Different mechanisms of action of agents acting on β -adrenoceptors in barium-stimulated and electrically-stimulated rat vas deferens

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1 The relaxation induced by β -adrenoceptor agonists in rat vas deferens was examined under two different experimental conditions: on electrically-induced twitch responses (35 V, 3 ms, 0.07 Hz), and on contractions induced by single doses of barium chloride (300 μ M). The experiments were performed in vasa of reserpine-treated rats, after blockade of α -adrenoceptors and extraneuronal uptake with dibenamine (10 μ M, 30 min), and neuronal uptake with cocaine (10 μ M).

2 When twitch responses were used, the values of pD_2 , interpolated from cumulative concentration-response curves for isoprenaline (Iso), adrenaline (Ad), and noradrenaline (NA) showed a rank order of potency consistent with the presence of β_2 -adrenoceptors (Iso > Ad $\gg$ NA).

3 When twitch responses were used, the non-selective β -antagonist, propranolol, caused a concentration-dependent parallel shift to the right of Iso concentration-response curves. Similar shifts were obtained by use of the β_2 -antagonist, isopropylmethoxamine (IMA), and higher doses of the β_1 -antagonist, practolol, according to the expectations from receptor occupation theory. Practolol presented the lowest value of pK_B , 5.03, corroborating the presence of β_2 -adrenoceptors.

4 When twitch responses were used, and Ad or NA employed instead of Iso, the antagonists produced shifts of concentration-response curves which were smaller than expected from theory, precluding the determination of pK_B values. This indicates that other mechanisms are involved besides an interaction with a single population of postsynaptic β_2 -adrenoceptors.

5 When barium chloride was used instead of twitch responses, although the potencies of Iso and Ad were increased respectively by about 30 fold and 5 fold, the rank order of potency was still consistent with an interaction with β_2 -adrenoceptors. In addition, the antagonists produced parallel and concentration-dependent shifts of the curves of all the agonists, as expected from receptor theory. The values of pK_B for a given antagonist were not modified by interchanging the agonists used, indicating a typical interaction with a single population of β_2 -adrenoceptors. When compared to the field-stimulated vas, the values of pK_B for propranolol and IMA against isoprenaline were respectively 1.3 and 0.6 log units larger. These results suggest the β -adrenoceptor agents act by different mechanisms of action in barium-stimulated and electrically-stimulated vas.

6 It is suggested that when barium is used, the effects of agents acting on β -adrenoceptors are mediated only by postsynaptic β_2 -receptors, while other complicating factors, probably nerve-dependent presynaptic mechanisms, may be involved with electrical stimulation.

Keywords: β -adrenoceptors; vas deferens; functional antagonism; barium chloride; isoprenaline; noradrenaline; adrenaline; propranolol; practolol; isopropylmethoxamine

Introduction

Previous studies have shown the presence of β_2 -adrenoceptors in rat vas deferens, mediating a relaxation of smooth muscle. Almost all the results are based on functional antagonism against electrically-induced twitches (Ganguly & Bhattacharya, 1969; Vohra, 1979; Lotti *et al.*, 1980; Diaz-Toledo & Jurkiewicz, 1980; 1990).

When the reported publications are analysed on the grounds of the two standard pharmacological procedures for receptor classification, namely the determination of the relative potencies of a series of agonists, and the determination of the potencies of competitive antagonists (Furchgott, 1972), at least two groups of incongruities are apparent: (a) there are very few quantitative informations about adrenaline (Ad) and noradrenaline (NA). As a consequence, our knowledge about the rank order of potency for Ad, NA and isoprenaline (Iso), which is one of the main tools for adrenoceptor classification, is still fragmentary. Although Vohra (1979) studied both agonists in the presence and absence of selective and non-selective β -blockers, concentration-response curves and determinations

of drug-receptor parameters were not performed, precluding a quantitative analysis and receptor characterization. (b) Data obtained by the use of the competitive antagonist propranolol vs. isoprenaline suggested that the β -agents could be interacting with two receptors (Lotti *et al.*, 1982). In addition, propranolol was also studied by Krstew *et al.* (1982), who were unable to antagonize the effects of NA with this drug. Similar results were reported by May *et al.* (1985) for propranolol against Ad and NA.

The objective of the present study was to obtain quantitative information about the effects and relative potencies of Ad, NA, and Iso, and about the potencies of β -adrenoceptor antagonists. Propranolol and practolol were used respectively as non-selective and β_1 -antagonists (Daly & Levy, 1979), and isopropylmethoxamine (IMA) as a β_2 -antagonist (Furchgott, 1972). Because of some atypical effects of these agents against electrically-induced twitches, we decided to repeat the experiments by using barium chloride as another functional antagonist.

