.017	0.021
2224	2,3-di-Br	0.047	0.058
2100	2-Cl,3-Br	0.05	0.078
St- 606	2-Me,3-Br	0.12	0.17
2169	2-Br,3-F	0.28	0.37
476	2,3-di-Cl	0.50	0.55
STH-2167	2-Cl,3-F	0.84	12.5[b]
St- 450	2-Me,3-Cl	1.0	0.68
608	2-Cl,3-Me	4.0	4.2
Di-substituted (2,4)			
St- 678	2-Br,4-Cl	0.11	0.20
375	2-Cl,4-Me	0.17	0.22
453	2-Cl,4-t-Bu	0.25	0.22
680	2-Me,4-Br	0.29	0.4
592	2,4-di-Br	0.90	0.66
363	2,4-di-Cl	1.05	0.75
692	2-Cl,4-NH_2	1.20	5.0
Kö- 1164	2-Cl,4-Br	2.8	3.0
St- 341	2-Me,4-Cl	3.8	2.8
612	2-Br,4-Me	4.5	3.8
893	2-F,4-Br	5.5	6.0
STH-2173	2-Br,4-F	5.6	7.7
St- 459	2-Cl,4-F	6.0	3.5
691	2-Cl,4-NO_2	7.5	9.0
604	2-F,4-Me	8.3	21.0[b]
486	2-F,4-Cl	9.6	13.0[b]
455	2-Et,4-Cl	15.5[b]	15.0[b]
1948	2,4-di-CF_3	>10.0	>10.0
467	2-Cl,4-CF_3	>10.0	>10.0
STH-2168	2-CF_3,4-F	>10.0	>10.0
Di-substituted (2,5)			
STH-2160	2-Br,5-F	1.35	2.15
2162	2-Cl,5-F	2.9	5.9
St- 585	2-Me,5-Cl	4.0	5.0
600	2-Me,5-F	4.5	10.0
603	2-Cl,5-Br	6.7	10.0
475	2,5-di-Cl	>10.0	>10.0
594	2-Br,5-Cl	>10.0	>10.0
1949	2-F,5-Me	>10.0	>10.0
Di-substituted (2,6)			
St- 155	2,6-di-Cl	0.033	0.043
93	2-Cl,6-Me	0.06	0.115
1962	2-Br,6-Cl	0.074	0.084
STH-2130	2-Br,6-F	0.079	0.115
St- 464	2,6-di-Br	0.1	0.18
1964	2-Cl,6-CF_3	0.11	0.185

Table 27 (continued)

Compound no.[a]	X	ED_{50} acid.	ED_{50} vol.
1923	2-Cl,6-F	0.13	0.13
1974	2-F,6-CF_3	0.155	0.17
1945	2,6-di-OMe	0.235	0.52
1959	2-Br,6-Me	0.235	0.52
454	2-Cl,6-Et	0.9	0.8
1697	2-Me,6-Et	1.04	51.0[b]
1957	2,6-di-CF_3	1.15	1.75
91	2,6-di-Et	1.2	47.0[b]
95	2,6-di-Me	2.5	6.3
Di-substituted (3,4)			
STH-2163	3-Br,4-F	1.2	1.5
St- 1943	3,4-di-OH	2.15	14.0[b]
473	3,4-di-Cl	>10.0	>10.0
2120	3-CF_3,4-Cl	>10.0	>10.0
2147	3-CF_3,4-Br	>10.0	>10.0
2108	3-F,4-Me	>10.0	>10.0
2121	3-Me,4-F	>10.0	>10.0
Di-substituted (3,5)			
St- 474	3,5-di-Cl	>10.0	>10.0
Tri-substituted (2,4,5)			
St- 739	2,4,5-tri-Cl	0.13	0.245
1915	2,5-di-Cl,4-Me	0.14	0.25
899	2,5-di-Cl,4-Br	0.3	0.52
1827	2-Cl,4-Me,5-NH_2	1.05	6.6
1824	2-Cl,4-Me,5-NO_2	>10.0	>10.0
Tri-substituted (2,4,6)			
St- 1942	2,6-di-Cl,4-Me	0.3	0.5
888	2-Br,4-Cl,6-F	0.42	0.56
886	2-Br,4-t-Bu,6-Cl	0.45	1.05
894	2,4-di-Br,6-Cl	0.48	0.6
884	2-Cl,4-Br,6-Me	0.52	0.75
871	2,6-di-Cl,4-Br	0.58	1.8
887	2,4-di-F,6-Br	0.82	0.68
1952	2,6-di-Cl,4-CH_2OH	1.05	2.65
896	2-Br,4-Cl,6-Me	1.1	2.1
89	2,4,6-tri-Me	1.15	>10.0
921	2-Br,4-F,6-Me	1.15	2.0
92	2,4,6-tri-Et	1.43	7.7
908	2-Br,4-F,6-Cl	1.6	1.4
881	2,6-di-Et,4-Br	1.65	5.25
922	2,4-di-Br,6-Me	1.9	2.5
906	2-Br,4-Cl,6-OMe	1.9	2.6
101	2,6-di-Me,4-t-Bu	1.9	10.0
882	2-Br,4-Cl,6-Et	2.0	1.95
732	2,4,6-tri-Cl	2.1	3.0
876	2-Br,4-Me,6-Cl	2.3	1.9

Table 27 (continued

Compound no.[a]
895
456
1988
1984
878
739
904
889
Hpt- 1127
St- 664
Hpt- 1128
1129
St- 666
Tri-substituted (3,4,
St- 2090
487
Tetra-substituted
St- 1961
1987
123
Penta-substituted
St- 1956
1924

[a] Numbering refers to tabl
[b] Extrapolated from the d

Antisecretory a
with permanent g
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(Jen et al., 1975).
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tested clonidine w
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stances within all

Table 27 (continued)

Compound no.[a]	X	$ED_{50\ acid.}$	$ED_{50\ vol.}$
895	2,6-di-Br,4-Me	3.2	4.2
456	2,4-di-Cl,6-Et	3.2	6.0
1988	2,4,6-tri-F	3.3	4.25
1984	2,6-di-Cl,4-COOH	4.1	5.2
878	2,4-di-Cl,6-Br	4.8	4.9
739	2,4,6-tri-Br	>10.0	>10.0
904	2,6-di-Br,4-Cl	>10.0	>10.0
889	2,4-di-Br,6-CF_3	>10.0	>10.0
Hpt- 1127	2,4-di-Cl,6-Me	>10.0	>10.0
St- 664	2,6-di-Cl,4-OMe	>10.0	>10.0
Hpt- 1128	2,4-di-Me,6-Cl	>10.0	>10.0
1129	2,6-di-Me,4-Cl	>10.0	>10.0
St- 666	2,6-di-Cl,4-OH	>10.0	>10.0
Tri-substituted (3,4,5)			
St- 2090	3,5-di-Br,4-NH_2	4.5	14.5[b]
487	3,4,5-tri-OMe	>10.0	>10.0
Tetra-substituted			
St- 1961	2,3,4,5-tetra-F	>10.0	>10.0
1987	2,3,5,6-tetra-F	>10.0	>10.0
123	2,3,5,6-tetra-Me	>10.0	>10.0
Penta-substituted			
St- 1956	2,3,4,5,6-penta-F	>10.0	>10.0
1924	2,3,4,5,6-penta-Cl	>10.0	>10.0

[a] Numbering refers to table 5.
[b] Extrapolated from the dose-response curve.

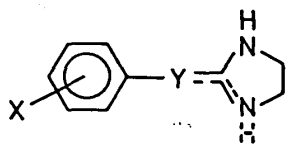
Antisecretory activity was evaluated in rats with permanent gastric fistulas by measuring pH and volume of gastric secretion for 11 chemical classes of compounds containing several features of the fundamental structure of clonidine (Jen et al., 1975). The potency of a compound was expressed in terms of the dose (mg/kg) elevating gastric pH by about 2 units and producing a decrease in secretion volume of about 50 %. Among the 2-(arylimino)imidazolidines tested clonidine was the most potent in this test system. If a methyl or and acetyl group was attached to one or to both imidazolidine nitrogen atoms the antisecretory activity dropped considerably. With a few exceptions the substances within all the other classes of structures such as 2-(arylimino)piperidines, pyrrolidines, perhydropyridines, 2-(benzyl)imidazolines, guanidines and amidines showed poor or very poor activity.

4.3.5. Effects on Histamine H_2-Receptors

Clonidine has been shown to possess agonistic actions at histamine H_2-receptors (see Chapter I). The effects of some imidazoline and imidazolidine compounds on histamine H_2-receptors in electrically driven guinea pig isolated ventricular strips (1 Hz, 2.5 msec, supramax. voltage) have been quantified by Kearney, Malta and Raper (1977). In the presence of propranolol and mepyramine (10^{-6}M of each)

the pD_2 value for histamine was 5.87. Table 28 shows the pD_2 values and intrinsic activities (α; histamine = 1) obtained with clonidine and a number of structurally related substances at these H_2-receptors. The actions of these drugs were competitively antagonized by metiamide. The highest activity is found for derivatives with halogen substituents at the 2 and 6 position as opposed to alkyl or hydrogen substitution at these ortho places.

Table 28: Intrinsic activity constants (α; histamine = 1) and pD_2 values for drug-induced increase in tension of electrically stimulated guinea pig isolated ventricular strips. Data from Kearny, Malta and Raper (1977).



Compd. no.[a]	(X)	Y	α	pD_2
Clonidine	(2,6-di-Cl)	N	1.10	5.45
St-1913	(2,6-di-Cl)	CH_2	1.13	5.30
464	(2,6-di-Br)	N	1.12	5.21
93	(2-Cl,6-Me)	N	1.03	5.15
91	(2,6-di-Et)	N	1.05	4.49
Tolazoline	(H)	CH_2	1.16	4.39

[a] Numbering refers to table 5.

The series of compounds studied with the aid of this model has been extended to 26 (Ong, Kearney, Malta, Vaughan and Raper, 1978). The compounds displayed a wide range of activity. The data indicate that the bridging nitrogen atom can be exchanged for a methylene group without substantial loss of potency. In addition, compounds substituted at both ortho postitions are the most active, di-halo substitution being more effective than di-alkyl substitution. Finally, introducing groups at other ring places leads to markedly decreased activities at cardiac H_2-receptor sites.

4.3.6. Presynaptic Activity

Noradrenergic varicosities contain receptive sites similar to the α-adrenoceptors at peripheral effector cells. Activation of these presynaptic receptors by released noradrenaline brings about an inhibition of further release. Thus, the amount of noradrenaline secreted per nerve impuls is physiologically controlled by a local, presynaptic α-receptor mediated negative feedback mechanism. A few remarks have been made in Chapter I with respect to the question as to whether or not a presynaptic mechanism is involved in the central sympathoinhibitory effect of clonidine. It is not the purpose of the present review to describe and explain the presynaptic effects of individual α-adrenoceptor agonists on all organs and tissues studied. The reader may consult excellent reviews on this subject (Langer, 1977; Starke et al., 1977; Westfall, 1977).

We will report in this review experiments with series of α-adrenoceptor-stimulating agents (phenylethylamines, imidazolidines and imidazolines) for which the relative presynaptic potencies have been determined and compared with the postsynaptic ones.

The rabbit pulmonary artery contains postsynaptic α-adrenoceptors which mediate smooth muscle contraction. Its noradrenergic nerves contain presynaptic α-adrenoceptors which mediate inhibition of the release of the transmitter evoked by nerve impulses. Dose-response curves for the pre- and postsynaptic effects of nine α-receptor agonists have been determined on superfused strips of the artery in the presence of cocaine, corticosterone and propranolol (Starke et al., 1974, 1975b). The concentrations of the drugs which caused 20 % of the maximal contraction ($EC_{20\ post}$) are compiled in table 29. The order of postsynaptic potency found is: adrenaline > noradrenaline > oxymetazoline > naphazoline > phenylephrine > tramazoline > α-methylnoradrenaline > clonidine > methoxamine.

At sufficient concentrations all the drugs reduced the stimulation-induced overflow of tritium from the arteries preincubated with ^{3}H-noradrenaline. The concentrations of the drugs diminishing the stimulation-induced overflow of

Table 29: I
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$EC_{20\ post}$: C
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$ED_{20\ pre}$: C
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naline by 2C

Compd.

Methoxamii
Phenylephri
Noradrenali
Adrenaline
Naphazolin
Oxymetazol
Clonidine
α-Methylnoradr
Tramazoline

tritium by
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Accordir
order of p
oxymetazo
noradrenali
naphazolin
As indices
presynaptic
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agonists w
Starke et

Table 30: R
pithed rat. D

Compd.

LSD
Xylazine (*Ba*
Clonidine
Oxymetazoli
Naphazoline
Methoxamin
Phenylephrin

Table 29: Pre and postsynaptic potencies of some α-adrenoceptor agonists in the rabbit pulmonary artery. $EC_{20\ post}$: Concentration (mol/l) causing 20% of the maximal contraction obtainable with the drug. $ED_{20\ pre}$: Concentration (mol/l) decreasing the stimulation-induced overflow of preincubated ^{3}H-noradrenaline by 20%. Data from Starke et al. (1974, 1975b).

Compd.	$EC_{20\ post}$ (mol/l)	$EC_{20\ pre}$ (mol/l)	$\frac{EC_{20\ pre}}{ED_{20\ post}}$
Methoxamine	7.4×10^{-7}	2.4×10^{-5}	32.5
Phenylephrine	5.4×10^{-8}	1.7×10^{-6}	30.9
Noradrenaline	7.3×10^{-9}	1.2×10^{-8}	1.6
Adrenaline	3.2×10^{-9}	1.9×10^{-9}	0.58
Naphazoline	3.8×10^{-8}	1.6×10^{-8}	0.41
Oxymetazoline	1.8×10^{-8}	3.1×10^{-9}	0.17
Clonidine	6.5×10^{-8}	1.0×10^{-8}	0.15
α-Methylnoradrenaline	6.1×10^{-8}	8.1×10^{-9}	0.13
Tramazoline	5.6×10^{-8}	3.7×10^{-9}	0.07

tritium by 20 % ($ED_{20\ pre}$) were used as an index of presynaptic activity (see table 29).

According to these $ED_{20\ pre}$ values the ranking order of presynaptic potency is: adrenaline > oxymetazoline > tramazoline > α-methylnoradrenaline > clonidine > noradrenaline > naphazoline > phenylephrine > methoxamine. As indices of the relation between post and presynaptic potencies, the ratios $EC_{20\ pre}/EC_{20\ post}$ are listed in table 29. Consequently, the nine agonists were divided into three groups by Starke et al. (1975b). For methoxamine and phenylephrine, the ratio is high, indicating a preferentially postsynaptic action. For noradrenaline, adrenaline and naphazoline, the ratio is not far from unity, indicating comparable potencies on pre- and postsynaptic α-adrenoceptors. For oxymetazoline, clonidine, α-methylnoradrenaline and tramazoline, the ratio is low, indicating a preferentially presynaptic activity.

The effects of some α-adrenoceptor agonists have been examined at pre- and postsynaptically located α-adrenoceptors in the pithed rat (Drew, 1976). The presynaptic receptors were those situated at the cardiac sympathetic nerve terminals and the postsynaptic sites were those present in the vascular smooth muscle. Changes in heart rate were used to measure the effects of the compounds at presynaptic α-adrenoceptors. Increases in diastolic blood pressure reflected the activity at postsynaptic α-adrenoceptors. The drugs produced dose-related increases in arterial pressure. Table 30 shows the mean dose (μg/kg of free base) of each compound required to increase diastolic pressure by 50 mm Hg. Also shown are the doses of the agents which reduced the stimulated heart rate by 50 beats/minute.

The data suggest that clonidine was approximately equipotent in reducing heart rate and elevating blood pressure, but LSD and xylazine *(Bay 1470)* were more effective in reducing heart rate than in increasing blood pressure. Oxymetazoline, naphazoline and methoxamine produced pressor responses at doses much lower than were required to reduce heart rate. Phenylephrine failed to lower heart rate even at high dose levels.

