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Steric Aspects of Agonism and Antagonism at β-Adrenoceptors: Experiments with the Enantiomers of Terbutaline and Pindolol

By

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Abstract: The enantiomers of terbutaline, a β_2-selective adrenoceptor agonist, and pindolol, an unselective antagonist with partial agonist activity, were examined with respect to their ability to react *in vitro* on adrenoceptors in the trachea (mostly β_2), the soleus muscle (β_2) and in the papillary muscle of the left ventricle (β_1) from the guinea-pig. (+)-terbutaline was more than 3,000 times less potent than (−)-terbutaline in relaxing the trachea and in depressing subtetanic contractions of the soleus muscle. (+)-terbutaline did not inhibit the effects of (−)-terbutaline in these tissues. The effect of (−)-terbutaline on the papillary muscle was about 200 times weaker than on the soleus. (+)-terbutaline had a negligible inotropic effect on the papillary muscle and it did not inhibit the effect of isoprenaline. The enantiomers of pindolol did not show any consistent agonistic activity under the present experimental conditions. (−)-pindolol inhibited competetively the effect of isoprenaline to the same extent in all three tissues. (+)-pindolol was about 200 times less potent in this respect. Our data do not reveal any qualitative differences in the pharmacological properties between the optical isomers of terbutaline and pindolol, respectively.

Key-words: Terbutaline – pindolol – stereoselectivity – β-adrenoceptors – guinea-pig – *in vitro*.

Stereoselectivity appears to be a universal property of β-adrenoceptor agonists and antagonists (for review see Patil *et al.* 1974; Ruffolo 1983). Thus the agonistic activity of adrenaline (Cushny 1908) and terbutaline (Wetterlin 1972; Jeppsson *et al.* 1982) resides mainly in the levorotatory isomer. Also for antagonists such as propranolol (Howe & Shanks 1966) and pindolol (Waite 1978; Clark *et al.* 1982), the levorotatory isomer is much more potent than its optical antipode. Besides these quantitative differences between optical isomers, qualitative pharmacological properties have been suggested. Thus Patil *et al.* (1971) postulated that if β-adrenoceptors are dissimilar in various tissues, the isomeric activity ratio of an agonist should be different. Further, it has been suggested that the nonagonist isomer of a β-adrenoceptor agonist may possess blocking activity resulting in partial agonist activity of the racemate (Al-Jeboory & Marshall 1978; Bowman 1981).

Another intriguing observation is that both enantiomers of pindolol, a potent β-adrenoceptor antagonist with a relatively high degree of intrinsic activity, are equally potent in relaxing tracheal (Waite 1978) and vascular (Clark & Bertholet 1983) smooth muscle although the (−)-isomer is considerably more potent than the (+)-isomer as a β-adrenoceptor antagonist in these tissues. This apparent dissociation between stimulation and blockade has been further evaluated by us. We measured the agonistic and antagonistic properties of the stereoisomers of terbutaline and pindolol on isolated tissues from the guinea-pig.

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Materials and Methods

Male guinea-pigs about 200 g were used in all experiments. The animals were stunned by a blow on the head and exsanguinated. The heart, the trachea and the soleus muscles were dissected out and transferred to dishes with oxygenated Krebs solution. The tissues were prepared as described below. The measurement of isometric contractions was made on a Grass model 7 D Polygraph using FT03 force transducers. All experiments were performed in jacketed organ baths (40 ml containing oxygenated Krebs solution maintained at 37°).

The trachea (mainly β_2-adrenoceptors) was freed from connective tissue and cut into rings over two segments. Cotton threads were fastened to both ends of the cartilage bridge which was then cut open in the middle. The preparation was mounted at a basal tone of 5 mN and allowed to stabilize for one hour. Additional tracheal tone was induced by carbachol 0.05 µmol/l (in experiments with terbutaline isomers) or 0.01 µmol/l (in experiments with pindolol isomers) 10–15 min. before the addition of the test substances. The effects were expressed in per cent of the maximum relaxation produced by 10 µmol/l isoprenaline added at the end of each cumulative addition of test substance.