Methods

Adult male Wistar rats (300–350 g, 6-months old), from our own colony, BAW-2 (Festing, 1980), were pretreated with reserpine (10 mg kg⁻¹, 24 h before death), and killed by a

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blow on the head. The vasa deferentia were removed, cleared of surrounding tissues, and mounted, under 1 g tension, in 10 ml organ baths containing heated (30°C) and aerated physiological solution of the following composition (mM): NaCl 138, KCl 5.7, CaCl₂ 1.8, NaH₂PO₄ · H₂O 0.36, NaHCO₃ 15.0 and dextrose 5.5. The isotonic responses to agonists were recorded with tangential writing levers, with a 6 fold amplification.

Concentration-response curves

Pretreatment of the isolated vas deferens In general, when β -agonists were added to the resting vas deferens, no relaxation was observed, except when a tonus was present, caused by an agonist, as for instance barium chloride, or by sequential electrically-induced twitches. In the latter case, Iso, Ad, and NA elicited a dose-dependent decrease of the amplitude of twitches, without affecting the baseline (Diaz-Toledo & Jurkiewicz, 1980; 1990). However, if the concentrations of these agonists were further increased, an undesirable rise in the baseline occurred, due to a well-known concentration-dependent interaction with postsynaptic α -adrenoceptors (Jurkiewicz & Jurkiewicz, 1976); the minimum concentrations of these agonists to start such an increase in the baseline were about 3×10^{-7} M for Ad and NA, and 3×10^{-5} M for Iso (Diaz-Toledo, 1983). To overcome this problem, α -adrenoceptors had to be inactivated in an essentially irreversible manner by a β -haloalkylamine. A single exposure to these agents is able to cause a persistent blockade of the contractile effects of noradrenaline, and also of dopamine, in rat vas deferens (Langeloh & Jurkiewicz, 1982; Busatto & Jurkiewicz, 1985). In the present work we used dibenamine as the alkylating agent (Furchgott & Bursztyn, 1967). This type of pretreatment of the isolated vas, which also included an inhibition of neuronal and extraneuronal uptake, since uptake is supposed to reduce the drug concentration in the vicinity of receptors (Furchgott, 1972; Furchgott *et al.*, 1974) was performed to avoid major sources of error in the estimation of drug-receptor parameters.

After a 30 min equilibrium period, the organs were incubated with dibenamine (10 μ M) for 30 min and then washed for 40 min. After this procedure, it was observed, in a group of control experiments, that Ad and NA were unable to cause any contraction of the vas, up to concentrations as high as 10^{-3} M, indicating the inactivation of postsynaptic α -adrenoceptors by dibenamine (Diaz-Toledo, 1983). As an additional control, it was observed that the presynaptic α -adrenoceptor agonist, clonidine, was unable to induce inhibitory effects upon electrically-induced twitches, when added up to a concentration of 3×10^{-5} M, while in preparations which were not treated with dibenamine, a complete inhibition of twitches was observed with a dose of clonidine about 300 times lower (Diaz-Toledo, 1983). To block neuronal uptake, cocaine (10 μ M) was added to the bath (Jurkiewicz & Jurkiewicz, 1976), 30 min before the beginning of concentration-response curves, and maintained thereafter. In relation to extraneuronal uptake, other drugs were not used, since dibenamine is known to be an irreversible blocker of this type of removal mechanism (Furchgott, 1972; Furchgott *et al.*, 1974).

The inhibitory effects of β -adrenoceptor agonists were studied in two series of experiments, on contractions elicited by electrical field stimulation, or by barium chloride, respectively.

Field stimulation The tissues were mounted between parallel platinum electrodes and continuously field stimulated with submaximal pulses of 35 V, 3 ms and 0.07 Hz. When twitches of constant amplitude were attained, cumulative concentration-response curves were obtained as described by Van Rossum (1963), by increasing agonist concentrations by steps of about 3 (i.e., 1; 3; 10; 30; etc). The inhibitory effects on the electrically evoked contractions were expressed as a percentage of the initial amplitude of twitches.