Table 30: Relative potencies of some α-adrenocepter agonists at pre and postsynaptic α-adrenoceptors in the pithed rat. Data from Drew (1976).

Compd.	Dose (μg/kg) producing Fall in heart rate of 50 beats/min.	Incr. in diast. blood press of 50 mm Hg	Ratio of doses (BP/HR)
LSD	25.5	>100	>4
Xylazine *(Bay-1470)*	153.9	553.2	3.64
Clonidine	7.5	5.5	0.73
Oxymetazoline	5.4	0.7	0.13
Naphazoline	94.9	3.3	0.03
Methoxamine	1061.7	46.0	0.05
Phenylephrine	> 100	4.8	<0.1

The effects of some α-adrenoceptor-stimulating substances on the twitch response of the rat isolated vas deferens to low frequency motor nerve stimulation have been studied by Drew (1977). Oxymetazoline (0.1 – 1.0 ng/ml), clonidine, xylazine *(Bay 1470)* and naphazoline (0.1 – 10 ng/ml) caused a concentration-dependent inhibition of the twitch response. However, methoxamine (0.1 – 30 ng/ml) and phenylephrine (0.1 – 10 ng/ml) had little or no inhibitory effect and in higher concentrations (> 30 and > 10 ng/ml, respectively) contracted the tissue and potentiated the twitch response. The amount of each drug, referring to the free base, producing 50% inhibition of the twitch response is given in table 31.

Table 31: Effects of α-adrenoceptor agonists on the twitch response to sympathetic nerve stimulation of the rat isolated vas deferens. Data from Drew (1977).

Compd.	Concentration (ng/ml) causing 50% reduction in twitch height
Oxymetazoline	0.19
Clonidine	0.47
Naphazoline	1.03
Xylazine *(Bay-1470)*	1.52
Phenylephrine	Contractile at > 10 ng/ml
Methoxamine	Contractile at > 30 ng/ml

The results indicate that oxymetazoline, clonidine, naphazoline and xylazine *(Bay 1470)* are potent presynaptic α-agonists, whereas phenylephrine and methoxamine are almost ineffective.

The results summarized in this section provide a basis for the conclusion that pre- and postsynaptic α-adrenoceptors differ in their sensitivity to drugs. The experiments on the rabbit pulmonary artery, the pithed rat and the rat isolated vas deferens indicate that clonidine, oxymetazoline and naphazoline behave as potent agonists at both pre- and postsynaptic α-adrenoceptors. Phenylephrine and methoxamine are relatively selective agonists at postsynaptic receptors and xylazine *(Bay 1470)* is a relatively preferential agonist at presynaptic ones. Dissimilarities between these two receptive sites can also be demonstrated on the basis of blocking potencies of α-sympatholytic drugs. Moreover, it may be concluded that the presynaptic α-adrenoceptors in the rat vas deferens resemble those in the rat heart and in the rabbit pulmonary artery.

Pre- and postsynaptic activities at peripheral adrenergic sites were investigated for clonidine, oxymetazoline, *St-91* (2,6-di-Et) as well as for methoxamine (Pichler and Kobinger, 1978). In spinal rats all drugs increased the blood pressure (postsynaptic action; order of potency: oxymetazoline > *St-91* > clonidine > methoxamine. The stimulation-induced tachycardia in pithed rats was diminished by all drugs (presynaptic action; order of potency: oxymetazoline > clonidine > St-91 >> methoxamine). From the quantitated data a pre-/postsynaptic activity ratio was calculated as follows: clonidine = 1.86; *St-91* = 0.69; oxymetazoline = 0.58; methoxamine = 0.13. The results show that the highest ratio, i. e. preferential presynaptic activity was calculated for clonidine.

Recently, the prejunctional α-receptor stimulant actions of clonidine-like drugs in the transmurally stimulated guinea pig ileum have been communicated (Kearney, Malta and Raper, 1978). In general, the highest potency was observed for compounds having 2,6-di-substitution on the phenyl ring (halide > alkyl). There was little difference in potency between compounds having a methylene or an imino bridge between the phenyl and the imidazolidine ring. With the exception of naphazoline, tramazoline and oxymetazoline, substitution at other than the 2 and 6 position markedly reduced activity. The general trends in the structure-activity relationship at these presynaptic α-adrenoceptors bear some resemblance to those obtained for other pharmacological actions reported in the course of this review. However, numerical values are not yet available so that a quantitative comparison cannot be made.

4.3.7. Miscellaneous Actions

A comparison has been made between the potencies of clonidine and 5 of its congeneric compounds in various pharmacological tests

(Hoefke et al., 1975). Two of them (prolongation of the sleeping time in mice and antisecretory activity) have already been treated above (sections 4.3.1. and 4.3.4.). Apart from several studies on circulatory parameters, which have been described separately, the substances were tested for mydriatic effects on conscious rats (absorption following gastric gavage), for hyperglycaemic action in rats and for their acute toxicity in mice.

Table 32: Dose range which showed mydriatic effect in conscious rats. Pupillary diameters were measured 10 minutes (i.v.) and 20 minutes (p.o.) after administration. From dose-response curves the ratio of equipotent doses i.v./p.o. was calculated as a measure of g.i. absorption. Data from Hoefke et al. (1975).

Compd. no.[a] (X)	Dose Range (mg/kg) i.v./p.o.	Ratio (%) i.v./p.o.
St-155 (2,6-di-Cl)	0.01 – 0.03 / 0.03 – 0.3	47
93 (2-Cl,6-Me)	0.03 – 0.33 / 0.1 – 1.0	31
375 (2-Cl,4-Me)	0.1 – 0.33 / 0.33 – 1.0	24
600 (2-Me,5-F)	0.1 – 10.0 / 1.0 – 10.0	22
608 (2-Cl,3-Me)	0.04 – 0.4 / 0.4 – 4.0	10
91 (2,6-di-Et)	0.1 – 1.0 / 3.0 – 30.0	6

[a] Numbering refers to table 5.

Mydriatic Effect on Conscious Rats; Absorption Following Gastric Gavage

The diameters of both pupils were measured 10–15 minutes (i. v.) and 20–25 minutes (p. o.) after the administration of various different doses of the drugs. Log dose-response curves were drawn. As a measure of absorption from the gastro-intestimal (g. i.) tract the distance between the two parallel lines (i. v.–p. o.) along the abscissa was calculated for each substance (ratio of equieffective dose i. v./p. o. × 100 = % g. i. absorption). Within the dose range given in table 32 all compounds exhibited a dose-dependent mydriatic effect. Table 32 also shows the absorption values for the substances investigated.

Table 34: LD_{50} values (mg/kg) of clonidine and some analogues estimated in mice after intravenous injection. Oberservation period 24 hours. Data from Hoefke et al. (1975).

Compd. no.[a] (X)	LD_{50} (mg/kg) (95% confidence limits)
St- 91 (2,6-di-Et)	2.4 (2.1 – 2.8)
155 (2,6-di-Cl)[b]	17.6 (14.2 – 22.0)
375 (2-Cl,4-Me)	22.3 (19.1 – 26.1)
608 (2-Cl,3-Me)	30.0 (27.0 – 33.3)
600 (2-Me,5-F)	31.0 (27.4 – 35.0)
93 (2-Cl,6-Me)	31.2 (27.4 – 35.6)

[a] Numbering refers to table 5.
[b] From Hoefke and Kobinger (1966).

Table 33: Blood glucose levels in rats in mg % (mean ± S.E.M.) after subcutaneous injection of 0.1 mg/kg test substance. Data from Hoefke et al. (1975).

Compd. no.[a] (X)	Hours after Administration 0	1	2	4
St-155 (2,6-di-Cl)	70 ± 2.2	117 ± 7.5	135 ± 7.1	125 ± 10.0
91 (2,6-di-Et)	70 ± 3.8	81 ± 1.7	85 ± 3.2	84 ± 2.9
93 (2-Cl,6-Me)	72 ± 2.3	115 ± 4.8	124 ± 4.8	115 ± 6.7
375 (2-Cl,4-Me)	62 ± 2.2	105 ± 3.1	93 ± 5.4	80 ± 3.9
600 (2-Me,5-F)	58 ± 1.5	91 ± 3.1	88 ± 2.6	69 ± 2.0
608 (2-Cl,3-Me)	74 ± 3.5	89 ± 3.1	91 ± 4.1	93 ± 3.1

[a] Numbering refers to table 5.

Hyperglycaemic Action

Blood glucose was determined enzymatically in starved rats before and after subcutaneous application of the drugs (0.1 mg/kg). All test substances increased blood glucose levels, which reached a maximum within 2 hours (table 33). At a dose of 0.1 mg/kg clonidine was most effective and *St-91* (2,6-di-Et) least effective in elevating blood glucose levels observed 2 hours after subcutaneous injection.

Acute Toxicity in Mice

Acute toxicity of the compounds was estimated in mice after intravenous administration. The observation period was 24 hours. The animals showed piloerection and exophthalmus following intravenous administration of all the derivatives. At toxic doses tonic-clonic convulsions developed. The LD_{50} values are reported in table 34. Clonidine and its phenyl-substituted derivatives showed no particularly high toxicity, *St-91* (2,6-di-Et) excepted, which appeared considerably toxic in mice.

V. Comparison Between Various Pharmacological Actions of Clonidine and Related Compounds

5.1. Introduction

The present Chapter is devoted to a comparison between the various pharmacological effects exerted by clonidine and related compounds reviewed in the preceding Chapter. As has been remarked in the foregoing, the use of clonidine in the treatment of hypertension is accompanied by some side-effects such as sedation and inhibition of secretion. When looking for new compounds, it is important to study whether a differentiation between therapeutic (antihypertensive) efficacy and side-effects may be possible. Moreover, the relationships between the various pharmacological actions are interesting. Upon comparing the relative potencies mediated by different receptive sites, indications may be obtained, whether the demands which are made upon the agonists by the receptors are comparable or differ mutually.

5.2. Hypotensive Activity Versus Sedation and Antisecretory Activity

Hoefke et al. (1975) have compared the ranking order of potencies in a number of pharmacological tests for clonidine and 5 of its phenyl-substituted analogues. The different potencies of the substances in these tests have been reported in ranking order in table 35. With some reservations, as to the biological variability of the test methods employed, a striking parallelism can be seen between cardiovascular, sedative and hyperglycaemic potencies.

The hyperglycaemic action of clonidine has been explained by a direct effect on α-adrenoceptors in the β-cells of the pancreas which results in a decrease in insulin activity in the blood (Senft et al., 1968). Additionally, Bock and van Zwieten (1971) have proposed a central nervous origin for the hyperglycaemia. The parallelism between the hyperglycaemic and sedative effects for this series of compounds may indicate that a central point of action may be of importance for the hyperglycaemia.

A less satisfactory correlation exists between the inhibitory action on gastric acid secretion and the other effects listed in table 35. This is not unexpected, since *St-91*, which has only a peripheral action, was included. The effect of clonidine on gastric secretion may be due to at least two components. There is a direct stimulating effect mediated by histamine H_2-receptors and a central inhibitory action (Karppanen and Westermann, 1973; Walz and van Zwieten, 1970).

As far as this inhibition of secretion by clonidine-like drugs is concerned, there exists an acceptable correlation between the dose (mg/kg) which reduced the titratable acidity to 50% ($ED_{50\ acid.}$) and the amount (mg/kg) decreasing

Table 35: Comparison between the potencies in a number of pharmacological tests of clonidine and some related imidazolidines. Data from Hoefke et al. (1975).

Compd. no.[a]	(X)	Ranking Order			
		Bradycardiç action[b]	Sedation[c]	Hyperglycaemic action[d]	Inhibition of gastric acidity[e]
St-155	(2,6-di-Cl)	1	1	1	1
91	(2,6-di-Et)	(6)	6	6	4
93	(2-Cl,6-Me)	2	2	2	2
375	(2-Cl,4-Me)	3	3	3	3
600	(2-Me,5-F)	5	5	5	6
608	(2-Cl,3-Me)	4	4	3	5

[a] Numbering refers to table 5.
[b] Decrease in heart rate in vagotomized rats (from table 15).
[c] Prolongation of hexobarbitone sleeping time in mice (from table 23).
[d] Blood glucose levels in rats (from table 33).
[e] Reduction of total gastric acidity in pylorus-ligated rats (from table 26).

the secretion volume by 50 % ($ED_{50\ vol}$) in pylorus-ligated rats:

$$ED_{50\ acid.} = 0.527\ ED_{50\ vol.} + 0.331 \quad \text{(eq. 13)}$$
$$n = 74;\ r = 0.798;\ s = 1.131;\ F = 127$$
$$(P<0.001)$$

The data used to generate equation 13 were taken from table 27. The values obtained by extrapolation were omitted.

Upon correlating $ED_{50\ acid.}$ and $ED_{50\ vol.}$ with the corresponding dose (mg/kg) diminishing arterial pressure by 20 mm Hg in the anaesthetized rabbit ($ED_{20\ blood\ pressure}$; data from table 5) the equations 14 and 15 resulted:

$$ED_{50\ acid.} = 2.882\ ED_{20\ blood\ pressure} + 1.133 \quad \text{(eq. 14)}$$
$$n = 74;\ r = 0.464;\ s = 1.984;\ F = 19.74$$

$$ED_{50\ vol.} = 2.367\ ED_{20\ blood\ pressure} + 1.915 \quad \text{(eq. 15)}$$
$$n = 66;\ r = 0.283;\ s = 2.668;\ F = 5.58$$

The equations 14 and 15 show no overall relationship between blood pressure lowering activity in the rabbit and inhibitory potency on gastric secretion in the rat. However, by calculating these correlations with the aid of Spearman's rank correlation test within series possessing an identical substitution at the phenyl ring statistically significant correlations have been found between $ED_{50\ acid}$ in rats and $ED_{20\ blood\ pressure}$ in rabbits (Hoefke, unpublished results). Significant relationships ($P < 0.05$) resulted for 2,3-, 2,4-, 2,5- and 2,6-disubstituted as well as for 2,4,6-trisubstituted derivatives.

In addition, when a comparison is made between $ED_{50\ acid.}$ (mg/kg) and $ED_{50\ vol.}$ (mg/kg) on the one hand and the blood pressure lowering activity in the rat (ED_{30}, µg/kg; data from table 6) one the other hand, relationships (equations 16 and 17) are obtained with much better correlation coefficients.

$$ED_{50\ acid.} = 0.0059\ ED_{30\ blood\ pressure} + 0.468 \quad \text{(eq. 16)}$$
$$n = 16;\ r = 0.877;\ s = 0.833;\ F = 47$$
$$(P<0.001)$$

$$ED_{50\ vol.} = 0.0083\ ED_{30\ blood\ pressure} + 0.867 \quad \text{(eq. 17)}$$
$$n = 15;\ r = 0.793;\ s = 1.677;\ F = 22$$
$$(P<0.001)$$

The result from the rank test investigations and the equations 16 and 17 point to the conclusion that generally these two actions are still somewhat inherent in each other, particularly when determined in the same animal species.

The results of Hoefke et al. (1975), treated above, strongly suggest that it seems unlikely that within the imidazolidine series hypotensive and sedative effects can be separated from each other. On the contrary, the results of Laverty (1969) indicated a differentiation between antihypertensive properties and sedation (data from

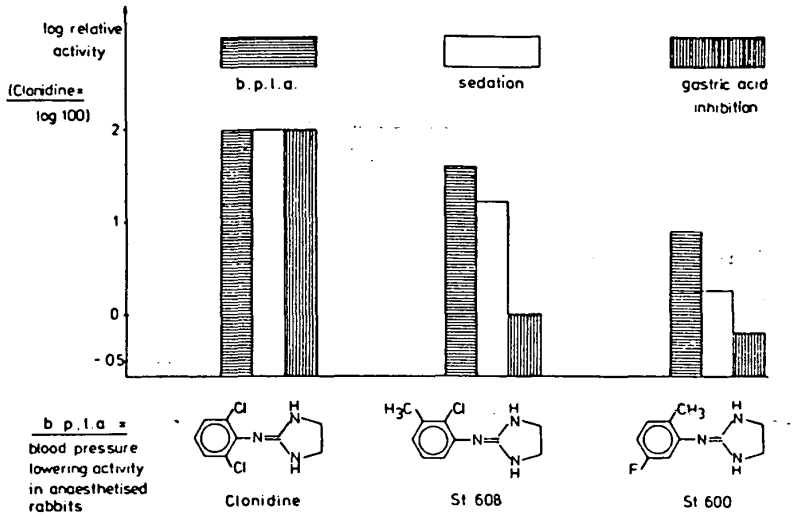

Fig. 47: Differentiation between hypotensive, sedative and gastric acid inhibitory properties in imidazolidines structurally related to clonidine. From Stähle (1974), with permission.

the tables 12 and 22) and also in the series of Hoefke et al. (1975) some examples were found. Figure 47 has been constructed by Stähle (1974) from the data of Hoefke et al. (1975) and shows 2 compounds with a more favourable ratio of blood pressure lowering activity over sedation or gastric acid inhibition than clonidine.

