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Some Pharmacological Properties of the Circular Smooth Muscle Layer of the Rat Vas Deferens

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Abstract—Vas deferens preparations were perfused in-vitro through the lumen and externally with a modified Tyrode solution alone or containing drugs. Contractions of the circular (internal) smooth muscle layer were recorded as changes in the pressure of internal perfusion. Contractions of the longitudinal (external) layer were simultaneously recorded through a tension transducer. When the organ was perfused through the lumen, the circular layer contracted after addition of methacholine ($pD_2 = 4{\cdot}13$), and noradrenaline ($pD_2 = 5{\cdot}00$), and relaxed after addition of isoprenaline ($pD_2 = 5{\cdot}22$). These effects were also observed when the drugs were perfused externally, although with lower values of pD_2 for noradrenaline and methacholine. The circular fibres were less sensitive when compared with the longitudinal fibres perfused externally with the above agonists. Methacholine-induced contractions of the circular layer were competitively antagonized by atropine ($pA_2 = 8{\cdot}53$), indicating the presence of muscarinic receptors. The effects induced by noradrenaline and isoprenaline were antagonized by indoramin ($pA_2 = 7{\cdot}78$), and timolol ($pA_2 = 8{\cdot}68$), respectively, indicating the presence of α- and β-adrenoceptors. The effect of noradrenaline was potentiated by cocaine and denervation, indicating the presence of neuronal uptake, and by corticosterone, indicating the presence of extraneuronal uptake in the circular layer.

It is surprising that much fundamental information is still lacking on the pharmacology of the circular smooth muscle of the vas deferens. The importance of this layer can be drawn from the fact that more than 50% of the wall of the vas deferens can be composed of circular fibres (Jurkiewicz et al 1977). From the physiological standpoint, the role of these fibres as a means for propulsion of spermatic fluid is also recognized (Batra 1974; Guha et al 1975).

Most of the pharmacological studies of the vas deferens are based on contractile responses to drugs, or to electrical stimulation, involving lengthwise mounting of the organ in a tissue bath, the vas being attached at one end to an isotonic or isometric recording system. Contractions of such a preparation are measured as a shortening of the organ, due to the shortening of longitudinal muscle fibres. Contractions of the cells of the circular layer are not supposed to alter the length of the vas (Busatto & Jurkiewicz 1985).

By the use of different procedures, the circular muscle has been previously investigated by Anstey (1971), Gosling & Dixon (1972), Dixon & Gosling (1972), Anton & McGrath (1977), Anstey & Birmingham (1978, 1980), Busatto & Jurkiewicz (1985) and Souza Brito & Jurkiewicz (1984). Those authors studied mainly the innervation, contractile characteristics, and the presence of α-adrenoceptors, in the organ. From their results, Anstey & Birmingham (1978) concluded that it is possible to record the response of the circular muscle of the guinea-pig vas deferens by techniques formerly used for other tubular organs containing smooth muscle. Busatto & Jurkiewicz (1985) described a method for perfusing the vas deferens internally and externally and simultaneously recording the contractions of the longitudinal and circular muscle layers. The main concern of the latter publication, in relation to the circular muscle, was to analyse this layer as a diffusion barrier for drugs to reach the longitudinal layer. Therefore, less attention was paid to its contractile response to drugs.

In spite of the publications reported above, much fundamental information is still lacking about the pharmacological properties of the circular layer; for instance, it is still unknown if this preparation has cholinergic receptors, since Anstey & Birmingham (1980) were unable to elicit contractions by using acetylcholine and other related agonists. In relation to the sympathetic system it is still unknown if the circular layer has β-adrenoceptors. There is no clear information about the presence, or not, of neuronal uptake, although it was demonstrated that cocaine increases the duration of the contractile response to transmural stimulation (Anstey & Birmingham 1980). Information about extraneuronal uptake is also absent. In addition, quantitative data as for instance pD_2 values for adrenergic and cholinergic agonists, and pA_2 values for the corresponding antagonists also ought to be determined. It is also unknown if the sensitivity of the circular layer, expressed through these parameters is, or is not, the same when the drugs are perfused through the lumen or externally, although some information has already been provided previously (Busatto & Jurkiewicz 1985). Finally, comparisons of drug parameters determined in the circular layer with those in the longitudinal layer are also missing.