The soleus muscle (β_2-adrenoceptors) was prepared essentially as described previously (Waldeck 1976; Holmberg & Waldeck 1980). Subtetanic contractions (S) were induced by electrical field stimulation (maximum pulses 0.5 msec., 12 Hz for 1.5 sec.) every 20 sec. Each train of pulses was followed, with 10 sec. delay, by a single pulse producing a twitch (T). The ratio S/T was used to calculate the reduction in the degree of fusion of the subtetanic contractions. Due to the complex effect kinetics of the β-adrenoceptor mediated effects on the soleus muscle (Holmberg & Waldeck 1980), the effects have been expressed in per cent of the maximum effect produced by 2.3 µmol/l of terbutaline given separately at the beginning of the experiment. This is also the reason why the cumulative concentration-effect curves for the agonists in some cases ended above 100 per cent.

The papillary muscle (β_1-adrenoceptors) from the left ventricle of the heart was dissected out, mounted on a holder with two ring-shaped platinum electrodes and attached to a force transducer. The basal tone was adjusted to 5mN and the muscle was paced with maximum pulses of 1 msec. duration (transmural field stimulation) at a frequency of 3 Hz. The positive inotropic effects were expressed in per cent of the maximum increase produced by 10 µmol/l isoprenaline added at the end of each cumulative addition of test substance.

The drugs used and their sources were: carbamylcholin chloride and (−)-isoprenaline. HCl (Sigma Chemical Co., St. Louis, Mo., U.S.A.), (−)-terbutaline. HBr and (+)-terbutaline·HBr (AB Draco, Lund, Sweden), (−)-pindolol and (+)-pindolol (Sandoz LTD; Basle, Switzerland), propranolol. HCl (ICI, Macclesfield, England). All drugs were dissolved in saline, the pindolol isomers after addition of an equivalent amount of tartaric acid.

Statistical evaluation was made using Student's t-test, in some cases preceded by analysis of variances.

Results

Terbutaline.

Both enantiomers of terbutaline caused a complete and concentration-dependent relaxation of the carbachol-contracted (0.05 µmol/l) trachea (fig. 1). However, (+)-terbutaline was about 3,000 times less potent than (−)-terbutaline ($P < 0.001$) (table 1). Propranolol, 0.1 µmol/l, shifted the concentration-response curve for (−)-terbutaline about two log units to the right, corresponding to a pA_2 value for propranolol of 8.91 ± 0.05 S.E. ($n = 3$) ($P < 0.001$). The effect of (+)-terbutaline was less efficiently blocked by propranolol and the concentration-ratio for this isomer was less than half of the value obtained for (−)-terbutaline ($P < 0.001$). Further, the presence of 5 µmol/l (+)-terbutaline, a concentration with a marginal effect per se but about 100 times above the EC50 for (−)-terbutaline, did not change the effect of (−)-terbutaline (table 1).

On the soleus muscle, (−)-terbutaline caused a concentration-dependent depression of the subtetanic contractions with a pD_2 equal to that obtained on the trachea (fig. 2 and table 1). Also in this tissue 5 µmol/l (+)-terbutaline failed to inter-

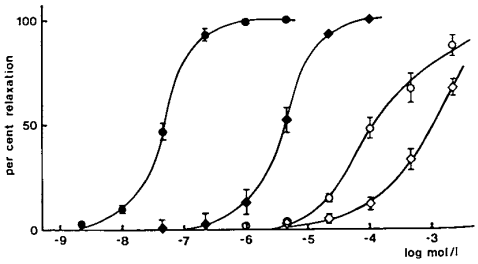

Fig. 1. Relaxation of the carbachol (5×10^{-8} mol/l) contracted guinea-pig trachea by the enantiomers of terbutaline and its inhibition by propranolol. Cumulative concentration-response curves were obtained in the absence (● ○ 9 and 8 experiments) or in the presence (◆ ◇, 3 experiments) of 10^{-7} mol/l propranolol, added 30 min. beforehand. Filled symbols: (−)-terbutaline. Unfilled symbols: (+)-terbutaline. The means ± S.E. are shown.

Table 1.

β-Adrenoceptor mediated effects of the enantiomers of terbutaline on isolated tissues from guinea-pig. pD_2-Values were calculated from cumulative concentration-response curves. The data are the means ± S.E. and the number of experiments.