For compound *St-608* (2-Cl, 3-Me) the quotient of hypotensive activity/antisecretory potency is 30 times better than for clonidine. This ratio is 20 times more favourable for *St-600* (2-Me, 5-F). Moreover, *St-600* possesses a 7 times more beneficial quotient of hypotensive activity over sedation than clonidine. Clinical trials will have to show whether these findings in animals also hold true for human patients.

A separation between the sedative and hypotensive activity in some substituted 6-phenyl-2,3,6,7-tetrahydro-5H-pyrrolo-[1,2-a]-imidazoles (see fig. 22) has been reported by Clough et al. (1978). The compound with X = 2,6-di-Cl, being equally potent as clonidine in lowering blood pressure, was found 1/10 as active as a sedative. The substance for which X = 2-Cl, 6-F was slightly less effective than clonidine in diminishing blood pressure, but was found only 1/80 as potent as a sedative.

In this connection it may also be mentioned that clonidine-like structures obtained by substituting the bridge nitrogen atom, especially by alkenyl residues, show a differentiation of the pharmacological spectrum in favour of analgetic properties. Stähle (1974) has given one example in order to illustrate these findings (fig. 48). The

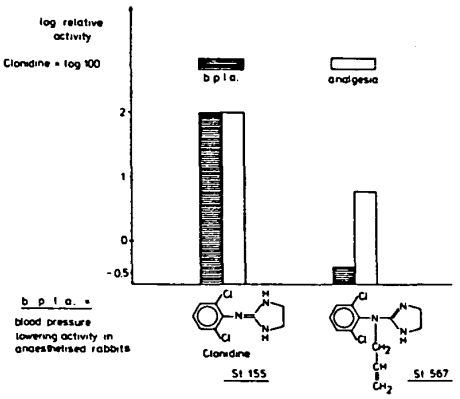

Fig. 48: Differentiation between hypotensive and analgetic activities by alkenyl substitution in clonidine analogues. From Stähle (1974), with permission.

ratio of hypotensive activity to analgesia is 8 times more pronounced in compound *St-567* than in clonidine.

5.3. Central Versus Peripheral α-Adrenergic Effects

The initial, transient hypertensive effect elicited by intravenous clonidine reflects the excitation of peripheral, vascular α-adrenoceptors. The subsequent, long lasting hypotensive phase, which is accompanied by bradycardia originates from a centrally mediated inhibition of peripheral sympathetic activity, brought about by stimulation of central α-adrenoceptors at medullary sites (also see Chapter I).

Attempts have been made at comparing peripheral and central α-adrenergic effects on the basis of stimulating potencies of agonists. Hoefke et al. (1975) have compared the activities of clonidine and 5 of its analogues with respect to their central sympathoinhibitory and peripheral α-adrenoceptor-stimulating (see table 18; page 49) effects. Bradycardia in vagotomized rats was taken for an estimation of central α-adrenergic activity, i. e. a decrease in sympathetic tone (see table 15; page 47). As can be seen from the tables 15 and 18 there is no simple, linear correlation between the central and the peripheral α-adrenergic effects of the drugs. Hoefke et al. (1975) have subsequently proposed that the central cardiovascular depressor effect (A) of a drug is a function of its transfer through the blood-brain barrier (B), described by the partition coefficient between octanol and aqueous phase (pH = 7.4) and of its α-adrenoceptor-stimulating potency (C). The natural logarithm of the product of the partition coefficient times relative hypertensive potency in the spinal rat (ln BC) was plotted on the x-axis and the natural logarithm of the relative bradycardic potency in the vagotomized rat (ln A) on the y-axis. The result is visualized in fig. 49. With the exception of *St-91* (2,6-di-Et) it supports the hypothesis that there exists an interrelationship between α-adrenoceptor-stimulating potency, lipophilicity and central cardiovascular depression.

This non-linear relationship indicates that to a certain degree the centrally mediated cardiovascular depression increases with increasing peripheral α-adrenoceptor-stimulating potency and lipophilicity. However, by multiplying lipophilicity with peripheral hypertensive potency, the solution of this relationship provides a single value only and there is no possibility to differentiate between the two factors. Therefore, each term should be present separately in the equation. Consequently, they can have their own weighting factor in determining central cardiovascular depression.

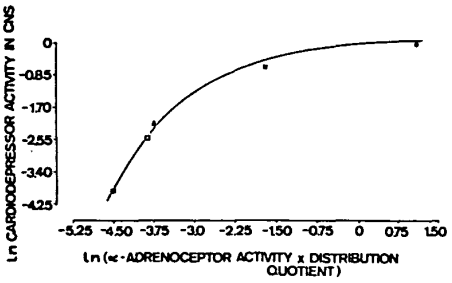

Fig. 49: Relationship between peripheral α-adrenoceptor activity, lipophilicity and central sympathoinhibitory activity.
Abscissa: Natural logarithm of the product of relative activity on peripheral α-adrenoceptors as derived from the hypertensive effect in spinal rats (see table 18) and the partition coefficient between octanol/buffer (pH = 7.4). *Ordinate:* Natural logarithm of the relative central cardiodepressor activity as derived from the bradycardic effect in vagotomized rats (see table 15). Clonidine (*St-155;* 2,6-di-Cl); × *St-93* (2-Cl,6-Me); Δ *St-375* (2-Cl,4-Me); □ *St-608* (2-Cl,3-Me); ■ *St-600* (2-Me, 5-F). From Hoefke et al. (1975), with permission.

Correlation studies have been reported in which the hypotensive activity of clonidine and 12 of its structurally, closely related imidazolidines was correlated with their peripheral, hypertensive potency. In these correlations the ability of the compounds to penetrate into the central nervous system was implicated separately by the octanol/buffer (pH = 7.4) partition coefficients (Timmermans and van Zwieten, 1977e). Dose-response curves were used in order to calculate the negative logarithm of the dose, μmol/kg, required to invoke a 30 % decrease in mean arterial pressure (pC_{30}) of anaesthetized rats as an index of central hypotensive activity (data from table 6). The negative logarithm of the dose, μmol/kg, associated with an increase in arterial pressure by

100 % (pC_{100}) in pithed rats was employed as a measure of peripheral hypertensive potency (data from table 17). The apparent partition coefficients (log P') used in this correlation study have already been listed in table 4.

The following equations were derived between the hypotensive (pC_{30}) and the hyptertensive (pC_{100}) activities of the imidazolidine derivatives (the figures in parentheses are the 95 % confidence intervals):

$$pC_{30} = 1.051\ (\pm 0.76)\ pC_{100} - 0.778 \quad \text{(eq. 18)}$$
$$n = 13;\ r = 0.674;\ s = 0.860;\ F = 9.15\ (P < 0.005)$$

$$pC_{30} = 0.627\ (\pm 0.29)\ \log P' + 0.837\ (\pm 0.46)\ pC_{100} - 0.302 \quad \text{(eq. 19)}$$
$$n = 13;\ r = 0.912;\ s = 0.501;\ F = 24.69\ (P < 0.001)$$

$$pC_{30} = 0.002\ (\pm 0.04)\ (\log P')^2 + 0.636\ (\pm 0.35)\ \log P' + 0.850\ (\pm 0.55)\ pC_{100} - 0.340 \quad \text{(eq. 20)}$$
$$n = 13;\ r = 0.912;\ s = 0.528;\ F = 14.85\ (P < 0.001)$$

The linear correlation between pC_{30} and pC_{100} is shown in equation 18. This relationship is significant, but is substantially improved upon the inclusion of lipophilicity (log P') (equation 19). In equation 19 the term in log P' is highly significant, whereas the incorporation of an additional squared term in log P' is not (equation 20). The positive signs of the coefficients of pC_{100} and log P' in the equations indicate that the hypotensive activity parallels the hypertensive potency and is also favoured by increasing lipophilicity of the compound. Equation 19 describes the central hypotensive activity as a linear combination of peripheral hypertensive potency and lipophilicity for a number of phenyl-substituted imidazolidines structurally closely related to clonidine. The correlation with multiplication of peripheral α-adrenoceptor activity with partition coefficient as well as the addition of peripheral α-adrenoceptor activity and partition coefficient resulted in a similar general conclusion.

In order to establish a more or less general applicablility of this relationship, similar studies have been performed with a number of structurally dissimilar α-adrenoceptor agonists (Timmermans and van Zwieten, 1977d; Timmermans, 1978). The structures used in this investigation are reported in table 36 and involved compounds possessing different ring junctions, hetero rings, aromatic and non-aromatic moieties. Also included were the classical α-adrenoceptor-stimulating agents naphazoline, tramazoline, tetryzoline and xylometazoline as well as 2 tri-substituted imidazolidines (*St-739* and *871*) with pronounced lipophilic character. The hypotensive activity was determined following intravenous administration to pentobarbitone-anaesthetized, normotensive rats. This sympathoinhibitory action was quantified by means of a pC_{25} calculated from dose-response curves (C_{25}: dose, μmol/kg, required to invoke a 25 % decrease in mean arterial pressure). The values have been listed in table 36. The peripheral hypertensive activity of the substances was established accordingly in pithed rats and characterized by means of a pC_{60} (C_{60}: dose, μmol/kg, associated with an increase in mean arterial pressure by 60 mm Hg). The data have been enumerated in table 36 (also see table 20). Apparent partition coefficients (log P') determined in the octanol/aqueous buffer system (pH = 7.4) at 37° were used as a measure of over-all lipophilic behaviour (see table 36).

Initially, correlation studies were performed to relate the central hypotensive activity, expressed by pC_{25}, with the peripheral hypertensive potency, described by pC_{60}, for 15 compounds. Compound no. 9 (*St-871*) and no. 15 (*St-739*) were omitted for reason of high lipophilictiy. The equations derived are summarized below:

$$pC_{25} = 1.011\ pC_{60} - 0.214 \quad \text{(eq. 21)}$$
$$n = 15;\ r = 0.821;\ s = 0.637;\ F = 26.85\ (P < 0.005)$$

$$pC_{25} = 0.522\ \log P' + 0.944\ pC_{60} - 0.439 \quad \text{(eq. 22)}$$
$$n = 15;\ r = 0.952;\ s = 0.357;\ F = 57.43\ (P < 0.001)$$

$$pC_{25} = -0.135\ (\log P')^2 + 0.678\ \log P' + 0.929\ pC_{60} - 0.340 \quad \text{(eq. 23)}$$
$$n = 15;\ r = 0.957;\ s = 0.353;\ F = 39.65\ (P < 0.001)$$

Equation 21 shows that for these 15 compounds there exists already an appreciable, posi-

Table 36: Central hypotensive activity (pC_{25}), peripheral hypertensive activity (pC_{60}) and octanol/aqueous buffer (pH = 7.4) partition coefficients (log P') at 37°C for a number of structurally dissimilar α-adrenoceptor agonists. The values reported were used to generate the equations 21 to 26. Data from Timmermans and van Zwieten, submitted for publication.

No.	Compd.	Central hypotensive activity in anaesthetized rats (μmol/kg) pC_{25}	Octanol/buffer (pH = 7.4; 37°C) partition coefficient (log P')²	log P'	Peripheral hypertensive activity in pithed rats (μmol/kg) pC_{60}
1	Compound *44–549*	*2.77*	*4.08*	*2.02*	*2.40*
2	*Bay a 6781*	2.32	1.93	1.39	2.11
3	Lofexidine	2.09	0.53	0.73	1.99
4	Clonidine	2.04	0.72	0.85	1.78
5	*Bay c 6014 (LD 2855)*	1.96	1.64	1.28	1.51
6	*UK-14,304-18*	1.55	0.10	0.31	1.56
7	Naphazoline	0.95	0.27	−0.52	1.83
8	*St-1967* (S-bridge)	0.88	1.85	1.36	1.24
9	*St- 871* (2,6-di-Cl,4-Br)	0.84	5.32	2.31	0.92
10	*St-1913* (CH_2-bridge)	0.68	0.28	−0.53	1.17
11	*KUM 32*	0.63	4.49	2.12	0.23
12	Xylazine *(Bay 1470)*	0.62	1.80	1.34	−0.02
13	Tramazoline	0.55	0.38	−0.62	1.80
14	Xylometazoline	0.26	0.16	0.40	1.12
15	*St- 739* (2,4,6-tri-Br)	−0.02	6.29	2.51	0.65
16	Tetryzoline	−0.16	0.81	−0.90	0.90
17	*St- 404*	−1.31	0.12	−0.34	−0.79

tive, linear correlation between the central hypotensive activity and the peripheral hypertensive potency. This relationship is most significantly improved upon the addition of lipophilicity (log P') (equation 22). Note the substantial increase in the correlation coefficient, r, and the F-value and the decrease in standard deviation, s, of the regression. However, the incorporation of an additional squared term in log P' did not provide a relationship statistically better (equation 23).

Upon including the very lipophilic compounds no. 9 *(St-871)* and no. 15 *(St-739)* the following equations were generated:

$$pC_{25} = 1.031\ pC_{60} - 0.257 \quad \text{(eq. 24)}$$
$$n = 17;\ r = 0.826;\ s = 0.605;\ F = 32.17\ (P < 0.001)$$

$$pC_{25} = 0.333 \log P' + 1.036\ pC_{60} - 0.532 \quad \text{(eq. 25)}$$
$$n = 17;\ r = 0.899;\ s = 0.486;\ F = 29.55\ (P < 0.001)$$

$$pC_{25} = -0.294\ (\log P')^2 + 0.787 \log P' + 0.950\ pC_{60} - 0.262 \quad \text{(eq. 26)}$$
$$n = 17;\ r = 0.942;\ s = 0.387;\ F = 34.05\ (P < 0.001)$$

Equation 24 is statistically as relevant as equation 21 and represents the interrelationship between pC_{25} and pC_{60} for these 17 α-adrenoceptor agonists. The introduction of log P' again enlarged the significance of the relationship (equation 25). However, the incorporation of an extra $(\log P')^2$ term improved the correlation even more (equation 26). Equation 26 is a most significant one. The hypotensive activity of of these 17 drugs is described as a function of peripheral hypertensive potency and lipophilicity. The latter is present in a parabolic form. Equation 26 provides calculated hypotensive activities which agree very well with the observed ones (see fig. 50).

The question now arises, whether this parabolic dependence on lipophilicity (log P') is

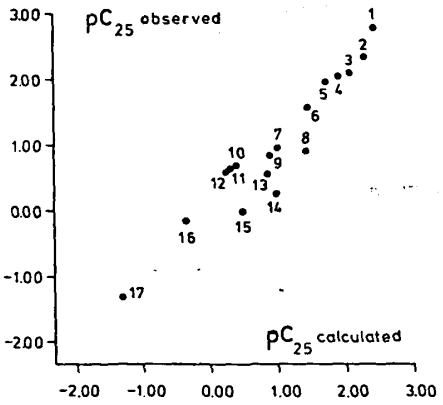

Fig. 50: Comparison between the hypotensive activities obtained experimentally from dose-response curves ($pC_{25\ observed}$; see table 36) and the values calculated by using correlation equation 26 ($pC_{25\ calculated}$) for 17 α- adrenoceptor agonists. The numbering refers to table 36. From Timmermans and van Zwieten, submitted for publication.

indeed related to the difference in accessibility of the agonists to the peripheral and central α-adrenoceptors and represents the transport process of the drugs from the blood to the brain. We now may return to fig. 8 (Chapter III). For clonidine and its structurally related imidazolidines it has been found that the fraction of the amount of drug, given intravenously, accumulating into the brain depends on the over-all lipophilic property (log P') of the substance involved. A parabolic description in log P' with very acceptable statistics resulted (equation 9; page 15). In particular the presence of *St-871* (2,6-di-Cl, 4-Br) and *St-739* (2,4,6-tri-Br) did make a parabolic dependence on log P' more significant than a linear description in lipophilicity (equation 8). Therefore, lipophilicity (log P') is indeed related to the transport process of the drugs from the blood to the brain.