Barium chloride Barium chloride (300 μ M) was used to induce a contraction of rat vas. This contraction, corresponding to less than 50% of barium maximal effect, usually reached a sustained plateau after about 2 min. When the response was steady, a single dose of agonist was added to the bath, and washed out after 2 min. This procedure was repeated at 20 min intervals, by adding the next doses of agonist, that were increased non-cumulatively by a factor of 10 (i.e., 1; 10; 100; etc). Mean concentration-response curves were obtained by measuring the inhibitory effects of the β -adrenoceptor agonist on barium-induced contractions. The effects were expressed as percentages of the barium-induced contraction.

Pharmacological parameters

pK_B determination In both experimental procedures, after determination of control concentration-response curves, rat vasa deferentia were incubated with competitive antagonists for 30 min. Then, concentration-response curves were obtained in the presence of an antagonist. Only two concentrations of each antagonist were used in each organ, to avoid tissue desensitization. The latter could also be detected by appropriate controls, in which the curves for agonist were repeated in the absence of antagonist. The pK_B value is assumed to have a theoretical significance, as an estimate of the drug-receptor dissociation constant ($-\log K_B$), and was determined directly from the equation based on the mass law (Arunlakshana & Schild, 1959; Furchgott *et al.*, 1974):

$$-\log K_B = \log(DR - 1) - \log[B]$$

using the values for each concentration of competitive antagonist, [B], and the corresponding dose-ratio for agonist, DR, according to the analytical method described by Mackay (1978). In the case of a typical competitive antagonism, the value of pK_B is expected to be constant in a given experiment, even by increasing the dose of antagonist, since according to the equation above, an increase of the dose of antagonist is expected to cause a proportional increase of $\log(DR - 1)$. If this criterion was not fulfilled, thus indicating a lack of theoretical significance, then the corresponding value of pK_B was not quoted.

pD_2 value The values of pD_2 were determined as $-\log ED_{50}$ by linear regression analysis, using all points between 20% and 80% of the maximal response.

Relative potency This parameter was determined as a ratio between ED_{50} values of agonists and that for NA. Thus, the value of 1.0 was arbitrarily assigned to the relative potency of NA.

Drugs

The following drugs were used: (+)-isoprenaline hydrochloride, (–)-noradrenaline ((–)-arterenol) hydrochloride, (–)-adrenaline ((–)-epinephrine) bitartrate, (±)-propranolol hydrochloride (all from Sigma Chemical Co. U.S.A.). Dibenamine hydrochloride (K & K lab. Inc., U.S.A.), cocaine (Merck, West Germany) and reserpine (Ciba-Geygi, Brazil) were also used. Practolol was kindly supplied by Dr B.B. Vargaftig (I. Pasteur, Paris), and isopropylmethoxamine by Prof. R.F. Furchgott (SUNY, U.S.A.).

Statistics

Results are expressed as mean values $\pm$ s.e. means. Student's *t* test, analysis of variance and Newman-Keuls' test were used for statistical comparisons. The significance level was considered as $P < 0.05$.

Results

Drug action in electrically stimulated vas

Figure 1 shows concentration-response curves of inhibitory effects of Iso, Ad, and NA on field-stimulated rat vas deferens. The three catecholamines produced concentration-dependent inhibition of the twitch responses and the maximal inhibition was nearly 100% of the twitch. From these data the pD_2 values and relative potencies were determined (Table 1). The concentration-response curves for Iso, Ad and NA, in the absence and presence of propranolol, are shown in Figure 2. The competitive antagonist induced a dose-dependent (10^{-8} M, 10^{-7} M) shift to the right of the concentration-response curve of isoprenaline, as expected from receptor theory. However, propranolol induced an unexpectedly small shift of concentration-response curves of Ad, even with larger doses than in the previous figure (10^{-7} M to 10^{-6} M). Similarly, propranolol (10^{-6} M, 10^{-5} M) was unable to shift the concentration-response curves of NA. When the antagonists were IMA (Figure 3), or practolol (Figure 4), a similar pattern against Iso, Ad, and NA was observed to that obtained by the use of propranolol. Table 1 shows the pK_B values of the different antagonists against the three agonists.

Drug action in rat vas stimulated by barium chloride

Figure 5 illustrates a typical experiment in the barium-stimulated vas deferens, in which the inhibitory effects of isoprenaline were tested in the absence and presence of propranolol. Figures 6, 7, and 8, show concentration-response curves for Iso, Ad and NA, in the absence and presence of two

Figure 1 Concentration-response curves obtained in field-stimulated rat vas deferens, for isoprenaline (●), adrenaline (○), and noradrenaline (Δ). The inhibitory effects are plotted as percentages of the amplitude of electrically-induced twitches immediately before adding the agonist. Vertical lines represent s.e.mean. Each curve is a mean of at least 8 experiments.

