Journal of Cardiovascular Pharmacology
16(Suppl. 5):S1–S9 © 1990 Raven Press, Ltd., New York

Pharmacology of β-Blockers: Classical Aspects and Recent Developments

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Summary: All clinically used β-blockers share the common feature of being competitive antagonists at β-adrenoceptors. They differ, however, in additional pharmacological properties, such as β_1/β_2-selectivity ratios, presence or absence of intrinsic sympathomimetic activity (ISA), and/or local anesthetic activity. Furthermore, β-blockers differ widely in their pharmacokinetic properties. The mammalian β_1- and β_2-adrenoceptors are the products of different genes but the receptor proteins show a certain degree of homology. Both span the cell membrane seven times. The cytoplasmic part of the receptor protein is the site of phosphorylations and hence involved in the process of receptor internalization. Upon exposure of tissues or organs to β-blockers, characteristic changes emerge at the cellular level. There is an increase in the density of β-adrenoceptors in the surface membrane, termed upregulation. This upregulation is subtype-specific, i.e., nonselective β-blockers increase the density of both β_1- and β_2-adrenoceptors whereas β_1-selective antagonists upregulate only the former subtype. In contrast, β-blockers with pronounced ISA downregulate β-adrenoceptors. β-Adrenoceptor density also changes in pathological situations. There is a downregulation of cardiac β-adrenoceptors in dilated cardiomyopathy, probably as a consequence of increased sympathetic tone. A rapid upregulation of β-adrenoceptors is characteristic of myocardial ischemia. This upregulation occurs in spite of a massive release of norepinephrine from cardiac adrenergic nerves during ischemia. Both norepinephrine release and upregulation of cardiac β-adrenoceptors lead to an adrenergic overstimulation of ischemic myocardium. Blockade of β-adrenoceptors inhibits the catecholamine component of this vicious circle and may explain part of the beneficial effects of β-blockers in coronary artery disease and myocardial infarction. **Key Words:** β-Adrenoceptors—β-Adrenoceptor subtypes—β-Adrenoceptor regulation—β-Blockers—Myocardial ischemia—Cardiomyopathy.

Research in the past 10–15 years has provided an impressive amount of new information on β-adrenoceptors and the pharmacology of their specific ligands. This includes the molecular biology of the β-adrenoceptor, the precise identification of β-adrenoceptor subtypes in human tissues, and the regulation of β-adrenoceptors under various conditions such as exposure to β-agonists and β-antagonists or in pathological situations, e.g., ischemia or heart failure. Finally, the regulation of β-adrenoceptors appears to have consequences for the density of other membrane receptors such as α_2-adrenoceptors and muscarinic receptors; this process is called transregulation.

MOLECULAR BIOLOGY OF THE β-ADRENOCEPTOR

The membrane system that is activated by β-agonists consists of three components: the β-adrenoceptor itself, a guanine nucleotide–binding regulatory protein (G protein), and the adenylate cyclase (Fig. 1) (1–3). Occupancy of the β-receptor protein by an agonist leads to coupling of the receptor to the adenylate cyclase through the G protein. The β-receptor is now in the high-affinity state (4,5). Because activation of the β-adrenoceptor leads to stimulation of adenylate cyclase, the involved G protein is of the "stimulatory" type (G_s) (6–9). The G_s protein is composed of three subunits, namely α, β, and γ, with α_s as the essential subunit. Prolonged exposure of the β-adrenoceptor to an agonist results in uncoupling of the receptor and the concomitant transition into the low-affinity state. Receptor uncoupling is the first step of receptor desensitization and subsequent internalization of β-adrenoceptors (10,11).