The main purpose of the present investigation is to obtain an initial quantitative information about the questions raised above, by using agonists and antagonists interacting with receptors of the parasympathetic and sympathetic systems, as well as drugs known to influence removal mechanisms of the latter system (Furchgott 1972).

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Methods

Animals

Pairs of vasa deferentia were removed from male adult Wistar outbred albino rats from our own colony, BAW-2 (Festing 1980), 250–300g, which were killed by ether inhalation. The vas deferens was cleared of the surrounding tissues and of its luminal secretion, with a nutrient solution of the following composition (mM): NaCl 138; KCl 5·7; $CaCl_2$ 1·8; NaH_2PO_4:H_2O 0·36; $NaHCO_3$ 15·0 and dextrose 5·5, prepared in glass distilled, deionized water, and bubbled with air.

Perfusion system

The perfusion system allowing the simultaneous recording of contractions of circular and longitudinal muscle layers of the vas deferens has been described previously by Busatto & Jurkiewicz (1985). In summary, two 10-gauge stainless steel cannulae were introduced into the lumen of the vas deferens, at the prostatic and epididymal ends, respectively. The first one served as an inlet, whereas the latter served as an outlet for the internal perfusion fluid. The vas deferens was firmly attached to the lower end of the glass chamber, through the prostatic cannula. The epididymal cannula was provided with a small hook, which was connected to a transducer for recording isometric contractions of the longitudinal layer. Changes in fluid resistance, due to circular muscle contractions, were recorded by a pressure transducer adapted to the prostatic tube. The flow rate was maintained constant at 2 mL min^{-1} and at a temperature of 30 ± 1°C. Simultaneously, the nutrient solution was perfused through the external surface of the vas by means of an independent channel of the perfusion pump. An initial equilibration period of 20–40 min was allowed before starting the experiment.

Dose-response curves of agonists

Drugs were added to the nutrient solution and perfused through the lumen. In a few experiments drugs were also perfused externally. The effects of various doses of methacholine (acetyl-β-methylcholine), noradrenaline, isoprenaline and adrenaline were studied. The perfusion time of agonists was 10 min and the interval between two applications was 30 min. In general, up to 14 doses could be given during a single experiment. Dose-response curves were drawn for each experiment for graphic interpolation of pD_2 values (Jurkiewicz et al 1977).

Blockade of uptake mechanisms

Neuronal uptake was blocked with cocaine (10^{-5}M), which was applied by the same route as noradrenaline, i.e., internally. Cocaine was perfused 10 min before and during the perfusion of noradrenaline. The same procedure was used for the extraneuronal uptake blocker corticosterone, the preliminary perfusion time for this drug being 30 min.

Denervated preparations (Kasuya et al 1969), which are devoid of neuronal uptake, were used instead of cocaine-treated organs in some of the experiments. In this case, 7-day denervated vasa deferentia and normal controls were perfused through the lumen with noradrenaline. Before being used, the organs were tested for denervation with tyramine (10^{-5}M), and discarded if a contraction was observed.

The changes induced in noradrenaline potency by these procedures were measured as dose-ratios (DR) calculated as the antilog of differences between pD_2 values.

Use of competitive antagonists

All the antagonists were applied by the same route as the agonists, i.e. internally, 15 min before and during the 10 min perfusion of agonists. A series of about six different single doses of the agonist was given to obtain individual control curves, and repeated in the presence of competitive antagonists. Control experiments were made by repeating the series of doses of agonist in the absence of antagonist. No significant variations of sensitivity were detected for the four agonists used in control experiments. The same dose of antagonist was given throughout an individual experiment. The following antagonists were used: indoramin (3×10^{-8} and 3×10^{-7}M), as an α-adrenoceptor antagonist against noradrenaline; timolol (3×10^{-9} and 10^{-8}M), as a β-adrenoceptor antagonist against isoprenaline and adrenaline; atropine (10^{-8}, 3×10^{-8} and 10^{-7}M), as a muscarinic antagonist against methacholine.