Figure 2 Concentration-response curves obtained in field-stimulated rat vas deferens: (a) for isoprenaline in the absence (○) and presence of propranolol 10^{-8} M (●) and 10^{-7} M (Δ); (b) for adrenaline in the absence (○) and presence of propranolol 10^{-7} M (●) and 10^{-6} M (Δ); and (c) for noradrenaline in the absence (○) and presence of propranolol 10^{-6} M (●) and 10^{-5} M (Δ). Vertical lines represent s.e.mean. Each curve is a mean of at least 8 experiments.

doses of respectively propranolol, IMA, and practolol, obtained from experiments similar to that shown in the previous figure. In contrast with Figures 2, 3, and 4, the shifts of concentration-response curves did not depart from the expectations from Arunlakshana & Schild's equation (1959), whichever agonist or antagonist was used, indicating a typical competitive antagonism. The pD_2 values for the relaxant effects of agonists, and the respective potency ratios (Table 2) were increased in relation to Table 1, but the rank order of potency was maintained. The pK_B values of propranolol, IMA and practolol, determined against Iso, Ad and NA, from the above experiments, are shown in Table 2. It is noteworthy

Table 1 Values of pD_2 and relative potencies (Rel Pot) for agonists and pK_B for antagonists, for relaxation mediated by β-adrenoceptors in electrically-stimulated rat vas deferens

Agonist	pD_2	Rel Pot	pK_B for antagonist		
			Propranolol	IMA	Practolol
(±)-Iso	7.25 ± 0.08	29	8.70 ± 0.07	6.70 ± 0.07	5.01 ± 0.04
(-)-Ad	7.10 ± 0.06	21	ND	6.10 ± 0.09	4.60 ± 0.13
(-)-NA	5.79 ± 0.08	1	ND	ND	ND

(±)-Iso, (±)-isoprenaline; (-)-Ad, (-)-adrenaline; (-)-NA, (-)-noradrenaline; IMA, isopropylmethoxamine.
n = at least 8 for each pD_2 and pK_B value. ND = not determined.

Figure 3 Concentration-response curves obtained in field-stimulated rat vas deferens: (a) for isoprenaline, (b) for adrenaline and (c) for noradrenaline in the absence (○) and presence of isopropylmethoxamine 10^{-6} M (●) and 10^{-5} M (△). Vertical lines represent s.e. mean. Each curve is a mean of at least 8 experiments.

that the pK_B values were not significantly modified by interchanging the agonists, indicating an interaction with a homogeneous receptor population.

Discussion

The results obtained either in field-stimulated vas deferens or in organs contracted by barium chloride, support previous findings that the relaxation induced by β -adrenoceptor agonists are mediated by β_2 -adrenoceptors (Ganguly & Bhattacharya, 1969; Vohra, 1979; Lotti *et al.*, 1980; Diaz-Toledo & Jurkiewicz, 1980; 1990). This was indicated, according to Lands *et al.* (1967), by the rank order of potencies for the agonists (Iso > Ad $\gg$ NA), when the organ was electrically stimulated (Table 1). The individual values of pD_2 were changed in barium-stimulated vas, leading to a relative

Figure 4 Concentration-response curves obtained in field-stimulated rat vas deferens: (a) for isoprenaline, (b) for adrenaline and (c) for noradrenaline in the absence (○) and presence of practolol 5×10^{-5} M (●) and 5×10^{-4} M (△). Vertical lines represent s.e. mean. Each curve is a mean of at least 8 experiments.

increase for isoprenaline and adrenaline potencies but the overall hierarchy was not substantially modified. In addition, the pK_B value for practolol was not very different from 5.0 in all circumstances, corroborating the presence of β_2 -adrenoceptors, since values higher than 6.5 would be expected for interactions with β_1 -adrenoceptors, as observed in several preparations (Daly & Levy, 1979).