The mammalian β_1- and β_2-adrenoceptors are the products of different genes but the receptor proteins show a certain degree of homology. Whereas the cDNA for the human β_1-adrenoceptor encodes a

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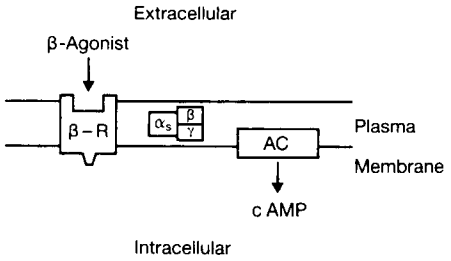

FIG. 1. Schematic representation of the membrane complex consisting of the β-adrenoceptor (β-R), a guanine nucleotide–binding regulatory protein that is composed of three subunits (α_s, β, γ) and the adenylate cyclase (AC). See the text for further details.

protein of 477 amino acid residues, the human β_2-adrenoceptor is a 413 amino acid glycoprotein. There is a 54% homology between the human β_1- and β_2-adrenoceptor (12–14). The receptor protein traverses or spans the cell membrane seven times. Such an arrangement is characteristic of several membrane receptors including the β-adrenoceptor subtypes, the porcine brain and cardiac muscarinic cholinergic receptors, and the visual pigment rhodopsin (12–15). The membrane-spanning regions of the receptor consist of clusters of 24 hydrophobic amino acids. There is some evidence that the cytoplasmic loops I–II and III–IV determine the ligand-binding properties (16–18). In other words, the hydrophobic amino acids within the membrane accommodate the β-agonist or -antagonist. The amino acid sequences 222–229 and 258–270 have been implicated in the coupling of the receptor to the guanine nucleotide binding regulatory protein (9). The rather large cytoplasmic part of the receptor protein is the site of phosphorylations and hence involved in the process of receptor internalization. There is a cAMP-dependent protein kinase that phosphorylates the β-adrenoceptor to a stoichiometry of 2 mol/mol. A region close to the carboxyl terminus is the target of another protein kinase, the β-adrenoceptor kinase, that preferentially phosphorylates the agonist-occupied form of the receptor to a stoichiometry of 8 mol/mol (19–24).

PHARMACOLOGY OF β-BLOCKERS

The prominent pharmacological property of β-adrenoceptor antagonists is the high affinity for β-adrenoceptors of the various organs, as can easily be demonstrated in binding experiments. The formation of the antagonist–receptor complex, however, is not followed by receptor activation, as occurs with agonists (Fig. 2A). All β-blockers in clinical use are competitive antagonists. The experimental equivalent is a parallel shift to the right of the concentration–response curve of a given agonist, e.g., isoprenaline, when increasing concentrations of an antagonist are added to a biological system, an isolated cardiac preparation (Fig. 2B).

As with antagonists for a variety of receptors, there are also β-blockers that upon binding to the β-adrenoceptor activate the receptor to a certain extent (Fig. 2C). They are called partial agonists or β-blockers with intrinsic sympathomimetic activity (ISA). Whereas a full agonist often needs to activate only a fraction of a given receptor population to elicit a maximal effect, a partial agonist always requires occupation of the total receptor population until its full effect is reached. This full effect may be less than that of a full agonist; at best, it may be similar. Beyond its intrinsic effect, a partial agonist antagonizes the effects of a full agonist (Fig. 2D) (25).

ISA can be directed toward β_2- as well as β_1-adrenoceptors, and the proportion may vary depending on the type of β-blocker. This explains both the vascular and the cardiac effects of β-blockers with ISA (26). The partial agonist activity is competitive and can be blocked with non-ISA β-blockers. Pindolol, alprenolol, oxprenolol, acebutolol, and the no longer available practolol are examples of β-blockers with ISA.

The terms β_1-selectivity and cardioselectivity are often used synonymously. The latter term is not appropriate for two reasons: (a) β_1-adrenoceptors are not confined to the heart but occur also in the kidney and in the brain; (b) the heart also contains β_2-adrenoceptors. Therefore, a β_1-selective blocker is not, and never can be, cardioselective.

β_1-Selectivity is not absolute and has to be viewed in relation to the doses of a β-blocker that affect β_1- and β_2-adrenoceptors and that allow the calculation of β_1/β_2-selectivity ratios (27–29). Wellstein et al. determined in humans the degree of occupation of β-adrenoceptors by various β-blockers over a wide range of plasma concentrations and related it to functional parameters, e.g., the reduction in exercise-induced tachycardia (30–32). Bisoprolol occupies 95% or more of human β_1-adrenoceptors at therapeutic doses but only less than 5% of β_2-adrenoceptors (30–32). As a consequence, the doses of bisoprolol can be raised three- to fourfold above the therapeutic range before antagonistic effects at bronchial or vascular β_2-adrenoceptors become manifest (33–36). Currently, bisoprolol is the most β_1-selective blocker in humans, followed in decreasing order by betaxolol, atenolol, metoprolol, and acebutolol (27–36) (Tables 1 and 2).