	Tracheal muscle* relaxation	Soleus muscle depression	Papillary muscle inotropy
(−)-Terbutaline	7.28 ± 0.06 (9)	7.28 ± 0.10 (7)	5.03 ± 0.08 (8)**
(+)-Terbutaline	3.76 ± 0.10 (8)	Undefinable	Nil
(−)-Terbutaline in the presence of 5×10^{-6} mol/l (+)-terbutaline	7.38 ± 0.03 (3)	7.44 ± 0.13 (3)	–

* Contracted with carbachol, 5×10^{-8} mol/l
** Partial agonist, $\alpha = 0.74 \pm 0.02$ (8)

fere with the effect elicited by (−)-terbutaline (table 1). (+)-terbutaline had the same qualitative effect as (−)-terbutaline on the soleus muscle but the potency appeared to be lower on the soleus muscle than on the trachea and a full concentration-response curve for (+)-terbutaline was not obtained within reasonable concentrations (compare fig. 1 with fig. 2).

The positive inotropic effect of (−)-terbutaline on the papillary muscle (fig. 2) was about 200 times weaker than the effect on the soleus muscle described above ($P < 0.001$). Moreover, (−)-terbutaline was a partial agonist on the papillary muscle when compared with isoprenaline (table 1). (+)-terbutaline had a negligible effect on the force of contraction of the papillary muscle also when compared with the effect on the soleus muscle elicited by this enantiomer (fig. 2).

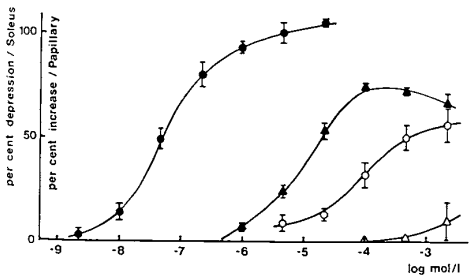

Fig. 2. β-Adrenoceptor mediated effects of the enantiomers of terbutaline on the isolated soleus muscle and on the papillary muscle from guinea-pig. Depression of the subtetanic contractions of the soleus muscle (● ○, 7 and 5 experiments) and increase in the force of contraction of the papillary muscle (▲ △, 8 experiments) were measured during cumulative addition of (−)-terbutaline (filled symbols) or (+)-terbutaline (unfilled symbols). The means ± S.E. are shown.

In another set of experiments, cumulative concentration-effect curves for isoprenaline were obtained on papillary muscles with or without 100 μmol/l of (+)-terbutaline. This concentration of (+)-terbutaline had no effect per se. The pD_2 values obtained for isoprenaline were 8.64 ± 0.10 S.E. (n = 8) and 8.62 ± 0.17 (n = 5), respectively.

Pindolol.

Pindolol did not show any consistent agonistic activity on the carbachol (0.01 μmol/l) contracted trachea. Both enantiomers caused a slight relaxation at 1 μmol/l (fig. 3), but the responses were too small to permit a further analysis. On the other hand, 20 min preincubation with (−)-pindolol or (+)-pindolol, inhibited the response to isoprenaline in a competitive way (fig. 3 and table 2). Three different concentrations of each inhibitor were used, but the estimations of pA_2 were based on the two highest concentrations only, since the low concentration of (+)-pindolol failed to give a significant agonist concentration-ratio (cf. MacKay 1978). The pA_2 for (−)-pindolol was significantly higher than that for (+)-pindolol ($P < 0.001$) and the enantiomeric potency ratio was about 150 (table 2).

Neither of the isomers of pindolol had any detectable effect on the contractions of the soleus muscle at any of the concentrations tested i.e. up

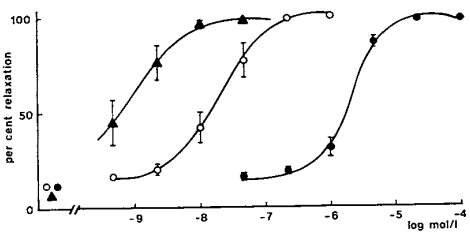

Fig. 3. Stereoselective inhibition by pindolol of the isoprenaline-induced relaxation of the carbachol (10^{-8} mol/l) contracted guinea-pig trachea. (−)-pindolol, 10^{-6} mol/l (●), (+)-pindolol, 10^{-6} mol/l (○) or the vehicle (▲), were added 20 min. before the cumulative addition of isoprenaline. The symbols close to the left show the effects of the preincubation per se. The data are the means ± S.E. from 5 experiments.