In spite of the similarity of both rank orders of potency, the individual differences on relative potencies and pK_B values (Tables 1 and 2) are an outstanding complication, indicating that the effects induced by β -agonists are mediated by different mechanisms, depending on whether the functional antagonist is barium or electrical stimulation. Furchgott (1972)

Table 2 Values of pD_2 and relative potencies (Rel Pot) for agonists and pK_B for antagonists, for relaxation mediated by β -adrenoceptors in barium-stimulated rat vas deferens

Agonist	pD_2	Rel Pot	pK_B for antagonist		
			Propranolol	IMA	Practolol
(±)-Iso	8.25 ± 0.13	812	9.96 ± 0.13	7.28 ± 0.08	5.12 ± 0.12
(-)-Ad	7.38 ± 0.15	109	10.10 ± 0.06	7.30 ± 0.09	5.07 ± 0.08
(-)-NA	5.34 ± 0.15	1	9.82 ± 0.11	7.39 ± 0.08	4.90 ± 0.12

Abbreviations as in Table 1.
 n = at least 8 for each pD_2 and pK_B value.

Figure 5 Typical kymograph recording of the relaxation induced by isoprenaline in contractions evoked by barium chloride ($300\ \mu\text{M}$) in rat isolated vas deferens. Isoprenaline was added at dots, and washed out after about 2 min (thin arrows). Intervals between doses were 20 min. Records were obtained in the absence (upper traces) or presence (lower traces) of propranolol ($10^{-8}\ \text{M}$), added 30 min before barium. In general, 3 to 5 doses of agonist/curve, and 2 doses of antagonist were used in each experiment, except when high doses of noradrenaline (above $10^{-3}\ \text{M}$) were needed (see Figures 6, 7 and 8). Isoprenaline concentrations are indicated as $-\log$ molar concentrations.

suggested that a greater than three fold difference, in the case of the relative potencies for the same agonist, or a difference of greater than 0.5 in the case of the pA_2 values for the same antagonist, should be considered preliminary evidence for different types of receptors and that a combination of two or more of such differences should be considered strong evidence. In our experiments, a difference larger than three fold was found for the relative potencies of isoprenaline (29:812) and adrenaline (21:109). In addition, the value of pK_B for propranolol against isoprenaline was about 1.3 log units greater, and for IMA almost 0.6 log units greater, when barium was used

Figure 6 Concentration-response curves obtained in rat deferens contracted by barium chloride $300\ \mu\text{M}$: (a) for isoprenaline, (b), for adrenaline, and (c) for noradrenaline in the absence (○) and presence of propranolol $10^{-8}\ \text{M}$ (●) and $10^{-7}\ \text{M}$ (Δ). Vertical lines represent s.e.mean. Each curve is a mean of at least 8 experiments.

Figure 7 Concentration-response curves obtained in rat vas deferens contracted by barium chloride $300\ \mu\text{M}$: (a) for isoprenaline, (b) for adrenaline and (c) for noradrenaline, in the absence (○) and presence of isopropylmethoxamine $10^{-6}\ \text{M}$ (●) and $10^{-5}\ \text{M}$ (Δ). Vertical lines represent s.e.mean. Each curve is a mean of at least 8 experiments.

as the functional antagonist. Therefore, these results strongly indicate that relaxations induced in both cases are mediated by different receptor populations.

The experiments with barium, unlike those with electrically-induced twitches, clearly indicated the presence of a homogeneous receptor population. The strongest indication stems from the pK_B values, obtained for each β -blocker, which were independent of the agonist used (Table 2). This is a *sine-qua-non* condition for receptor homogeneity, based on expectations from receptor theory (Arunlakshana & Schild, 1959), as well as on results obtained in a series of pharmacological preparations (Furchgott, 1972; Daly & Levy, 1979).

The atypical results observed when electrically-induced twitches were used instead of barium, indicate the presence of distorting factors. This is probably due to the complexity of the events leading from transmural stimulation to contraction. The involvement of nerve terminals can be demonstrated by the use of tetrodotoxin, which inhibits the electrically-induced twitch in vas (Krstew *et al.*, 1982; May *et al.*, 1985). Electrically-induced twitches depend on the release of reserpine-resistant neurotransmitters, followed by an interaction of these transmitters with muscle receptors. The reduction of the amplitude of twitches, mediated by β -receptors, is supposed to be due to a blockade of this chain of events. On the basis of this assumption it can be hypothesized that two populations of receptors, one of them presynaptic, located in nerve cells, and the second postsynaptic, in muscle cells, would be responsible for the relaxation caused by β -agonists. An interaction with presynaptic receptors would provoke a perturbation of nerve activity, and as a consequence, of transmitter release and contractions. In addition, postsynaptic β -receptors would antagonize the contraction induced by the transmitter-receptor interaction in smooth

Figure 8 Concentration-response curves obtained in rat vas deferens contracted by barium chloride 300 μ M: (a) for isoprenaline, (b) for adrenaline and (c) for noradrenaline, in the absence (○) and presence of practolol 5 $\times 10^{-5}$ M (●) and 5 $\times 10^{-4}$ M (Δ). Vertical lines represent s.e.mean. Each curve is a mean of at least 8 experiments.

muscle. In barium-stimulated vasa, the interaction of β -agonists with nerve receptors is useless, because the effect of barium is independent of transmitter release. In other words, the presynaptic receptors would also be reached by β -agonists, but this would result in an ineffective, or 'silent' interaction (Jurkiewicz *et al.*, 1971). Therefore, only the interaction of β -agonists with the postsynaptic β_2 -adrenoceptors would be effective in barium-stimulated organs.