A number of β-blockers—propranolol, alprenolol, oxprenolol, pindolol, and timolol—have a direct effect on cell membranes called local anesthetic, membrane-stabilizing, or quinidine-like. Although the affinity to the β-adrenoceptor is determined by the configuration of the OH-group–carrying carbon atom, the local anesthetic activity resides in both isomers.

In cardiac muscle, it finds expression in a decrease in resting membrane potential and upstroke velocity of the action potential as well as in changes in action

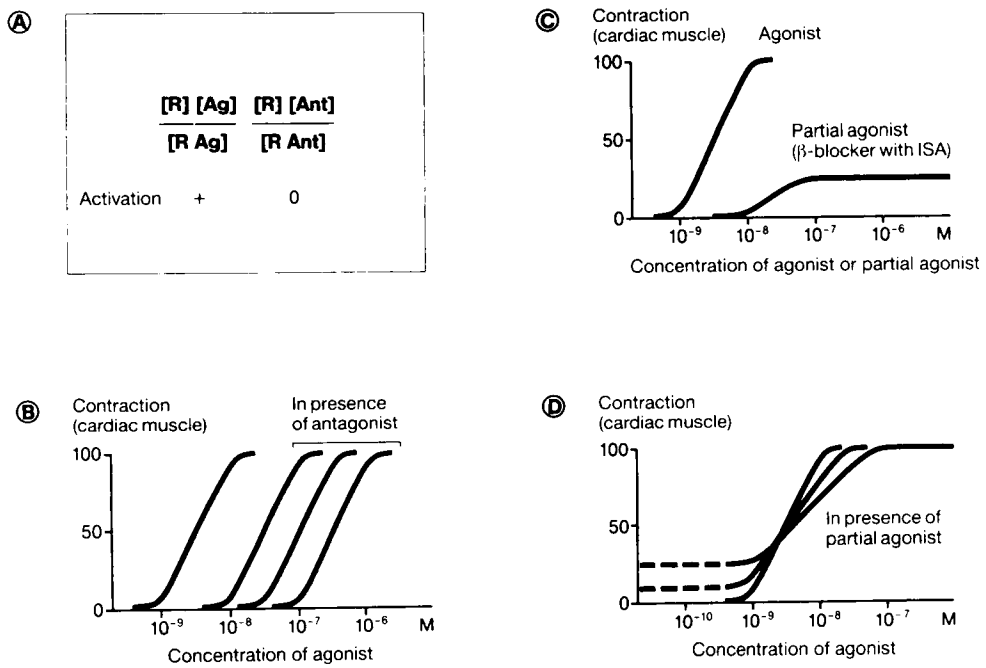

FIG. 2. The formation of the receptor–agonist complex [R Ag] leads to activation of the receptor. In the case of a pure antagonist, the formation of the corresponding complex [R Ant] is not followed by activation. At constant receptor density, the formation of [R Ag] or [R Ant] depends upon the agonist [Ag] or antagonist [Ant] concentration in close proximity of the receptor [R] as predicted by the law of mass action (**A**). **B** shows the parallel rightward shift of the concentration–response curve of an agonist by increasing concentrations of a competitive antagonist. (**C**) In contrast to a full agonist (for instance, isoproterenol), a partial agonist (for instance, a β-blocker with intrinsic sympathomimetic activity) is capable of only partially activating a given system. As a result, the maximum of its concentration–response curve is lower than that of a full agonist. (**D**) Beyond its intrinsic effect (dotted part of the concentration–response curve), a partial agonist antagonizes the effect of a full agonist.

potential duration and effective refractory period. However, these effects are observed only at distinctly higher concentrations than those required for β-adrenoceptor blockade. Local anesthetic activity does not contribute to the antihypertensive and antiarrhythmic effects of β-blockers (37,38).