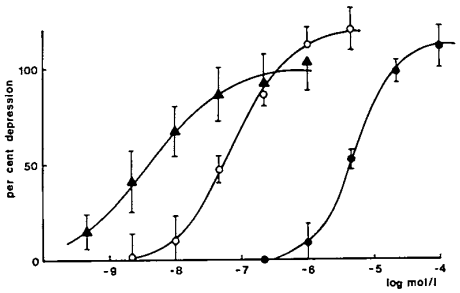

Fig. 4. Stereoselective inhibition by pindolol of the isoprenaline-indued depression of the subtetanic contractions of the isolated guinea-pig soleus muscle. (−)-pindolol, 10^{-6} mol/l (●), (+)-pindolol, 10^{-6} mol/l (○) or the vehicle (▲) were added 20 min. before the cumulative addition of isoprenaline. The data are the means ± S.E. from 5–6 experiments.

to 1 μmol/l for (−)-pindolol and up to 10 μmol/l for (+)-pindolol. However, the depression of the subtetanic contractions by isoprenaline was competetively inhibited by both isomers of pindolol when added 20 min. beforehand (fig. 4 and table 2). As was the case with the trachea (−)-pindolol was about 150 times more potent than (+)-pindolol as an antagonist ($P < 0.001$), but the estimated pA_2-values appeared to be slightly lower in the soleus than in the trachea.

Both enantiomers of pindolol caused a depression of the contractions of the papillary muscle by about 30 per cent. This negative inotropic effect, developed within 2–3 min., was observed throughout the whole concentration range investigated and did not appear to be concentration dependent. The vehicle, tartaric acid, was without effect. Both enantiomers of pindolol also inhibited the effect of isoprenaline on the papillary muscle. Cumulative concentration-response curves were constructed for the positive inotropic effect of isoprenaline with and without various concentrations of pindolol (fig. 5). The depression by pindolol described above, and which developed during the

Table 2.

Stereoselective inhibition by pindolol of β-adrenoceptor mediated effects on isolated tissues from guinea-pig. Isoprenaline was added cumulatively without or with various concentrations of (−)-pindolol or (+)-pindolol. The pA_2-values were calculated from the concentration-ratios (CR) obtained at the two highest antagonist concentrations. The mean ± S.E. and the number of experiments are shown.

	Tracheal muscle*	Soleus muscle	Papillary muscle
Isoprenaline, pD_2	9.16 ± 0.20 (5)	8.47 ± 0.27 (6)	8.48 ± 0.10 (4)
(−)-Pindolol, pA_2	9.59 ± 0.06 (10)	9.17 ± 0.10 (11)	9.68 ± 0.08 (11)
Log CR at 10^{-8} mol/l	1.32 ± 0.34 (5)	1.26 ± 0.22 (5)	1.74 ± 0.12 (5)
Log CR at 10^{-7} mol/l	2.71 ± 0.02 (5)	2.22 ± 0.18 (6)	2.72 ± 0.17 (5)
Log CR at 10^{-6} mol/l	3.46 ± 0.08 (5)	3.11 ± 0.09 (5)	3.59 ± 0.07 (6)
(+)-Pindolol, pA_2	7.42 ± 0.,11 (10)	6.97 ± 0.16 (10)	7.22 ± 0.13 (13)
Log CR at 10^{-7} mol/l	0.39 ± 0.17 (5)	0.06 ± 0.25 (5)	0.66 ± 0.16 (5)
Log Cr at 10^{-6} mol/l	1.40 ± 0.16 (5)	1.07 ± 0.18 (5)	1.15 ± 0.22 (7)
Log CR at 10^{-5} mol/l	2.47 ± 0.15 (5)	1.95 ± 0.25 (5)	2.38 ± 0.08 (6)