This finding emphasises the relevance of the appearance of log P' in the relationship between central hypotensive and peripheral hypertensive activity enumerated above. Moreover, if very lipophilic α-adrenoceptor agonists are included a parabolic description in log P' is necessary.

It may be supposed then that the over-all lipophilic behaviour of an α-adrenoceptor-stimulating drug, expressed by log P', will cause the relative difference between peripherally mediated pressor activity and centrally induced depressor potency. For instance, the peripheral hypertensive activities (pC_{60}) of *St-871* and tetryzoline are approximately the same (0.92 and 0.90, respectively; see table 36; page 67. However, their log P' values differ by more than 2 logarithmic units, a difference which causes the great disparity in central hypotensive activity (pC_{25}) of these two compounds (0.84 and −0.16, respectively: see table 36).

Based on these results it is most tempting to speculate upon a possible resemblance between the α-adrenoceptors located at medullary cardiovascular centres and those present in the periphery at the vascular wall. There may only be a difference in the accessibility to these two types of receptive sites.

As a result of a possible similarity between peripheral, vascular and central, medullary α-adrenoceptors, proposed above, any α-sympathomimetic drug eliciting peripheral hypertensive effects will also display central sympathoinhibitory actions, provided that the drug is able to reach its receptive site in a sufficient amount. However, it has already been mentioned in the preceding Chapter that the 2,5-dichloro derivative and some other analogues of clonidine were found ineffective by Rouot et al. (1976, 1977; see table 8). On the other hand, these compounds showed readily measurable hypotensive effects according to other authors (Timmermans and van Zwieten, 1977a, 1977b; Stähle, 1974; Hoefke, 1976; see tables 5, 6 and 9). The reason for the discrepancy between these results is not clear. Furthermore, no cardiovascular inhibition was observed in dogs following intracisternal administration of 3–50 μg/kg of the classical α-sympathomimetics naphazoline, oxymetazoline and tramazoline (Schmitt and Fénard, 1971). Besides, direct injections of naphazoline, xylometazoline, oxymetazoline *St-91* (2,6-di-Et) and *St-1913* (CH_2-bridge) into the anterior hypothalamic area of rats did not effect blood pressure and heart rate (Struyker Boudier et al., 1975; see table 14; page 47. These results have been challenged by Kobinger and Pichler (1975, 1976). Following intravenous adminis-

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tration to vagotomized rats, naphazoline, oxymetazoline and *St-91* (2,6-di-Et) did not display the sympathoinhibitory actions typical of clonidine. On the other hand after intracisternal injection into dogs and vagotomized cats these compounds elicited a cardiovascular depression similar to that following clonidine (Kobinger and Pichler, 1975, 1976). In addition, hypotensive and bradycardic effects were observed after systemic application of naphazoline, tramazoline, tetryzoline and xylometazoline to anaesthetized, normotensive rats (Timmermans et al., 1978; also see figs. 35 and 45). Moreover, also *St-1913* was found effective in this animal model (see fig. 42, page 25 and table 36, page 67).

The central α-stimulatory effect of clonidine can readily be antagonized by piperoxan and yohimbine and in a far less convincing manner by other α-sympatholytic agents such as phenoxybenzamine and phentolamine, which are active blockers of noradrenaline and clonidine at peripheral sites (Schmitt et al., 1971, 1973; cf. van Zwieten, 1975a). This observation may indicate a difference between peripheral, vascular and central, medullary α-adrenoceptors. However, phentolamine,when administered centrally, is an effective antagonist of the sympathoinhibitory effects of clonidine (Kobinger and Walland, 1971) and of naphazoline applied intracisternally (Kobinger and Pichler, 1975). The over-all lipophilic behaviour of α-sympatholytic drugs has never been taken into account quantitatively with respect to the interpretation of these results. In fact the lipophilic character of piperoxan and yohimbine is about 30 times more pronounced than that of phentolamine and phenoxybenzamine (Timmermans, unpublished results). Consequently one should consider the possibility that differences in penetration abilities of these compounds discriminate between peripheral and central α-blocking potencies. Finally, the differences in blocking activities at pre- and postsynaptically located α-adrenoceptors between piperoxan and yohimbine on the one hand and phentolamine and phenoxybenzamine on the other hand (Cubeddu et al., 1974; Starke et al., 1975a; Borowski et al., 1976; Drew, 1976) may also be interpreted as a possible additional factor.

5.4. Hypotensive Activity Versus Bradycardic Potency

It has been reported by Hoefke et al. (1975) that the estimation of the hypotensive potency in cats (see table 7; page 40) of clonidine and some analogues is comparable with the results in the test for bradycardia in vagotomized and atropinized rats (see table 15; page 47). This qualifies the latter as being representative for a determination of the typical clonidine-like hypotensive effect, i. e. a decrease in peripheral sympathetic tone. The compounds also facilitated the vagally mediated cardiodepressor reflex. It seems, therefore, that a decrease in sympathetic and an increase in vagal activity are linked together for this series of derivatives.

Following intravenous administration of 27 imidazolidines, including clonidine, to anaesthetized, normotensive rats the reduction in cardiac frequency was measured at the moment of maximal decrease in mean arterial pressure (Timmermans and van Zwieten, 1977a) and quantified by means of an ED_{25} (see table 6; page 37). Equation 27 was formulated from the hypotensive (ED_{30}) and the bradycardic (ED_{25}) data (Timmermans and van Zwieten, 1977e):

$$\log ED_{30} = 1.212 \log ED_{25} - 0.494 \quad \text{(eq. 27)}$$

$$n = 26,\ r = 0.960;\ s = 0.248;\ F = 279.22\ (P < 0.001)$$

The unsubstituted derivative no. *15* was not included in the development of equation 27, since this compound appeared only moderately active in decreasing heart rate. Equation 27 which is visualized in fig. 51 shows a linear relationship between blood pressure lowering and bradycardic activities of the substances in the anaesthetized, normotensive rat.

It has also been found that in this same animal species the ranking order of bradycardic activities of naphazoline, tramazoline, xylometazoline and tetryzoline is comparable with that of the hypotensive potency (Timmermans et al., 1978b; cfr. fig. 35 with fig. 45). Apparently, these two pharmacological actions run parallel, i. e. when an imidazolidine derivative is more active with respect to a reduction in arterial pressure, it is also more potent in lowering cardiac frequency.

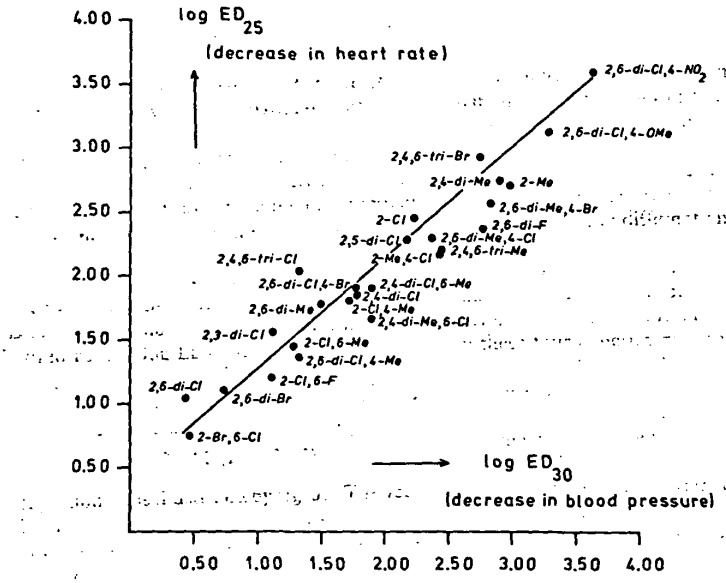

Fig. 51: Relationship between the hypotensive activity (log ED_{30}) and the bradycardic potency (log ED_{25}) in the anaesthetized, normotensive rat for clonidine and its structurally related imidazolidines. The relationship is described mathematically by means of equation 27. From Timmermans and van Zwieten (1977e), with permission.

An even more significant relationship (equation 28) proved to exist between the antihypertensive activity of imidazolidines, log ED_{20}(BP), (data from table 11; page 44) and the potency in reducing heart rate, log ED_{20}(HR), (data from table 13; page 46) measured in the conscious, spontaneously hypertensive rat (Timmermans and van Zwieten, 1977b):

$$\log ED_{20}(BP) = 1.041 \log ED_{20}(HR) - 0.186 \quad \text{(eq. 28)}$$
$$n = 7;\ r = 0.989;\ s = 0.079;$$
$$F = 221.69\ (P < 0.001)$$

The linear relationship is visualized in fig. 52 and shows that, similarly to the anaesthetized, normotensive rat, bradycardia parallels hypotension in the conscious, spontaneously hypertensive rat as well.

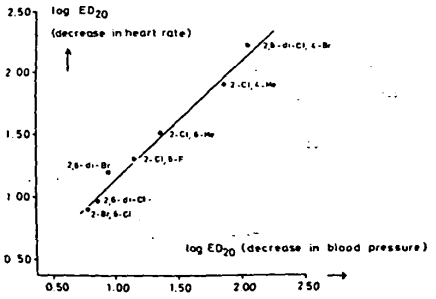

Fig. 52: Relationship between the antihypertensive activity (log ED_{20}) and the bradycardic potency (log ED_{20}) in the conscious, spontaneously hypertensive rat for clonidine and some phenyl-substituted imidazolidines. The relationship is described mathematically by means of equation 28. From Timmermans and van Zwieten (1977b), with permission.

5.5. Hypotensive Activities in Various Animal Species

The hypotensive effect of clonidine and 26 of its congeneric analogues has been measured in anaesthetized, normotensive rats following intravenous administration (Timmermans and van Zwieten, 1977a). Dose-response curves were used in order to quantify the hypotensive activities of the drugs by means of ED_{30} values (see table 6; page 37). A procedure similar to the one described above has been followed to establish the antihypertensive action of a selected number of derivatives in the conscious, spontaneously hypertensive rat (Timmermans and van Zwieten, 1977b), which yielded an ED_{20} for the antihypertensive activity of the substances (see table 11; page 44). By means of infusions into the left vertebral artery of anaesthetized cats the central mode of action of a number of clonidine-like imidazolidines has been investigated (Timmermans and van Zwieten, 1977b). The central hypotensive activity was quantified with the aid of an ED_{25} value (see table 9; page 42). Finally, at the pharmacological research department of C. H. Boehringer Sohn, Ingelheim (Hoefke and coworkers) the anaesthetized rabbit has been used in order to investigate the hypotensive effects of an impressive number of imidazolidines. An ED_{20} was used as a measure of the hypotensive efficacy of the compounds (see table 5; page 31).

Equation 29 (also see fig. 53) correlates the hypotensive activity in the anaesthetized, normotensive rat (log ED_{30}; μg/kg) with the hypotensive potency induced centrally in the anaesthetized cat (log ED_{25}; μg/kg) of 8 imidazolidines studied in these two animal species:

$$\log ED_{30} = 0.891 \log ED_{25} + 0.702 \quad \text{(eq. 29)}$$
$$n = 8;\ r = 0.974;\ s = 0.284;$$
$$F = 109.07\ (P < 0.001)$$

The relationship is linear and shows that compared to the rat the cat is somewhat more sensitive to these hypotensive drugs.

Equation 30 (also see fig. 54) relates the depressor activities in the anaesthetized, normotensive rat (log ED_{30}; μg/kg) with the antihypertensive potencies in the conscious, spontaneously hypertensive rat (log ED_{20}; μg/kg) of 7 compounds for which both parameters were determined:

$$\log ED_{30} = 1.083 \log ED_{20} - 0.340 \quad \text{(eq. 30)}$$
$$n = 7;\ r = 0.978;\ s = 0.127;$$
$$F = 110.07\ (P < 0.001)$$

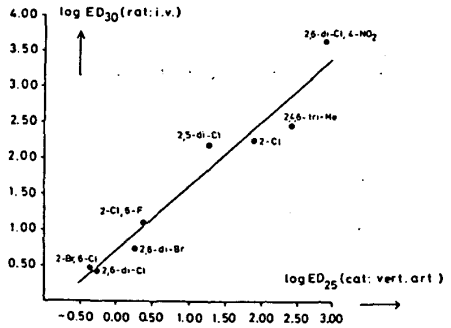

Fig. 53: Relationship between the hypotensive activity in the anaesthetized, normotensive rat (log ED_{30}) and the hypotensive potency induced centrally in the anaesthetized cat (log ED_{25}) for clonidine and a number of structurally related imidazolidines. The relationship is described mathematically by means of equation 29. From Timmermans and van Zwieten (1977b), with permission.

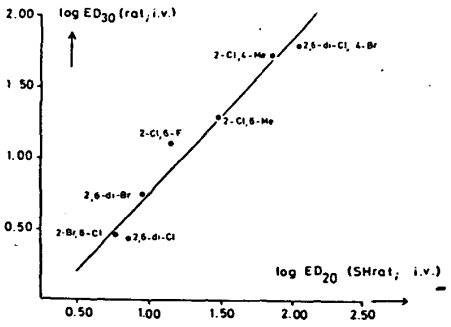

Fig. 54: Relationship between the depressor activity in the anaesthetized, normotensive rat (log ED_{30}) and the antihypertensive potency in the conscious, spontaneously hypertensive rat (log ED_{20}) for clonidine and a number of structurally related imidazolidines. The relationship is described mathematically by means of equation 30. From Timmermans and van Zwieten (1977b), with permission.

In accordance with equation 29 this relationship is also linear. In this particular case the conscious, spontaneously hypertensive rat is less sensitive than the anaesthetized, normotensive rat towards a reduction in arterial pressure brought about by these clonidine-like hypotensive agents.

Equation 31 was generated between the corresponding hypotensive data obtained with the aid of the anaesthetized, normotensive rat (log ED_{30}; µg/kg) and the ones from the anaesthetized rabbit (log ED_{20}; µg/kg):

$$\log ED_{20} = 0.581 \log ED_{30} + 1.040 \quad \text{(eq. 31)}$$

$$n = 27;\ r = 0.739;\ s = 0.522;\ F = 30.11\ (P < 0.05)$$

Equation 31 is illustrated by fig. 55. The vertical arrows indicate that these particular compounds were found only moderately active in the rabbit at the dose given (see table 5; page 31). Consequently, their ED_{20} values will be higher in this animal species.

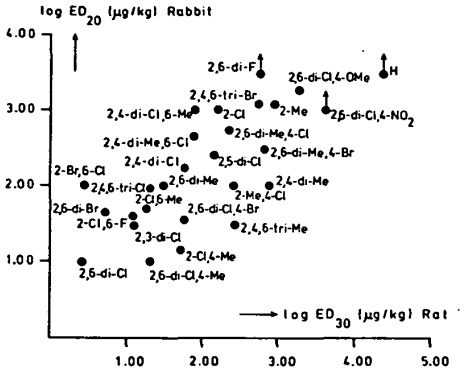

Fig. 55: Relationship between the hypotensive activities established in the anaesthetized, normotensive rat (log ED_{30}; µg/kg) and the anaesthetized rabbit (log ED_{20}; µg/kg) for clonidine and a number of congeneric substance. The relationship is described mathematically by means of equation 31.