COMPARISON OF β-BLOCKERS

From a pharmacological point of view, β-blockers can be ranked according to potency, β_1-selectivity, ISA, and local anesthetic activity and from a pharmacokinetic point of view according to bioavailability, plasma half-life, and clearance. Because local anesthetic activity does not contribute to the therapeutic effect, it was omitted from Table 1, which gives a comparison of commonly used β-blockers. The first column of Table 1 shows equipotent single oral doses in humans. The rank order does not reflect the affinity of the various β-blockers for the β-adrenoceptor because it is modified through the bioavailability of the compounds. Because of its limited bioavailability,

TABLE 1. *Comparison of commonly used β-blockers*

Compound	Equipotent single oral doses in men (mg)	Compound	Usual therapeutic dose range (mg/day)	Compound	β_1-Selectivity	Compound	ISA
Bopindolol	1	Bopindolol	1–2	Bisoprolol	+++	Pindolol	+++
Pindolol	5	Bisoprolol	5–10	Betaxolol	++	Alprenolol	++
Bisoprolol	10	Pindolol	5–30	Atenolol	++	Oxprenolol	++
Betaxolol	10	Betaxolol	10–20	Metoprolol	+	Acebutolol	+
Timolol	10	Timolol	10–40	Acebutolol	+		
Atenolol	100	Atenolol	50–100				
Metoprolol	100	Nadolol	80–240				
Oxprenolol	100	Oxprenolol	80–320				
Propranolol	100	Propranolol	80–320				
Nadolol	120	Metoprolol	100–200				
Acebutolol	200	Sotalol	160–320				
Sotalol	200	Acebutolol	400–800				

TABLE 2. *Comparison of β_1-selective blockers*

Compound	Usual therapeutic dose range (mg/day)	β_1-/β_2-adrenoceptor subtype selectivity (ref. 32)	Bioavailability (%)	Mean plasma half-life (h)	Clearance (renal %:hepatic %)	Protein binding (%)
Bisoprolol	5–10	75/1	90	10–12	50:50	30
Betaxolol	10–20	35/1	80	12–16	15:85	50
Atenolol	50–100	35/1	50	6–9	95:5	<5
Metoprolol	100–200	20/1	40	3–4	5:95	12
Acebutolol	400–800	Not determined	50	3–4	20:80	84

propranolol, for instance, ranks rather low, although it is very potent in in vitro experiments or after i.v. injection. Drugs with rather long plasma half-life, such as nadolol and bisoprolol, ascend in rank order when equipotent single oral doses and the therapeutic daily dose range are compared. Metoprolol descends due to its short plasma half-life.

Among the β_1-selective blockers, bisoprolol not only has the highest β_1-selectivity but is also the most potent one (27–36). Bisoprolol also stands out in terms of pharmacokinetics: it has the highest bioavailability and a long plasma half-life (Table 2). One-half of a given dose of bisoprolol is excreted unchanged through the kidneys; the other half is broken down in the liver to metabolites that are devoid of affinity for the β-adrenoceptor. This balanced clearance of bisoprolol is particularly relevant in the case of a reduced function of one of the two clearance organs (39). In such situations, an adaptation of dose of bisoprolol is rarely necessary. Bisoprolol has a low protein binding of only 30%; however, the binding of atenolol and metoprolol is even lower (Table 2).

β-ADRENOCEPTOR SUBTYPES IN HUMAN TISSUES

Until a few years ago, it was common understanding that the heart contains exclusively or overwhelmingly β_1- and the lungs β_2-adrenoceptors. The results of more recent biochemical and pharmacological studies, including those on human tissues, point to the existence of the respective other β-adrenoceptor subtypes in each of the two organs.