* Contracted with carbachol, 10^{-8} mol/l

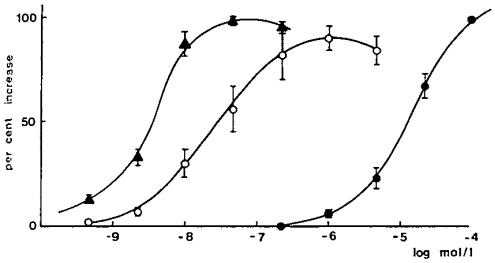

Fig. 5. Stereoselective inhibition by pindolol of the positive inotropic effect of isoprenaline. (−)-pindolol, 10^{-6} mol/l (●), (+)-pindolol, 10^{-6} mol/l (○) or the vehicle (▲) were added 20 min. before the cumulative addition of isoprenaline. The data are the means ± S.E. from 4–7 experiments.

20 min. preincubation period, was not included in the calculation. The pA_2-values for the steric isomers of pindolol obtained on the papillary muscle were similar to those obtained on the trachea (table 2), the (−)-isomer being much more active than the (+)-isomer ($P < 0.001$). The enantiomeric potency ratio was almost twice that obtained in the trachea and in the soleus but this difference is within the experimental error.

Discussion

Terbutaline showed a very high degree of stereoselectivity thus confirming previous observations (Wetterlin 1972). However, the effect curves of the (+)-isomer extended into a concentration range where unspecific effects may occur. Thus unspecific relaxation may explain why propranolol was less efficient in blocking the effect of (+)-terbutaline than that of (−)-terbutaline on the trachea. In the soleus and in the papillary muscle it was not even possible to obtain complete concentration effect curves for the less active enantiomer.

A large excess of the (+)-isomer of terbutaline did not inhibit the effects of (−)-terbutaline, on the trachea or on the soleus muscle, nor did it inhibit the inotropic effect of isoprenaline on the papillary muscle. These results show that (+)-terbutaline, in comparison with (−)-terbutaline, has virtually no affinity for either β_1- or β_2-adrenoceptors. Thus the functional β_2-selectivity of racemic terbutaline cannot be attributed to β_1-adrenoceptor blocking properties of the inactive isomer. Brittain *et al.* (1973) arrived at a similar conclusion concerning the β_2-selectivity of salbutamol.

On the contrary, our results as well as previous data (Wetterlin 1972) show that the (−)-isomer of terbutaline per se acts as a β_2-selective adrenoceptor agonist. There are no data available on the receptoragonist dissociation constant for the stereoisomers of terbutaline, but ligand binding studies with the racemate indicate a pK_D of about 5.0 with little or no difference between β_1- and β_2-adrenoceptors (U'Prichard *et al.* 1978; Minneman *et al.* 1979). We believe that the functional β_2-selectivity of terbutaline demonstrated here and elsewhere (Persson & Olsson 1970; O'Donnell & Wanstall 1974) is due mainly to partial agonist activity at β_1-adrenoceptors and to a lesser extent to difference in affinity for the two receptor types.

Based on i.a. experiments on guinea-pig trachea (Waite 1978) and canine vascular smooth muscle (Clark & Bertholet 1983), pindolol has been described as a non-selective β-adrenoceptor antagonist with partial agonist activity at β_2-adrenoceptors (Clark *et al.* 1982). An inconsistent finding by these authors was that whereas the antagonistic effect of pindolol was stereoselective, both optical isomers showed the same agonistic activity. An even more complex relation between stimulation and blockade with the enantiomers of pindolol has been described by Walter *et al.* (1982).

We were unable to find any consistent relaxation of the guinea-pig trachea by either isomer of pindolol. Nor was there any sign of agonistic activity on the soleus muscle. Perhaps the efficacy of pindolol is too low to produce a significant response in these tissue preparations. Nor did prenalterol, another partial agonist, show any detectable response on the isolated guinea-pig soleus muscle (Johansson & Waldeck 1980) but caused a significant β_2-adrenoceptor mediated relaxation of the rat uterus (Mattsson *et al.* 1982). It cannot be excluded, however, that a substantial part of the non-stereospecific smooth muscle relaxant effect of pindolol observed by others (Clark *et al.* 1982) is unrelated to β-adrenoceptors.

The inotropic and chronotropic effects of pindolol on the heart are complex (Kaumann &

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