Equation 31 shows a significant, linear relationship between the hypotensive activities in the rat and the rabbit. The results are of a lower level of significance than those of equations 29 and 30, but are acceptable in view of the fact that the data obtained in the rabbit have been gathered with various different rabbit strains during many years.

The three equations listed above interrelate the hypotensive properties of clonidine and its analogues in four different animal models. The equations formulated demonstrate that generally all of the factors determining the relative differences in depressor activity within this series of derivatives display a similar character in these four experimental animal models.

The relative differences in hypotensive potencies of clonidine and its derivatives were found comparable in the anaesthetized, normotensive rat, the anaesthetized cat, the conscious, spontaneously hypertensive rat and in the anaesthetized rabbit. This observation indicates that the mechanism, underlying the depressor response, is similar in these four animal models. Moreover, it leads to the speculation that possibly the central α-adrenoceptors of these animals possess identical character. The afore-mentioned observation also stresses the opinion that the penetration processes of the compounds into the central nervous system are not fundamentally different from each other in these animals. Therefore, after application ot the drugs via the vertebral artery the demands which are made upon the lipophilicity of the compounds are just as stringent as after their systemic application.

The conscious, spontaneously hypertensive rat proved less sensitive towards the clonidine-like substances than the anaesthetized, normotensive rat. This may be attributed to the anaesthesia and the difference in basal blood pressure. Acute intravenous administration of small doses of clonidine only rarely provokes a hypotensive effect in conscious, normotensive rats (Trolin, 1975; Timmermans, personal observations), while rats under pentobarbitone anaesthesia and also conscious, spontaneously hypertensive rats readily respond by a fall in blood pressure. It has been suggested that the hypertensive effect of clonidine is not solely limited to the periphery, but that it also depends on intact structures rostral to the hypothalamus (Trolin, 1975; Henning et al., 1976). According to these authors anaesthetics, like pentobarbitone, suppress this pressor effect centrally. On the other hand, barbiturates induce an enhance-

ment in peripheral sympathetic tone. For these reasons it seems likely that the hypotensive response will be less pronounced in conscious rats than in pentobarbitone anaesthetized animals. In the spontaneously, hypertensive rat an increased peripheral resistance, possibly brought about by a centrally induced increase in peripheral sympathetic activity, seems an important cause of this form of hypertension. Drugs, like the present imidazolidines, possessing a sympathoinhibitory action will display a greater effect on such animals than on normotensive ones. Consequently, clonidine and its related derivatives should rather be considered antihypertensive agents than hypotensive ones.

VI. Quantitative Structure-Activity Relationships in Clonidine-Like Imidazolidines

6.1. Introduction

In the present Chapter the studies will be reported which aimed at a quantitative description of some pharmacological actions of clonidine-like imidazolidines in terms of molecular properties. The Hansch model proving a useful method of obtaining a quantitative relationship between molecular structure and biological activity, is concisely outlined in section 6.2. This will permit a better understanding of the correlation equations presented in this Chapter. The substituent and molecular parameters used troughout this Chapter are compiled in section 6.3. The results of regression analyses for the agonistic activity of 11 imidazolidines and imidazolines on the isolated rabbit intestine (data from table 24; page 52) are treated in section 6.4. Section 6.5. is concerned with the quantitative relationships generated between the peripheral hypertensive activity of 22 imidazolidines (data from table 17; page 49) and physicochemical parameters. The correlation equations relating molecular features with the hypotensive activity following intravenous administration of imidazolidine compounds to anaesthetized, normotensive rats and rabbits are considered in section 6.6. In addition, the results of structure-activity relationship studies at the level of the central α-adrenoceptor are reviewed in section 6.7. The hypothetical mechanism proposed for the mode of interaction between imidazolidines and central α-adrenoceptor is dealt with in section 6.8.

6.2. Quantitative Approach to Structure-Activity Relationship Studies: The Hansch Model

The ultimate goal of inquirements into a quantitative treatment of the molecular features determining biological activity is obvious: predicting the biological activity of a substance prior to synthesizing and screening the molecule. Knowledge of the molecular basis of drug action will be of a great economic value, since a more rational approach to drug design will reduce the expensive and time-consuming synthetic efforts, biological testing and diminishing the trial and error factors. Information concerning structure and activity is also fruitful from a mechanistic point of view; it may give fundamental insight into the mode of interaction of small molecules with a biological system. For discussions of early attempts at quantitative structure-activity relationships (QSAR), the reader is referred to other reviews (Hansch, 1969 and 1971; Purcell et al., 1970).

Based on the original Hammett equation (see Chapter III, section 2.2.) several investigators have made efforts in establishing quantitative correlations between physicochemical properties of molecules and their biological activities (Aldridge and Davison, 1952; review by Purcell et al., 1970). However, the «biological Hammett equation» has met with but limited success (Purcell et al., 1973), which can be attributed to the compulsory use of a single parameter only.

Being aware of the physicochemical nature of

biological reactions and recognizing the importance of partitioning in the transport of a drug to its ultimate site of action, Hansch and co-workers expanded the Hammett equation to include additional physicochemical parameters (Hansch, 1969; Hansch and Fujita, 1964; Hansch et al., 1963). As a result the $\varrho - \sigma - \pi$ equation was derived for the correlation of biological activites in congeneric series of drugs with their molecular structures (Hansch, 1969):

$$\log 1/C = k_1\pi + \varrho\sigma + k_2$$

In this equation C represents the molar concentration of an analogue in a congeneric series of molecules necessary to elicit a defined biological response; π is the hydrophobic substituent constant (see Chapter III, section 3.2.), σ is the Hammett substituent parameter (see Chapter III, section 3.2.2.) and k_1, ϱ and k_2 are constants for the particular selection of substances generated by regression analyses of the data. The relation is said to be «extrathermodynamic» and since Hansch pioneered this approach, it is often called the Hansch model. This basic equation has been modified by adding or replacing a variety of parameters in attempts to find better and more meaningful correlations. Hansch's postulate that the biological response to a drug is parabolically rather than linearly related to its partition properties (Hansch and Fujita, 1964; Hansch et al., 1963 and 1968) resulted in the inclusion of the π^2 term, thus leading to the following equation:

$$\log 1/C = k\pi^2 + k_1\pi + \varrho\sigma + k_2$$

An important advantage of this extrathermodynamic or linear free-energy-related approach is its flexibility to modification by incorporation or deletion of physicochemical parameters to supply a more adequate description of a particular biological phenomenon in a series of structurally related substances, which is modulated by variation in substitution.

The influence of substituents on biological activity can be partly due to steric effects. Hansch (1969) has introduced the Taft steric parameters (E_s, Taft, 1956) to allow for this, resulting in the following equation:

$$\log 1/C = k\pi^2 + k_1\pi + \varrho\sigma + k_2E_s + k_3$$

In addition to the original hydrophobic, electronic and steric approximations the use of many other and most diverse sets of such parameters has been suggested. The integration of quantum chemical calculations has been resulted in several «theoretical» constants derived from quantum mechanical indices. Finally, various miscellaneous properties or combinations of parameters have been explored in the Hansch analysis (for reviews see Hansch, 1971 and 1973; Verloop, 1972; Purcell et al., 1973; Martin, 1978).

The Hansch approach is now accepted by many workers in the field of drug design as a standard method for the optimization of bilogical activity in congeneric classes of bioactive drugs.

6.3. Parameters

The dependent biological variables (log 1/C) in the equations reported in the following sections are all in terms of the molar concentrations of the compounds connected with a well-defined, standard response. A variety of substituent constants associated with electronic effects of the phenyl-attached substituents has been considered: σ (σ_{meta} and σ_{para}: Ritchie and Sager, 1966; σ_{ortho}: Barlin and Perrin, 1966), F and R (Norrington et al., 1975), $\mathcal{F}$ and $\mathcal{R}$ (Swain and Lupton, 1968). These parameters have already been met and explained in Chapter III (section 2.2.). In addition, ΔpK°_a ($= pK^\circ_{a\,X} - pK^\circ_{a\,H}$; see Chapter III, section 2.2.) referring to the substituent effect on the dissociation of the compounds was also utilized. The pK'_a value as such has found application too. Moreover, the use of atomic π-electronic charge densities at the skeletal positions was also explored for the free bases, q(B), as well as for the protonated species, q(P), (Chapter III, section 4.3.). The calculated energy of the highest occupied molecular orbital of bases and protonated forms, HOMO(B) and HOMO(P), respectively, as well as the one of the lowest empty molecular orbital, LEMO(B) and LEMO(P) (Chapter III, section 4.3.), was applied in the correlation studies. These would be the relevant orbitals involved in any donation or acceptance of charge. The applicability of the difference between the HOMO and LEMO energies, EE(B) and EE(P), which corresponds to

the lowest π-electronic excitation energy of the molecules, was also studied. In order to estimate hydrophobic interactions and/or transport processes the apparent partition coefficient, log P', the true partition coefficient, log P (Chapter III, section 3.1.), π (phenoxyacetic acid series, Fujita et al., 1964) and parachor, Par (Ahmad et al., 1975), were employed. To account for steric effects of the substituents the Taft steric constant, E_s (Taft, 1956; compiled and expanded by Hansch, 1973) and the molar refraction at the wavelength of the sodium D line, MR (Norrington et al., 1975), which represents the volume of the substituents, were systematically explored. Both aforecited parameters have been scaled so that the value of the hydrogen substituent was zero. In the E_s constant of the methoxy function only the first atom was considered and for the nitro substituent the value associated with the half-width of the group was used. The van der Waals volumes of the fragments of the molecules (Bondi, 1964) have also been used. The molar volume, MV, of each molecule has been calculated by summation of the volumes of the fragments. Log MV has then been employed as an approximation of the total steric effect of the molecule.

Statistical correlations were carried out by a stepwise multiple regression analysis using a regression computer program. The method of least squares was used in deriving the equations. The correlation coefficient, r, the standard deviation, s, and the result of an F-test, from which the significance of the correlation was calculated, are given. The figures in parentheses are the 95 % confidence intervals. Stepwise inclusion of parameters was justified by application of the F-test ($P < 0.05$).

6.4. QSAR in Imidazolidines and Imidazolines with Respect to α-Adrenergic Activity on Isolated Rabbit Intestine

The peripheral α-adrenergic agonistic activity (pD_2; table 24; page 52) of a number of imidazolidines and imidazolines on the isolated intestinal smooth muscle of the rabbit has been correlated with several physicochemical parameters (Struyker Boudier et al., 1975). The biological data of 11 compounds were analysed, since for 3 substances (*St-666*, *St-1943* and oxymetazoline) the essential experimental constants could not be obtained. The equations correlating pD_2 with one or more parameters are given in table 37.

In correlating pD_2 with one parameter the most significant relationship was obtained with pK'_a (equation 35). The significance of equation 35 could be slightly improved by adding log P (true partition coefficient of the uncharged molecule from the system chloroform/aqueous buffer at pH = 7.4) or log MV (equations 36 and 37). The incorporation of log MV (equation 37) was significant at the 90 % level. The addition of both log MV and log P to equation 35 did not further improve the significance neither linearly nor parabolically (equation 38).

Table 37: Correlation equations obtained from regression analyses describing the relationship between pD_2 values from rabbit intestine studies and physicochemical properties of 11 imidazolidines and imidazolines. Data from Struyker Boudier et al. (1975).

	r	s	P	eq. no.
$pD_2 = 3.626 \log MV - 1.238$	0.636	0.341	≤ 0.05	32
$pD_2 = 0.456 \log P + 4.624$	0.668	0.329	< 0.05	33
$pD_2 = -0.295 \log P' + 5.613$	0.517	0.378	> 0.05	34
$pD_2 = 0.362\ pK'_a + 2.088$	0.837	0.242	< 0.01	35
$pD_2 = 0.164 \log P + 0.298\ pK'_a + 2.347$	0.858	0.241	< 0.01	36
$pD_2 = 1.876 \log MV + 0.299\ pK'_a - 0.870$	0.888	0.216	<0.001	37
$pD_2 = 3.468 \log MV + 0.324\ pK'_a$ $-0.119 (\log P)^2 + 0.320 \log P - 4.212$	0.900	0.236	<0.001	38

From this analysis it may be concluded that the variance in pD_2 of the compounds at the α-adrenoceptors of the rabbit intestine can be explained at least partly on the basis of the difference in pK'_a, i. e. the degree in dissociation of the imidazolidine(imidazoline) free base at physiological pH. Moreover, within the series of substances studied an increase in molar volume also seems to contribute to an enhanced activity.

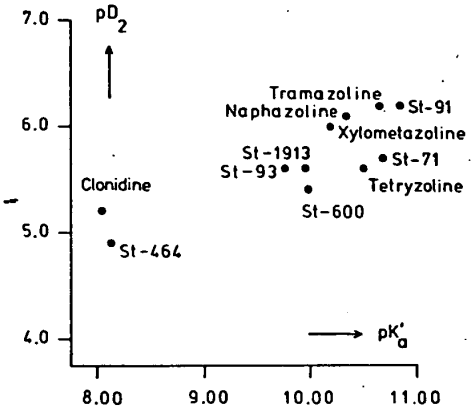

Fig. 56: Relationship between the pD_2 on rabbit intestine α-adrenoceptors and the pK'_a value of imidazolidines and imidazolines. The relationship is described mathematically by equation 25 (table 37). Data from Struyker Boudier et al. (1975).

Equation 35 is illustrated by fig. 56. The figure shows the positive relationship between pD_2 and pK'_a. However, it should be noted that the compounds used do not comprise a true data set. The independent variable, pK'_a, does not cover the range between 8 and 10 uniformly. For this reason the influence of clonidine and *St-464* on the quality of the correlation can be expected to be particularly great. Upon omitting these two compounds with a low pK'_a value, equation 39 resulted.

$$pD_2 = 0.498\ pK'_a + 0.677 \quad \text{(eq. 39)}$$
$$n = 9;\ r = 0.624;\ s = 0.253;\ F = 4.46\ (P>0.05)$$

This relationship 39 is statistically of no importance and shows that the meaning of correlation equation 35 too heavily depends on the inclusion ot these two compounds. Therefore, structures possessing pK'_a values between 8 and 10 should be added to the data set in order to confirm its validity.