Binding studies with β-adrenoceptor ligands have yielded the following β_1:β_2-ratios (%) for the human heart: right atrium, 80:20 to 65:35 (40–43); left atrium, 65:35 (43,44); right ventricle, 87:13 to 74:26 (42,43,45); and left ventricle, 86:14 to 69:31 (41–45). Thus, all major parts of the human heart contain a sizable proportion of β_2-adrenoceptors, the atria being apparently somewhat richer in this receptor subtype than the ventricles (43). In both atria and ventricles, β_1- and β_2-adrenoceptors are functionally coupled to the adenylate cyclase, suggesting that in the human heart both β-adrenoceptor subtypes play a physiological role in regulating heart rate and/or contractile force (43,46,47). However, in weighing the functional impact of β_2-adrenoceptors, one has to consider that the physiological transmitter of adrenergic nerves, i.e., norepinephrine, has a higher affinity for β_1- than for β_2-adrenoceptors. Therefore, one has to assume that regulation of heart rate and myocardial contractile force through cardiac sympathetic nerves is mostly, if not entirely, mediated by β_1-adrenoceptors. This, however, may be different at elevated levels of circulating epinephrine, which has high affinity for β_2-adrenoceptors.

In lung tissue, the majority of β-adrenoceptors belongs to the β_2 subtype; 20–30% have been identified as β_1-adrenoceptors (48–50). Their precise location is not clear. Although β_1-adrenoceptors may reside predominantly on lung parenchyma and mucous glands, the possibility cannot be entirely dismissed that some of them subserve bronchodilator function. In this case, even highly β_1-selective blockers would be expected to suppress some bronchodilator component, of whatever magnitude it ultimately may be. However, β_1-selective blockers will not blunt the bronchodilator effect of β_2-agonists.

REGULATION OF β-ADRENOCEPTORS

Prolonged activation of the β-adrenoceptor leads to fading of the biological response despite the continuous presence of the agonist. This phenomenon is referred to as tachyphylaxis, desensitization, adaptation, tolerance, or refractoriness. Only recently have we begun to understand desensitization at the molecular level (1,2,10,19,51). One of the first events is functional uncoupling of the receptor from the G protein and the transition from the high- to the low-affinity state. The β-adrenoceptors are capable of existing in two discrete states having either high or low affinity for agonists but equivalent affinity for antagonists. At this stage, the number of receptors in the surface membrane is not changed (4–6,11,51,52).

If agonist exposure is continued, some of the β-adrenoceptors disappear from the surface membrane, a phenomenon that is called sequestration, internalization, or downregulation of receptors (53–56). Phosphorylation of the receptor protein is the critical step in this process. Both phosphorylation by a cAMP-dependent protein kinase as well as by a cAMP-independent kinase appear to be involved, and there is evidence that phosphorylation through the two kinases occurs at distinct sites of the receptor protein (16,19–23). The cAMP-independent protein kinase has been identified recently and distinguished

from other known kinases, particularly from protein kinase C and Ca/calmodulin kinase (20–23,52). The new kinase has been named β-adrenoceptor kinase. It is a ubiquitous soluble, cytosolic enzyme. It is unlikely that the β-adrenoceptor kinase is activated in the process of desensitization. The sequence of events is rather such that the conformational change that is induced by the binding of an agonist to the receptor protein converts the β-adrenoceptor into a much better substrate for the kinase than is the unstimulated receptor (52). With phosphorylation of the receptor, the process of internalization is initiated. The receptors in the internalized, sequestered compartment are removed from contact with the G protein and the adenylate cyclase; however, they appear to be structurally intact and upon dephosphorylation recycle to the cell surface and become recoupled to the adenylate cyclase. If agonist-induced desensitization is permitted to continue, then part of the internalized receptors are destroyed, presumably by lysosomal proteases (52–54).

Downregulation of β-adrenoceptors through prolonged receptor stimulation is of a clinical relevance in the treatment with β-agonists of obstructive airway disease or of heart failure (2,10,49,57,58). Furthermore, β_1- but not β_2-adrenoceptors are downregulated in the pathological situation of dilated cardiomyopathy, probably as a consequence of increased cardiac sympathetic tone (43,45,59–61).

ALTERATIONS OF β-ADRENOCEPTOR DENSITY BY β-BLOCKERS

At therapeutic plasma levels, some β-blockers induce distinct changes in β_2-adrenoceptor density as measured in human lymphocytes (62,63). These changes occur within 2–3 days of treatment and their magnitude is an increase or decrease by approximately 50%. Although nonselective β-blockers, such as propranolol, increase β_2-adrenoceptor number, β_1-selective blockers, such as bisoprolol, leave it unaffected. Pindolol, a β-blocker with pronounced ISA, decreases β_2-adrenoceptor density in human lymphocytes (63–65). From a variety of similar studies, the general rule is derived that β-adrenoceptor antagonists without ISA upregulate and those with ISA downregulate β-adrenoceptors (62–66). The upregulation occurs in a subtype-specific way such that β_1-selective blockers upregulate only β_1-adrenoceptors (60–68).