At least three other publications have advanced the possibility of multi-receptor interaction by β -agonists in the vas: Lotti (1983) suggested the presence of a postsynaptic β_2 -adrenoceptor and of a presynaptic β -adrenoceptor, based on anomalous lines obtained in Schild plots, while Krstew *et al.* (1982) and May *et al.* (1985) proposed an interaction with α -adrenoceptors. In relation to our experiments, it is unlikely that α -adrenoceptors are involved, because of at least two pieces of experimental evidence: (a) the inactivation of these receptors by dibenamine was endorsed by the fact that clonidine, a typical presynaptic agonist, was unable to cause a relaxation of the electrically-stimulated vas, as described in Methods, and (b) the difference in pD_2 values, when compar-

ing electrically-stimulated with barium-stimulated vasa, was found to be largest for Iso (compare Tables 1 and 2); if α -adrenoceptors were responsible for this difference, one would expect the pD_2 values for NA and Ad to be most affected, because of their higher affinities for α -adrenoceptors.

Although many precautions have been taken to ensure satisfactory experimental conditions and to exclude the influence of α -adrenoceptors, neuronal and extraneuronal uptake, and release of endogenous noradrenaline, we cannot be sure that this receptor population in nerve tissue, if present, is of β -receptors. In fact, Furchgott (1972) has shown that receptors which cannot be characterized as either α - or β -adrenoceptors can also interact with the so-called β -agonists. From the standpoint of adrenergic transmission, there are a number of publications describing, besides the well-known presence of catecholamines and ATP, the existence, or release, of [Met]enkephalin, neuropeptide Y, and calcitonin gene-related peptide, in vas deferens of different animal species (DeP Potter *et al.*, 1987; Maggi *et al.*, 1987; Kasarov *et al.*, 1988). Therefore, this two-adrenoceptor hypothesis can only be considered a suggestion rather than conclusive. We believe that a clear answer to this question will only be obtained after a better understanding of at least three inter-related pertinent issues: (a) the role, releasing mechanisms, and interactions of the endogenous substances present in nerve terminals of the vas deferens; (b) a model for functional antagonism (Mackay, 1981) accounting for the hypothesis that we have a double site of action for the β -agents; and (c) a model for competitive antagonism explaining the changes observed for pD_2 and pK_B values (Tables 1 and 2) and the anomalous shifts of dose-response curves due to competitive antagonists (Figures 2, 3, and 4). Although some models have been published in the past (see, for instance, Furchgott *et al.*, 1974) they do not account for the experimental conditions involved here. On the other hand, the presence of two different sites interacting with β -agonists and antagonists has been clearly shown by means of non-functional methods, such as radioligand binding assays (May *et al.*, 1985).

The high pK_B value for propranolol, obtained in organs contracted by barium chloride (Table 2), is in agreement with the pA_2 value obtained by Daly & Levy (1979) in rat aorta (9.45), using the same contracting agent. In relation to the high values for the relative potency of isoprenaline (Table 2), corresponding to a difference larger than 2.5 log units between pD_2 values for Iso and NA, it is noteworthy that when Iso and NA were used against barium in rat aorta and in guinea-pig uterus, similar values were obtained (Daly & Levy, 1979). Further facts on the use of barium chloride as a functional antagonist against other β -agents in vas deferens, have not been reported, to our knowledge. In relation to the pK_B values for propranolol, IMA and practolol against isoprenaline in field-stimulated vas, our data agree with results previously presented for other preparations (Furchgott, 1972; Daly & Levy, 1979).

In summary, it is concluded that the inhibitory effects of β -adrenoceptor agonists on rat vas deferens contracted by barium chloride are mediated by β_2 -adrenoceptors. In addition, it is suggested that the inhibitory effects in electrically stimulated vas are mediated by the same receptors, in addition to a second receptor population, the main characteristics of which are still unknown.

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