6.5. QSAR in Clonidine-Like Imidazolidines with Respect to Peripherally Mediated Hypertensive Activity

Quantitative correlations between the peripheral activity of 22 phenyl-substituted imidazolidines and physicochemical parameters have been presented by Rouot et al. (1976, 1977). The hypertensive activity was expressed by the pD_2 value obtained from dose-response curves following intravenous administration of the compounds to pithed rats (see table 17). The authors considered that the excitation of this peripheral, vascular α-adrenoceptor is brought about by the protonated imidazolidine. Consequently, they used the pD_2 value corrected for ionization, $pD^*_2 = \log 1/C^+$, where C^+ represents the molar concentration of the amount of protonated form at pH = 7.4. The equations in pD^*_2 were slightly improved over those in which uncorrected pD_2 values were employed. With respect to the steric constant, E_s, a partial summation over certain positions at the phenyl ring was studied, e. g. E_s-2,6. The E_s-2 was attributed to the smaller substituent at an ortho position, whereas E_s-6 referred to the larger substituent at the other ortho position. The following equations were found:

$$pD^*_2 = -0.49\ (\pm 0.24)\ (E_s\text{-}2,6)^2 - 1.70\ (\pm 0.78)\ E_s\text{-}2,6 + 5.95 \quad \text{(eq. 40)}$$
$$n = 17;\ r = 0.78;\ s = 0.45;\ F = 11.1\ (P < 0.005)$$

$$pD^*_2 = -0.59\ (\pm 0.20)\ (E_s\text{-}2,6)^2 - 1.57\ (\pm 0.62)\ E_s\text{-}2,6 - 0.84\ (\pm 0.58)\ E_s\text{-}2 + 6.03 \quad \text{(eq. 41)}$$
$$n = 17;\ r = 0.88;\ s = 0.36;\ F = 15\ (P < 0.005)$$

$$pD^*_2 = -0.82\ (\pm 0.17)\ (E_s\text{-}2,6)^2 - 2.08\ (\pm 0.45)\ E_s\text{-}2,6 - 1.28\ (\pm 0.42)\ E_s\text{-}2 - 0.48\ (\pm 0.22)\ \mathscr{F} + 6.11 \quad \text{(eq. 42.)}$$
$$n = 17;\ r = 0.96;\ s = 0.22;\ F = 35.8\ (P < 0.005)$$

$$pD^{*}_{2} = -0.77\ (\pm 0.20)\ (E_s\text{-}2,6)^2 - 1.93\ (\pm 0.50)\ E_s\text{-}2,6 - 1.19\ (\pm 0.52)\ E_s\text{-}2 - 0.70\ (\pm 0.26)\ E_s\text{-}3 - 0.38\ (\pm 0.36)\ \mathcal{F} + 6.17 \quad \text{(eq. 43)}$$
$$n = 22;\ r = 0.93;\ s = 0.29;\ F = 19.3\ (P < 0.005)$$

The involvement of steric features of the substituents attached at both ortho positions is pointed at in equation 40. This relationship could be improved by the incorporation of an E_s-2 term (equation 41). The statistically most relevant relationship (equation 42) contains 4 terms and resulted from the inclusion of the inductive component of the electronic effect of the substituents, $\mathcal{F}$. It appeared that replacing E_s-2 (Equations 41 and 42) by E_s-6 yielded identical levels of significance. This finding has been explained in the sense that the E_s-2 or E_s-6 term differentiates the di-ortho-substituted substances from the mono-ortho-substituted ones. This means that the variance observed in pD^{*}_{2} not solely depends on the steric bulk at the ortho position alone, but is also determined by the distribution of the substituents on both ortho positions. The steric bulk of the 4-substituent apparently plays no important role, since an addition of a term in E_s-4 did not improve the correlation. However, its electronic effect is incorporated in $\mathcal{F}$ (equation 42). A comparison of equation 43 with 42 shows that an additional E_s-3 term in order to accommodate the meta-substituted compounds does not adequately explain the contribution of these substituents to the variance in pD^{*}_{2}.

The presence of a $(E_s\text{-}2,6)^2$ term in all of the equations indicates the existence of an optimal steric influence in the ortho position. The steric hindrance of the chlorine substituent is close to this ideal value, but its electronic effect is unfavourable. A better candidate is, therefore, the methyl group. Rouot et al. (1976, 1977) synthesized the 2,4,6-tri-methyl, the 2,6-dimethyl, 4-hydroxy and the 2-ethyl compounds. Their calculated and observed α-sympathomimetic activities agreed very well, supporting the applicability of the equations mentioned above in predicting this biological action in congeneric series of (arylimino)imidazolidines.

6.6. QSAR in Clonidine-Like Imidazolidines with Respect to the Centrally Mediated Hypotensive Activity Following Intravenous Administration to Rats and Rabbits

Whatever the mechanism of initiation of a biological effect at a molecular level may be, in order to elicit any effect at all, the drug in question must in its active form reach a sufficiently high concentration in the compartment where its sites of action are located. After intravenous administration of bioactive drugs a complex event, constituting the pharmacokinetics, gets under way. Protein binding, distribution, elimination, biotransformation and excretion will play a prominent part in the pharmacokinetics following systemic application of the present imidazolidines. As a result of these processes a certain limited amount of the dose injected will eventually occupy the receptor compartment, representing the central nervous system in the case at issue. These molecules induce a stimulus based on the interaction with the central α-adrenoceptors, which finally leads to the hypotensive effect (also see Chapter I). Consequently, the central hypotensive activity of the clonidine-like drugs is the result of a chain of complex events, which for simplicity may be divided into the actual receptor interaction and the processes determining the concentration in the target (brain) tissue prone to excitation of the receptors.

It has been demonstrated in Chapter III (section 3.3.) that the transport phenomenon from the blood the the brain, i. e. more specifically the fraction of the dose administered intravenously accumulating in the rat brain, could be described by a parabolic relationship in log P'. Thus lipophilic behaviour connected to transport, which will be an important factor for central hypotensive activity, can be represented satisfactorily by log P'. The following two equations (Timmermans and van Zwieten, 1977f) were derived in order to study to what extent log P' (table 4) predicts the hypotensive activity of 27 imidazolidines (table 6) following intraven-

ous administration to anaesthetized, normotensive rats**:

$$\log 1/ED^*_{30} = 0.451\ (\pm 0.30) \log P' + 0.456 \quad \text{(eq. 44)}$$
$$n = 27;\ r = 0.529;\ s = 0.864;\ F = 9.70\ (P < 0.005)$$

$$\log 1/ED^*_{30} = -0.253\ (\pm 0.22)\ (\log P')^2 + 0.491\ (\pm 0.28) \log P' + 0.789 \quad \text{(eq. 45)}$$
$$n = 27;\ r = 0.647;\ s = 0.793;\ F = 8.62\ (P < 0.002)$$

The parabolic relationship (equation 45) is statistically somewhat better than the linear one (equation 44). This equation 45 only explains 42% ($= r^2$) of the variance in hypotensive activity and shows that lipophilicity in relation to transport is an important parameter in the structure-activity relationship, although additional molecular properties will be involved.

Lipophilicity as expressed by octanol/water (pH = 7.4) partition coefficients has been found to represent the major important parameter for determining hypotensive activity in anaesthetized rabbits within a limited series of imidazolidines (Stähle and Hoefke, unpublished data, 1975). For 13 substances, mainly substituted at both ortho positions, the relationship between log $1/ED_{20}$ (μmol/kg) and log P' is formulated by equation 46:

$$\log 1/ED_{20} = 0.307 \log P' + 0.576 \quad \text{(eq. 46)}$$
$$n = 13;\ r = 0.532;\ s = 0.488;\ F = 4.34\ (P > 0.05)$$

The data used to generate equation 46 have been listed in table 38. As can be deduced from this correlation, there exists no appreciable linear relationship between the blood pressure lowering activity following intravenous administration to anaesthetized rabbits and the overall lipophilic behaviour of the derivatives.

** The biological parameters in this section obtained in anaesthetized normotensive rats (table 6) have been corrected for ionization (log $1/ED^*_{30}$). The total dose injected to invoke 30% decrease in mean arterial pressure has been recalculated for the amount of protonated form, presumably prevailing under physiological conditions, with the aid of the percentages listed in table 2 (see Chapter III). It appeared that by using these corrected ED_{30} values correlations slightly better than those in which the total doses were employed could be generated (Timmermans and van Zwieten, 1977f).

Table 38: Hypotensive activity, log $1/ED_{20}$ (μmol/kg), octanol/water (pH = 7.4) partition coefficients, log P', and two quantum chemical energetic indices, HOMO and LEMO, of 13 structurally closely related phenyl-substituted imidazolidines. The values have been used to generate the equations 46 and 47. From Stähle and Hoefke (unpublished data, 1975).

Compd. no.[a]	(X)	log $1/ED_{20}$	log P'	HOMO	LEMO
St- 1945	(2,6-di-OMe)	−0.59	−1.56	0.097	−1.013
1697	(2-Me,6-Et)	−0.10	−0.98	0.385	−0.588
93	(2-Cl,6-Me)	0.69	−0.52	0.254	−0.954
92	(2,4,6-tri-Et)	0.97	−0.24	0.103	−0.892
1923	(2-Cl,6-F)	0.80	0.02	0.321	−0.952
STH-2130	(2-Br,6-F)	1.47	0.33	0.764	−0.950
St- 155	(2,6-di-Cl)	1.43	0.48	0.393	−0.000
1974	(2-F,6-CF_3)	0.85	0.75	0.177	−0.597
1962	(2-Br,6-Cl)	0.49	0.98	0.391	−0.513
464	(2,6-di-Br)	0.90	1.17	0.389	−0.814
1964	(2-Cl,6-CF_3)	0.70	1.24	0.172	−0.791
1957	(2,6-di-CF_3)	0.74	1.39	0.190	−0.190
732	(2,4,6-tri-Cl)	0.52	1.44	0.401	−0.928

[a] Numbering refers to table 5.

The shape and the statistics of equation 46 are very similar to those of equation 44. However, in the case at issue the introduction of a squared term in log P′ resulted in a very significant improvement of correlation 46 (Stähle and Hoefke, unpublished data, 1975):

$$\log 1/ED_{20} = -0.484\ (\log P')^2 + 0.372 \log P' + 1.019 \quad \text{(eq. 47)}$$
$$n = 13;\ r = 0.903;\ s = 0.260;\ F = 22.09\ (P < 0.001)$$

Equation 47 now accounts for 81.5 % of the variance in hypotensive data in the rabbit, which is very acceptable. The parabolic dependence of log $1/ED_{20}$ on log P′ is visualized by fig. 57 (also see page 15).

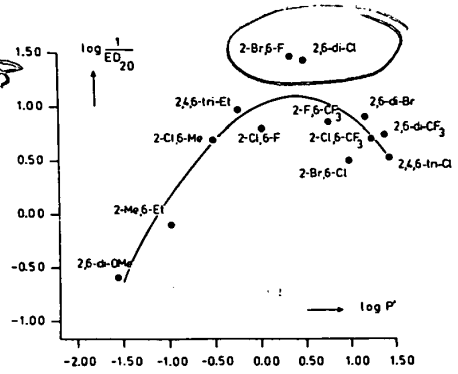

Fig. 57: Relationship between the hypotensive activity following intravenous adminstration to anaesthetized rabbits, log $1/ED_{20}$, and the octanol/buffer (pH = 7.4) partition coefficient, log P′, for a limited series of clonidine-like imidazolidines. The correlation is mathematically described by equation 47. The numerical values have been reported in table 38. Unpublished data from Stähle and Hoefke (1975).

In the attempts at improving the correlation further, the HOMO and LEMO energy levels (see table 38) have been included. However, within the present selection of derivatives the inclusion of these quantum chemical indices did not result in better relationships. Equation 47 stresses the significance of lipophilicity as an important determinant of hypotensive activity. This parameter seems of particular value, especially for structurally very closely related substances in the imidazolidine series.

Table 39: Hypotensive activity, log $1/ED_{20}$ (μmol/kg), octanol/buffer (pH = 7.4) partition coefficients, log P′, of 9 structurally closely related 2,3-disubstituted phenyliminoimidazolidines. Data from Hoefke et al. (1979).

Compd. no.[a]	(X)	log $1/ED_{20}$	log P′
STH-2224	(2,3-di-Br)	1.82	0.89
2100	(2-Cl,3-Br)	1.55	0.67
St- 606	(2-Me,3-Br)	1.46	−0.19
STH-2165	(2-Br,3-Cl)	1.32	0.47
St- 476	(2,3-di-Cl)	0.95	0.53
STH-2169	(2-Br,3-F)	0.47	−0.02
2167	(2-Cl,3-F)	0.40	−0.49
St- 450	(2-Me,3-Cl)	0.43	−0.79
608	(2-Cl,3-Me)	−0.27	−0.57

[a] Numbering refers to table 5.

A similar analysis was also made in a group of 2,3-disubstituted imidazolidines (table 39). A significant linear correlation was found between log $1/ED_{20}$ and log P′ (Hoefke et al., 1979):

$$\log 1/ED_{20} = 0.905 \log P' + 0.853 \quad \text{(eq. 48)}$$
$$n = 9;\ r = 0.798;\ s = 0.443;\ F = 12.26\ (P < 0.01)$$

The introduction of a squared term in log P′ did not further improve the correlation:

$$\log 1/ED_{20} = 0.160\ (\log P')^2 + 0.889 \log P' + 0.801 \quad \text{(eq. 49)}$$
$$n = 9;\ r = 0.800;\ s = 0.477;\ F = 5.33\ (P < 0.01)$$

This observation contrasts to the results obtained with the 2,6- and the 2,4,6-substituted analogues (equations 46 and 47). It may depend on the fact that in the group of 2,3-disubstituted congeners the spread in log P′ is not wide enough (see table 39). In both groups the log $1/ED_{20}$ values have a comparable range of variation.

By stepwise inclusion and/or deletion of the substituent and molecular parameters enumerated in the foregoing section 6.3. the following equation was obtained mathematically comprising a relationship between hypotensive activity (table 6) and molecular structure of 27 imidazolidines following intravenous applica-

tion to anaesthetized, normotensive rats (Timmermans and van Zwieten, 1977f):

$$\log 1/ED^*_{30} = -0.00032\ (\pm 0.00008)\ (\Sigma\ Par)^2 + 0.105\ (\pm 0.03)\ \Sigma\ Par - 0.695\ (\pm 0.17)\ \Delta\ pK^\circ_a + 5.333\ (\pm 1.89)\ HOMO(P) + 6.752\ (\pm 2.25)\ EE(P) + 2.494 \quad \text{(eq. 50)}$$

$$n = 27;\ r = 0.952;\ s = 0.341;\ F = 40.34\ (P < 0.001)$$

Equation 50 is most significant and accounts for 91% of the variance in the log $1/ED^*_{30}$ values.

Looking at the structure of equation 50 stepwise, the following order was found:

$$\log 1/ED^*_{30} = -0.451\ \Delta\ pK^\circ_a - 0.013 \quad \text{(eq. 51)}$$
$$r = 0.482;\ s = 0.892;\ F = 7.58$$

$$\log 1/ED^*_{30} = -0.00035\ (\Sigma\ Par)^2 + 0.117\ \Sigma\ Par - 8.844 \quad \text{(eq. 52)}$$
$$r = 0.656;\ s = 0.784;\ F = 9.08$$

$$\log 1/ED^*_{30} = -0.713\ \Delta\ pK^\circ_a + 7.473\ HOMO(P) + 9.350\ EE(P) + 15.768 \quad \text{(eq. 53)}$$
$$r = 0.777;\ s = 0.669;\ F = 11.65$$

$$\log 1/ED^*_{30} = -0.00040\ (\Sigma\ Par)^2 + 0.129\ \Sigma\ Par - 0.534\ \Delta\ pK^\circ_a - 9.933 \quad \text{(eq. 54)}$$
$$r = 0.853;\ s = 0.554;\ F = 20.43$$

In correlating log $1/ED^*_{30}$ with one parameter, $\Delta\ pK^\circ_a$ is the major single variable (equation 51). The next major important variable is the combination of a linear and a squared term in Σ Par (= the summation over the parachor values of the phenyl-attached substituents including the hydrogens) (equation 52). The quantum chemical parameters HOMO(P) and EE(P) are inseparably inherent in each other. Solely the linear combination of these terms significantly improved the correlation (cfr. equation 53), in spite of their high collinearity. The best equation in three terms is formed by $\Delta\ pK^\circ_a$ and a parabolic dependence on Σ Par (equation 54).

Parachor is defined as the product of the molar volume and the fourth root of the surface tension (Sugden, 1924). When the surface tension of the compounds in an analogues series is numerically similar, the parachor values of the congeners are a good measure of their relative molecular sizes (Ahmad et al., 1975). Surface tension itself may be related to an over-all lipophilic behaviour of the molecules (cfr. Hellenbrecht at al., 1973). It can be anticipated that parachor represents a variable containing lipophilic as well as steric properties. When parachor (equation 50) was replaced by the hydrophobic substituent constant π ($\Sigma\pi$ = summation over the substituent π values) or by the steric substituent parameter E_s ($\Sigma\ E_s$ = summation over the substituent E_s values) the equation 55 and 56 resulted:

$$\log 1/ED^*_{30} = -0.865\ (\pm 0.35)\ (\Sigma\pi)^2 + 2.433\ (\pm 0.94)\ \Sigma\pi - 0.670\ (\pm 0.21)\ \Delta\ pK^\circ_a + 6.926\ (\pm 2.47)\ HOMO(P) + 8.886\ (\pm 2.92)\ EE(P) + 11.576 \quad \text{(eq. 55)}$$
$$n = 27;\ r = 0.912;\ s = 0.455;\ F = 20.85\ (P < 0.001)$$

$$\log 1/ED^*_{30} = -0.509\ (\pm 0.15)\ (\Sigma\ E_s)^2 - 2.434\ (\pm 0.69)\ \Sigma\ E_s - 0.440\ (\pm 0.18)\ \Delta\ pK^\circ_a + 3.023\ (\pm 2.33)\ HOMO(P) + 5.124\ (\pm 2.62)\ EE(P) - 6.145 \quad \text{(eq. 56)}$$
$$n = 27;\ r = 0.943;\ s = 0.369;\ F = 33.80\ (P < 0.001)$$

These two equations show that the substitution of parachor by parameters standing for lipophilic or steric features alone, is accompanied by a loss of significance of the correlation and strongly indicate that parachor contains both these properties. Although the collinearity between Σ Par and $\Sigma\pi$ and also between Σ Par and $\Sigma\ E_s$ is considerable, it seems safe to conclude that in this structure-activity relationship significant roles can be attributed to lipophilic properties (transport) and steric features, partly determining the interaction of the imidazolidine with the central α-adrenoceptor. Both phenomena together are best described by parachor.