UPREGULATION OF β-ADRENOCEPTORS IN MYOCARDIAL ISCHEMIA

There is one pathological situation that does not obey to the general rule of up- and downregulation of β-adrenoceptors by antagonists and agonists, respectively. In severe myocardial ischemia progressing to myocardial infarction there is a rapid upregulation of cardiac β-adrenoceptors, although at the same time the release of norepinephrine from cardiac adrenergic nerves is greatly enhanced. The various phases of this enhanced release have been studied in detail by Schömig et al. (69–71) and are compiled in Fig. 3.

The first indications for an upregulation of cardiac β-adrenoceptors in ischemia came from studies by Mukherjee et al. (72,73). In 1985, Maisel et al. (74) postulated an increased externalization of receptors during myocardial infarction. Strasser et al. (52) studied changes in β-adrenoceptor number in isolated

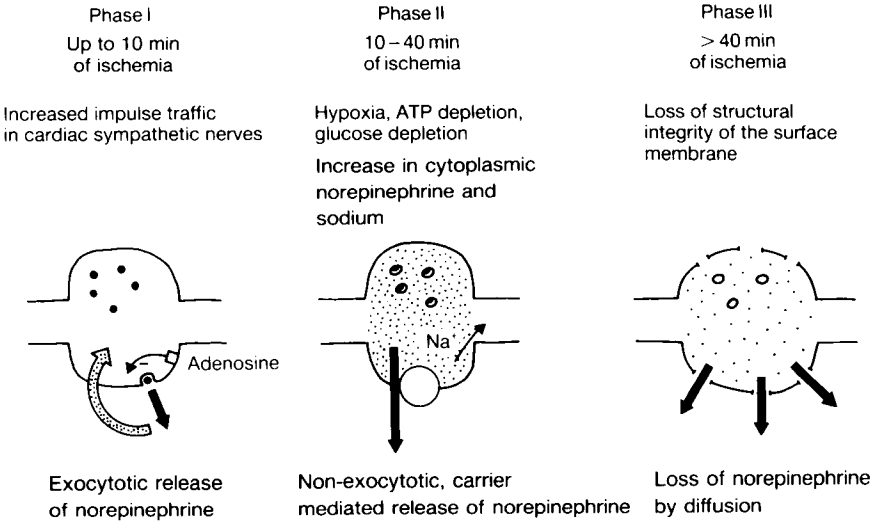

FIG. 3. Schematic drawing of adrenergic varicosities showing the release of norepinephrine during progression of cardiac ischemia (phase I–III). **Left:** Within the first minutes of ischemia, impulse traffic in cardiac sympathetic nerves is elevated, leading to exocytotic release (black arrow) of norepinephrine. Therefore, the release is still subject to modulation by inhibitory presynaptic receptors, for instance presynaptic adenosine receptors, which are certainly stimulated in ischemia. Furthermore, the norepinephrine uptake mechanism is still intact (dotted arrow). **Middle:** Longer-lasting ischemia leads to ATP depletion and to an impairment of the storage mechanism of the norepinephrine-containing granules. As a consequence, norepinephrine leaks from the granules into the cytoplasm of the adrenergic varicosity (illustrated by numerous dots in the varicosity). Because of ATP depletion, Na^+/K^+-ATPase activity decreases, resulting in elevated cytoplasmic sodium. Both elevated cytoplasmic norepinephrine and sodium provide the conditions under which the norepinephrine uptake mechanism operates in the reverse fashion and extrudes norepinephrine. **Right:** If ischemia lasts even longer, the surface membrane of the adrenergic varicosity loses its structural integrity, becomes leaky, and passive norepinephrine efflux occurs. The sequence of events was reconstructed from data published by Schoemig et al. (69–71).

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