The appearance of $\Delta\ pK^\circ_a$ in the quantitative structure-activity relationship can be ascribed to

...ts profound influence on lipophilicity of the ...midazolidines, since the degree of dissociation ...epends on the pK°_{a} of the molecules. Additionally, it may also reflect electronic effects playing ... part in the drug-receptor interaction. The par...ial correlation to the excitation energy (EE) in ...combination with the HOMO energy, an index ...f electron donor ability (Pulmann and Pul...mann, 1963) may be interpreted as an indication ...hat a charge-transfer complex is formed at the ...eceptor site. Equation 50 predicts the hypoten...sive activities of the imidazolidines (log $1/ED^{*}_{30}$) quite satisfactorily (see fig. 58). All the ...experimental values are predicted within the ...limits of ± 2s (s = standard deviation of the ...regression). The difference between the observed ...and the calculated hypotensive activities is great...st for compound no. *11* (2,5-di-Cl).

It is obvious that the use of biological data ...btained in vivo in order to correlate with ...olecular structure is strictly limited by the ...complexity of the events, giving rise to the ...response ultimately measured in vivo. The equations presented in this section demonstrate that a proper choice of parameters succeeds in generating acceptable correlations. It should be noted, however, that the mathematical description of such a complex system urged the use of over-all molecular parameters probably comprising various properties. The equations only provide a faint working model for speculations on the mode of action of the imidazolidines at the level of the central α-adrenoceptor.

6.7. QSAR in Clonidine-Like Imidazolidines at the Level of the Central α-Adrenoceptor

It has been reported in the preceding section that the variance in the hypotensive data of clonidine-like imidazolidines obtained following intravenous administration to anaesthetized, normotensive rats could be described in terms of molecular properties. However, no clear picture of the actual mechanism of action at a molecular level could be obtained which is owed to the fact

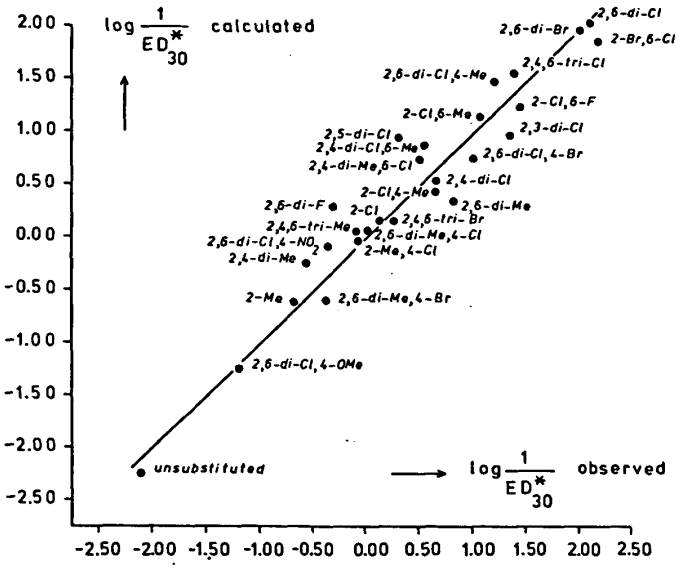

Fig. 58: Comparison between hypotensive activities obtained experimentally following intravenous administration to anaesthetized, normotensive rats and values calculated by using equation 50 for 27 imidazolidines, including clonidine.

that in addition to the receptor interaction the «kinetics» are also to be accounted for. As far as these «kinetics» in anaesthetized, normotensive rats are concerned 42% of the variance in hypotensive activity can be attributed to them (see eq. 45). This part-process has been avoided in order to study those molecular properties of the imidazolidines and, consequently, those structural moieties in these compounds essential to the interaction with the central α-adrenoceptor (Timmermans and van Zwieten, 1977f). The results reported in Chapter III (section 3.3.) have been used to achieve this, viz. separation of the «kinetics» from the receptor occupation. It has been shown that the tendency of the imidazolidine compounds to accumulate in the rat brain can be represented by a parabolic relationship in log P'(equation 9; page 15). For all the member imidazolidines from section 6.6. this dose-independent equation 9 has been employed to calculate the rat brain concentration (nmol/g of brain tissue w. w.) associated with a reduction in mean arterial pressure by 30%. This new ED_{30} can be considered to be a measure of the concentration of the drugs at the level of the central α-adrenoceptor. It no longer depends on the «kinetics» ultimately providing this concentration in this compartment, since they are already accounted for by equation 9, the new ED_{30} value is obtained from. As a result, this new biological parameter, log $1/ED_{30}$(C), will be potentially more suitable in studying a relationship between structure and hypotensive activity.

The following equation 57 (Timmermans and van Zwieten, 1977f) was derived from the biological data at the central α-adrenoceptor level, relating the chemical structure of the drugs to their hypotensive activities*:

$$\log 1/ED_{30}(C) = -0.401\ (\pm 0.12)\ (\Sigma E_s)^2 - 1.771\ (\pm 0.56)\ \Sigma E_s + 1.898\ (\pm 1.09)\ \Sigma R + 5.129\ (\pm 1.67)\ HOMO(P) + 6.771\ (\pm 1.96)\ EE(P) + 8.026 \quad \text{(eq. 57)}$$

$$n = 27;\ r = 0.941;\ s = 0.326;\ F = 32.20\ (P < 0.001)$$

* The use of the total rat brain concentration, i. e. not corrected for ionization, in the equations resulted in slightly better correlations compared to the one corrected for ionization.

This equation is most significant and explains 89% of the variance in hypotensive activity. The development of this quantitative structure-activity relationship is given below.

$$\log 1/ED_{30}(C) = -0.069\ (\Sigma E_s)^2 + 1.083$$
$$r = 0.385;\ s = 0.812;\ F = 4.34$$

$$\log 1/ED_{30}(C) = -0.406\ (\Sigma E_s)^2 - 1.678\ \Sigma E_s - 0.677$$
$$r = 0.668;\ s = 0.668;\ F = 9.65$$

$$\log 1/ED_{30}(C) = -0.512\ (\Sigma E_s)^2 - 2.163\ \Sigma E_s + 1.308\ EE(P) - 11.095$$
$$r = 0.791;\ s = 0.562;\ F = 12.78$$

$$\log 1/ED_{30}(C) = -0.378\ (\Sigma E_s)^2 - 1.560\ \Sigma E_s + 4.636\ HOMO(P) + 6.650\ EE(P) + 2.902$$
$$r = 0.901;\ s = 0.406;\ F = 23.83$$

The hypotensive activity of the imidazolidines at central α-adrenoceptor level is expressed in terms of the resonance contribution, R, of the phenyl-attached substituents, Σ R, a parabolic dependence on the over-all steric factors of these substituents, Σ E_s, and the two quantum chemical indices HOMO(P) and EE(P), already met in the foregoing section. In addition to Σ E_s the involvement of Σπ and Σ MR in the quantitative structure-activity relationship was also studied. It was found that E_s models substituent effects better than π or MR, although there is a high collineartiy among these vectors. Apparently, steric effects are involved and E_s is the parameter of choice. Equation 57 is the «best» equation which could be generated from the available data. Of the other relationships derived, equation 58 possesses statistics of comparable quality to the ones of equation 57:

$$\log 1/ED_{30}(C) = -0.555\ (\pm 0.11)\ (\Sigma E_s)^2 - 2.347\ (\pm 0.54)\ \Sigma E_s - 0.590\ (\pm 0.19)\ \Sigma F - 48.694\ (\pm 40.01)\ q_{C_s}(P) + 1.432\ (\pm 0.52)\ EE(P) + 34.122 \quad \text{(eq. 58)}$$

$$n = 27;\ r = 0.935;\ s = 0.340;\ F = 29.35\ (P < 0.001)$$

The inclusion of the inductive component of the electronic effect of the substituents at the phenyl ring, Σ F, as well as the π-electron charge density at the guanidine carbon atom of the protonated imidazolidines, q_{C_s} (P), was the cause that HOMP(P) ceased to be a significant

parameter. One may conclude from this relationship 58 that electrostatic forces possibly play a part in the binding between imidazolidine and central α-adrenoceptor.

Equation 57 provides calculated hypotensive activities which agree well with the observed ones. In the same manner as in equation 50, no. *11* (2,5-di-Cl) possessed the widest deviation and is not predicted within the limits ±2s. Apparently, meta substituents are not adequately accounted for, although compound no. 9 (2,3-di-Cl) fits excellently in the regression. Omission of both meta-substituted derivatives (no. 9 and *11*) yielded equation 59:

$$\log 1/ED_{30}(C) = -0.439\ (\pm 0.10)\ (\Sigma E_s)^2 - 1.939\ (\pm 0.48)\ \Sigma E_s + 2.180\ (\pm 0.91)\ \Sigma R + 4.719\ (\pm 1.40)\ HOMO(P) + 6.249\ (\pm 1.64)\ EE(P) + 7.190 \quad \text{(eq. 59)}$$

$$n = 25;\ r = 0.965;\ s = 0.262;\ F = 50.78\ (P < 0.001)$$

This equation relates the hypotensive activity of the unsubstituted derivative as well as the depressor potencies of 2-, 2,4- and 2,4,6-substituted congeners to their chemical structures. It explains 93 % of the variance in blood pressure lowering activity. In fig. 59 the hypotensive activities calculated according to equation 59 are plotted against those actually determined in animal experiments. This figure shows that equation 59 provides calculated activities which correspond satisfactorily with the experimental ones.

The positional dependence of the steric effect was examined for each ring position separately. To the larger ortho substituent at the phenyl ring figure 2 was given. Groups at the para position received figure 4 and the smaller ortho substituent figure 6. Upon factorizing the steric involvement in the structure-activity relationship the equations 60–62 resulted (Timmermans and van Zwieten, 1977f) in which the figures attached to E_s refer to the position of the substituent(s) at the phenyl ring:

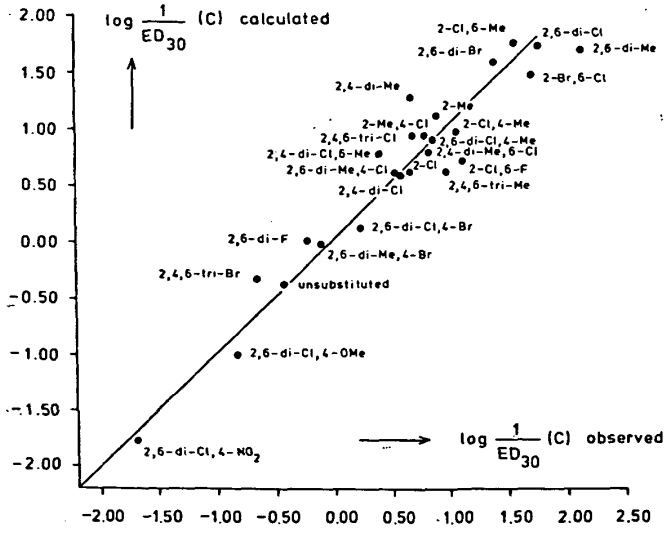

Fig. 59: Comparison between hypotensive activities at central α-adrenoceptor level obtained experimentally and values calculated by using equation 59 for clonidine and its structurally related imidazolidines. The meta-substituted derivatives (no. 9 and *11*) were omitted. From Timmermans and van Zwieten (1977f.), with permission.

$$\log 1/ED_{30}(C) = -0.756\ (\pm 0.29)\ (E_s\text{-}2,4)^2 - 2.383\ (\pm 0.98)\ E_s\text{-}2,4 + 2.141\ (\pm 1.24)\ \Sigma R + 3.812\ (\pm 2.31)\ HOMO(P) + 5.323\ (\pm 2.65)\ EE(P) + 3.879 \quad \text{(eq. 60)}$$

$$n = 25;\ r = 0.923;\ s = 0.383;\ F = 21.81\ (P < 0.001)$$

$$\log 1/ED_{30}(C) = -0.575\ (\pm 0.32)\ (E_s\text{-}4,6)^2 - 1.513\ (\pm 0.99)\ E_s\text{-}4,6 + 2.088\ (\pm 1.66)\ \Sigma R + 3.621\ (\pm 2.89)\ HOMO(P) + 5.045\ (\pm 3.30)\ EE(P) + 4.624 \quad \text{(eq. 61)}$$

$$n = 25;\ r = 0.894;\ s = 0.445;\ F = 15.13\ (P < 0.001)$$

$$\log 1/ED_{30}(C) = -2.431\ (\pm 1.36)\ (E_s\text{-}2)^2 - 4.402\ (\pm 1.90)\ E_s\text{-}2 - 0.391\ (\pm 0.20)\ (E_s\text{-}4,6)^2 - 0.881\ (\pm 0.65)\ E_s\text{-}4,6 + 2.778\ (\pm 1.27)\ \Sigma R + 5.217\ (\pm 2.16)\ HOMO(P) + 6.659\ (\pm 2.35)\ EE(P) + 9.817 \quad \text{(eq. 62)}$$

$$n = 25;\ r = 0.967;\ s = 0.266;\ F = 35.48\ (P < 0.001)$$

When equation 60 is being compared with equation 61 the better statistical quality of the former stresses the major importance of E_s-2 (larger substituent) over E_s-6 (smaller substituent). The addition of E_s-6 to equation 60 did not result in a significant improvement of the correlation. Therefore, it seems likely that, as far as the steric features of the interaction between imidazolidine and central α-adrenoceptor are concerned, only one of the ortho substituents (2-position) is involved in this process. In equation 62 all of the terms are statistically justified. This relationship also points to the steric attribution of position 2 at the phenyl ring, the interaction with the central α-adrenoceptor is probably most dependent on. Optimal values of the steric involvement for position 2 (E_s-2°) and for positions 4 and 6 in a composite sense (E_s-4,6°) were calculated by using this equation 62. The optimal E_s values found were: E_s-2° = − 0.91 and E_s-4,6° = − 1.13. It is interesting to note that the value of E_s-2° is very close to the steric constant of the chlorine substituent (E_s = − 0.97; Hansch, 1973). From similar regression equations, it could be concluded that the optimal value of E_s-6 amounts to approximately − 0.9, almost leaving no space to the 4-position.

The information which could be obtained from the regression equations presented in this section emphasizes the usefulness of investigating a structure-activity relationship at the level where the actual drug-receptor complexes are formed. By avoiding the kinetic aspects of drug transport the features of the central α-adrenoceptor could be studied. In the following section the attempts which have been made to speculate on the mode of action of imidazolidines are being reported. The possible features of the α-adrenoceptors involved in peripheral hypertensive and central hypotensive effects postulated with the help of quantitative correlation equations are being treated.

6.8. The α-adrenoceptor; speculations concerning its properties on the basis of structure-activity relationship studies

6.8.1. Considerations about the nature of the peripheral, vascular α-adrenoceptor

Experiments with various derivatives of noradrenaline allowed Belleau (1963, 1967) and Barlow (1964) to suggest that the engagement of this α-adrenoceptor is brought about by means of three major forms of interaction: an ionic type between the protonated nitrogen atom of the side chain and a negative site of the receptor; a hydrogen bond between the alcoholic hydrogen atom of the side chain and an acceptor situated at the receptor and, finally, a charge transfer interaction through electron donation of the aromatic ring of the agonists to an electron deficient area of the receptor. From a quantum mechanical study of the conformational properties of a number of phenethylamines, including adrenaline and noradrenaline, Pullman et al. (1972) postulated the α-adrenergic pharmacophore. The structural requirements presumed to be most important for α-sympathomimetic activity are visualized in fig. 60.

According to Pullmann et al. (1972) the compounds should have their nitrogen atoms

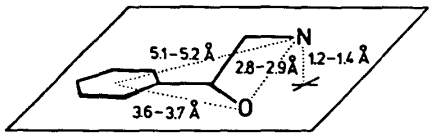

Fig. 60: The α-adrenenergic pharmacophore as postulated by Pullman et al. (1972).

located at a distance (A) of 1.2–1.4 Å above the plane of the aromatic moiety. The distance of this nitrogen atom from the centre of the aromatic portion (B) should amount to 5.1–5.2 Å. The distance between the nitrogen atom and the oxygen atom of the side chain is suggested to be 2.8–2.9 Å. Finally, this oxygen atom is 3.6–3.7 Å removed from the centre of the aromatic nucleus. The principal aspects of this model do not differ fundamentically from those postulated by Kier (1968, 1969).

Being aware of the α-sympathomimetic property of clonidine and its congeneric derivatives the question arises whether these particular molecules can meet these structural demands of the α-adrenoceptor. In the calculated ground state equilibrium geometry of the protonated clonidine molecule (Timmermans et al., 1977e) the distances A and B amount to 1.2 and 4.9 Å, respectively (see fig. 61).

These values are in reasonable accordance with those suggested by Pullman et al. (1972). An inspection of molecular models led Wermuth et al. (1973) to drawing the same conclusion. Clonidine does not possess an alcoholic hydroxyl group which is presumably involved in hydrogen bond formation in noradrenaline (adrenaline). It is possible that the NH function of the bridge occupies this place. The distances between the bridge nitrogen atom and the centre of the phenyl ring on the one hand and the imidazolidine nitrogen atom on the other hand amount to 2.8 and 2.4 Å, respectively. These values are considerably smaller than in noradrenaline and adrenaline. However, potent α-sympathomimetic drugs like naphazoline, xylometazoline, oxymetazoline and others (see section 4.2.5.) are not capable of forming a hydrogen bond due to the presence of a methylene bridge. Therefore, it may be stated that for α-adrenoceptor stimulation hydrogen bond formation is not demanded in advance. As a result, it seems plausible to accept that 2-(arylimino)imidazolidines in general are permitted to interact with the α-adrenoceptor on account of their skeletal structure.

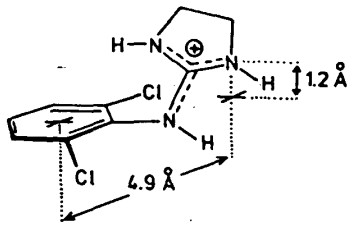

Fig. 61: Interatomic distances in the calculated, prefered conformation of protonated clonidine (Timmermans et al., 1977e), between centres considered to be of importance for the interaction with the α-adrenoceptor.

The result of structure-activity relationship studies on the hypertensive effect of clonidine and a number of its congeners in the pithed rat (Rouot et al., 1976, 1977; see section 6.5.) points in a qualitative sense to certain features of the peripheral, vascular α-adrenoceptor which agree with the model given above. The appearance of the inductive component of the electronic effect of the phenyl-attached substituents in the equations indicate that electron-donating substituents increase hypertensive activity. This may be interpreted in the sense that the formation of a charge transfer complex is favoured by these substituents. However, the major contribution to the quantitative structure-activity relationship is made by the steric properties of the substituents, especially by those located at both ortho-positions. Apparently, binding places for these groups are present at the α-adrenoceptor. The space available is limited, since a parabolic dependence on steric factors is found for these positions.

6.8.2. The nature of the central α-adrenoceptor; mode of interaction

The regression equations reported in the foregoing section 7. have been translated into a hypothetical working model, which may provide insight into the mode of action of clonidine

and its related imidazolidines at the central level (Timmermans and van Zwieten, 1977f). It should be regarded a speculative basis for further investigations.

The model emerging consists in a receptor site which has the ability of accepting electrons from an electron-donating drug. The appearance of the HOMO(P) energy in the correlation equations indicates that such an interaction between a protonated imidazolidine and the central α-adrenoceptor probably occurs. Attention may be focused on the HOMO energy as a calculable index of the spontaneously donor ability of a molecule. High-lying HOMO energy levels favour the electron donation and, consequently, hypotensive activity increases in the case at issue. The partial correlation to the first excitation energy, EE(P), being an intramolecular promotion of an electron from the highest occupied molecular orbital (HOMO) to the lowest empty molecular orbital (LEMO), is somewhat difficult to interpret. For the present series of compounds a high collinearity between HOMO(P) and EE(P) exists.

It appears that hypotensive activity is advantaged by high EE(P) values. Probably the possibility of an intramolecular electron promotion harms the electron donation to the central α-adrenoceptor. It must be argued that rather specialized conditions must be met to induce some electron exchange between drug and receptor. The first condition is that the geometry of the donor and the acceptor should be such that a very intimate fit ensues between these molecules. The overlapping of appropriate orbitals between the two will then permit the exchange with a minimum energy requirement. The second condition requires that the levels of donor and acceptor are so disposed that again a minimum expenditure of energy is necessary for the transfer of an electron. This means that the donor molecule must have a high-lying HOMO energy and the acceptor a low-lying LEMO energy. When these conditions are met, the need for significant amounts of external energy is obviated and the electron exchange becomes relatively facile.

Another site of interaction is suggested by the significant contribution of q_{C_4}(P) to hypotensive activity. This charge index may be considered to reflect the charge density at the imidazolidine portion of the molecules. From the correlation equation it emerges that the increase of a positive charge at this moiety parallels an increase in depressor activity. Presumably a positively charged nitrogen atom interacts with a negatively charged site of the central α-adrenoceptor. Upon including q_{C_4}(P) in the equation the parameter HOMO(P) was no longer significant, whereas the electronic effect of the substituents by inductive forces (Σ F) appeared in the regression. This seems to indicate that the Σ F term explains part of the variance in the HOMO(P) relevant to its effect on log $1/ED_{30}$(C), since electron donation by the substituents results in high-lying HOMO energy levels. From the incorporation of Σ R in the structure-activity relationships it can be deduced that electronic effects by resonance factors lower the hypotensive activity. This may be interpreted in the sense that resonance interaction between the aromatic portion and the imidazolidine moiety of the molecules is not permitted for high depressor activity. Interaction of this type may partly compensate the positive charge at the imidazolidine ring in the case of electron-repelling substituents and electron-attracting groups will hinder the electron donation to the receptor.

Rather stringent demands are made upon the steric occupation at the phenyl ring of the imidazolidines. For each position a parabolic dependence on steric factors was found. Factorizing the steric involvement revealed that probably one side of the aromatic nucleus determines the fitting with the central α-adrenoceptor. This side appears to be the one which bears the larger ortho substituent. It seems therefore plausible to postulate that in the conformation in which the protonated molecules interact with this receptive site, the smaller ortho substituent is directed to the imidazolidine portion, so that the larger substituent is situated at the site of the molecule, the interaction with the central α-adrenoceptor is most dependent on. It is tempting to suggest that when there is a possibility for this receptor to choose between ortho substituents, preferably chlorine or the substituent whose steric bulk is close to that of chlorine, shall be selected for this fit. The other (smaller) ortho substituent may then possibly determine the orientation of the residual imidazolidine ring. This group should not be too small, since

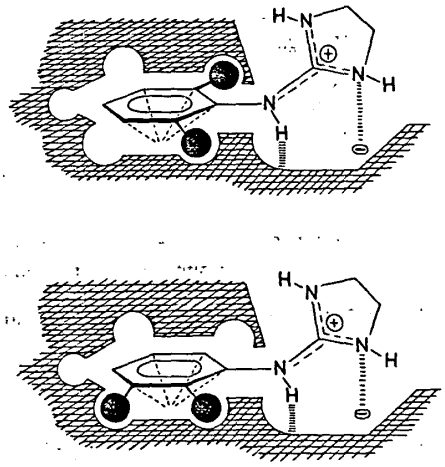

Fig. 62: Hypothetical working model visualizing the mode of interaction between clonidine-like imidazolidines and the central α-adrenoceptor. In view of the approximately equal hypotensive potency of 2,3- and 2,6-disubstituted imidazolidines the engagement of the central α-adrenoceptor by these two types of molecules is illustrated. For explanation see text.

coplanarity between aromatic portion and imidazolidine moiety will increase the resonance interaction between these two systems, which is not permitted for high hypotensive activity. Although apparently the demands made upon the steric bulk of the para substituent are less stringent, hypotensive activity is favoured when this position is either left unsubstituted or small groupes are attached to this ring position. Other effects, exerted by these groups, are probably of more importance.

Although not studied quantitatively, it may be accepted that a formation of a hydrogen bond between central α-adrenoceptor and imidazolidine is possible. However, as has been remarked with respect to the excitation of the peripheral, vascular α-adrenoceptor (section 6.8.1.), hydrogen bond formation seems also of minor importance for the engagement of the central α-adrenoceptor in view of the observation that in many centrally acting hypotensive drugs (section 4.2.18.) such an interaction cannot be accomplished. Based on studies with group selective reagents on tissues containing α-adrenoceptors, Salman et al. (1976) have proposed the presence of thiol groups at such selective sites. One may speculate upon the involvement of this group in the formation of a hydrogen bond.

The major aspects of this hypothetical model of interaction are schematically illustrated by fig. 62. It should be stressed that in essence this concept is identical to the one proposed for the mode of interaction of α-sympathomimetic drugs with the peripheral, vascular α-adrenoceptor (section 6.8.1.).

6.9. Concluding Remarks

The attempts to establish a mathematical relationship between a number of pharmacological actions of imidazolidines and their molecular structure, reviewed in the previous sections, revealed the complexity of the in vitro and even more the in vivo systems for performing such studies. To a certain extent these investigations point to some structural features for optimal activity at the appropriate α-adrenoceptor.

A very interesting facet in the structure-activity relationship of these phenyl-substituted imidazolidines is the influence of substituents located at the 3 and/or 5-position. It has been shown in section 6.5. that these meta-substituted compounds could not simply be incorporated in the structure-activity relationship with respect to the peripheral, hypertensive activity. Moreover, it strikes that the hypotensive activity of compound no. *11* (2,5-di-Cl) forms an outlier at central α-adrenoceptor level, whereas the potency of no. *9* (2,3-di-Cl) is accounted for by the regression equations. It should be added that the depressor activity of compound no. *11* (2,5-di-Cl) at central α-adrenoceptor level is far less than that of no. *16* (2-Cl). On the other hand no. *9* (2,3-di-Cl) is more potent than no. *10* (2,4-di-Cl).

Similar observations were made in derivatives of noradrenaline and adrenaline. Substitution on the phenyl ring with a hydroxyl group at the 3-position is more effective in providing vasopressor action than substitution at the 4-postion (for review see Lands and Brown, 1967). Addi-

tionally, the presence of a 3-hydroxyl substituent at the aromatic ring of oxymetazoline is an enhancing factor for sympathomimetic activity in pithed rats (section 4.2.5.) and the isolated rabbit intestine preparation (section 4.3.2.) when compared to that of xylometazoline in which this substituent is missing. Moreover, 2,5-di-substituted 2-(phenylimino)imidazolidines in all cases display a higher hypertensive activity than isomeric 2,4-di-substituted compounds (table 17).

The electronic, quantum chemical and lipophilic properties of isomeric 2,3- and 2,5-di-substituted imidazolidines are virtually similar. Therefore, the differences in activity found may be related to steric effects. Proper substitution at the 3-position favours hypotensive activity, but substitution at the 5-position diminishes depressor potency. This conclusion is in agreement with the suggestion made in the previous section that probably one side of the phenyl ring determines the fit with the central α-adrenoceptor. Apparently, an additional binding place is present for a 3-substituent at this receptive site (see fig. 62). However, the results obtained in the rabbit (see fig. 38) indicate that very limited space is available for a 5-substituent.

A careful inspection of table 17 suggests that a 5-substituent affects the peripheral, hypertensive activity to a much smaller extent than observed for the hypotensive activity at the central α-adrenoceptor level. Consequently, it may be stated that the peripheral, vascular and the central, medullary α-adrenoceptors still discriminate between their agonists in a very subtle manner. A discrepancy may arise between 5-substituted imidazolidines of the clonidine-type in which this substituent represents a bulky group (Timmermans et al., 1978e). This suggestion contrasts ot the proposition about a possible similarity between these two types of α-adrenoceptors put forward in section 5.3. However, no compound possessing a bulky 5-substituent has been included in these studies. In order to put these postulates on a sound basis more meta-substituted derivatives would have to be incorporated in quantitative analyses. At present, this is the subject of further detailed investigations (Timmermans and van Zwieten and Hoefke et al., to be published).

From a mechanistic point of view it is most tempting to speculate on new and promising clonidine-like drugs possessing high hypotensive activity which is predictable on account of the present insight into the structure-activity relationship in this series of molecules. However, from a therapeutic standpoint high hypotensive activity is far less interesting than a favourable therapeutic range. From the majority of investigations aimed at separating side-effect from biological activity desired it may be concluded that for derivatives of clonidine the side-effects presumably run parallel with the hypotensive activity. Moreover, since therapeutically useful, antihypertensive drugs are preferably administered by mouth, the problem becomes even more complicated, because then resorption and first-pass effects might be serious draw-backs in the applicability of potentially active antihypertensive agents which is not the case with clonidine. The penetration of the compounds from the blood into the brain and the actual receptor-pharmacon complex formation are again superimposed upon the aforementioned processes. In order to solve this complex chain of events quantitatively each part-process should be studied separately.

To some extent the studies reviewed provide useful information concerning blood-brain transport phenomena and indicate a number of properties for optimal activity at central α-adrenoceptor level. The features emerging do not allow any definite conclusions with respect to a pre- or postsynaptic nature of this central α-adrenoceptor (also see Chapter I). Principally it would apply to both types of receptors. Imidazolidines of high hypotensive activity should be capable of forming a charge transfer complex and a high positive charge at the imidazolidine portion should be ensured. This should be achieved by substituents donating electrons by inductive forces. Preferably these compounds should be 2,6-di-substituted with groups whose steric dimensions are comparable to those of chlorine and the para position should be left unsubstituted. In view of these criteria methyl groups are the best candidates. However, methyl-substituted derivatives poorly penetrate into the central nervous system, as a result of the high pK_a value of these compounds. Consequently, the hypotensive activity of imidazolidines administered intravenously is

enhanced by substituents like chlorine or bromine. Finally, meta substitution is still a rather unexplored area and looks most promising for further investigations.

In active hypotensive imidazolidines the substitution at the phenyl ring should be such that it meets the demands for optimal receptor engagement and that it ensures a favourable penetration ability into the central nervous system. The optimal balance between these two mutually conflicting requirements might yield new, highly active hypotensive drugs in the imidazolidine series structurally related to clonidine. On the other hand it has been demonstrated that replacement of the 5-membered imidazolidine portion by other hetero rings also yield very effective hypotensive compounds (section 4.2.1.). At present, the variation in hypotensive activity due to these structural changes has not yet been subjected to a quantitative analysis. However, the possibility that compounds will be developed which are even more potent than clonidine, cannot be ruled out.

The studies described in the present review paper have demonstrated that a meaningful comparison of physicochemical and pharmacological characteristics of a well-defined series of homologues can lead to the presentation of a quantitative structure-activity relationship, which may even have some use for the design of new, active compounds in the imidazolidine series. Furthermore, it should be noted that this type of studies has been extended to experiments in intact animals; previous quantitative structure-activity relationship studies had been limited to isolated organ preparations.

The present analyses have enabled us to gain some insight into the properties of the central α-adrenoceptor. Concomitantly, it has become possible to develop concrete ideas concerning the interaction between this central receptor and centrally acting antihypertensive drugs.

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