The isoprenaline curves were obtained after an initial tonus induced by 3×10^{-3}M barium chloride. At the beginning of these experiments, the irreversible α-adrenoceptor blocker dibenamine (10^{-5}M) was perfused for 15 min.

The blockade induced by the competitive antagonists was expressed by means of log dose-response curves, which were shifted to the right along the dose axis. These shifts were also analysed by means of Schild plots (Arunlakshana & Schild 1959), from which the values of pA_2 were extrapolated.

Results

Effects of full agonists

Contractile effects of noradrenaline and methacholine, and the inhibition of barium chloride contractions by isoprenaline are shown in Fig. 1. Maximal effects were clearly reached with lower doses when noradrenaline and methacholine were

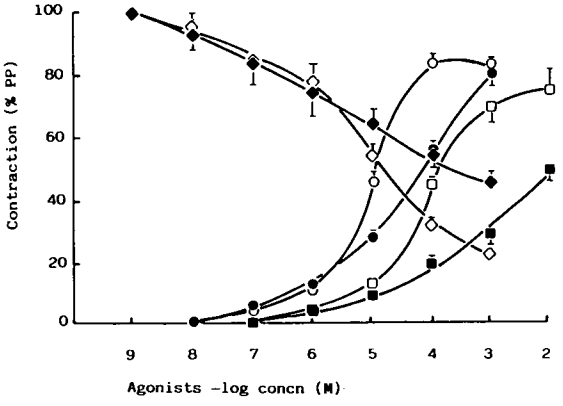

FIG. 1. Effect of methacholine (□,■), noradrenaline (○,●) and isoprenaline (◊,♦) on the circular smooth muscle of the rat vas deferens, after perfusing the agonists through the lumen (open marks) or externally (full marks). Contractions are expressed as percent values of the effects (PP = perfusion pressure) induced at the beginning of each experiment by a maximal dose (10^{-2}M) of barium chloride. Relaxation induced by isoprenaline is expressed as percent value of the initial tonus induced by 3×10^{-3} M barium chloride. Each point is a mean of at least 7 experiments. Vertical lines show the s.e.m.

perfused through the lumen instead of externally. In addition, according to the respective pD_2 values (Table 1), the circular layer showed a 5 to 7 fold difference in sensitivity according to the route of perfusion, with best responses when these agonists were applied internally. In the case of isoprenaline, the maximal relaxation was also more pronounced after internal perfusion. A relatively large fluctuation of pD_2 values was observed when the β-agonist was perfused externally (Table 1), and no significant differences could be detected, in relation to internal perfusion. From these results, since in general the circular smooth muscle was more sensitive to drugs applied by internal than by external perfusion, most of the experiments were made using the internal route.

Table 1 also shows pD_2 values for the longitudinal layer. This layer was more sensitive to all the agonists studied, if the drugs were applied externally.

Effects of competitive antagonists

Competitive antagonists were used to obtain additional information about the receptors interacting with the agonists listed in Table 1. The dose-response curves obtained in the presence of antagonists are shown in Figs 2, 3. Atropine induced a parallel shift to the right of the dose-response curves of methacholine (Fig. 2A). Similar shifts were induced by indoramin in relation to noradrenaline (Fig. 2B), and by timolol in relation to isoprenaline (Fig. 3A). In the latter case, although the maximal effects of the agonist were not clearly reached after timolol, the shifts were parallel at the dose range used for isoprenaline. The pA_2 values, calculated from these experiments, are shown in Table 2. Contrary to the results observed for pD_2 values (Table 1), no significant differences of pA_2 values were detected between circular and longitudinal smooth muscles for a given antagonist perfused internally (Table 2).

Experiments with adrenaline and timolol show that the β-antagonist potentiated the effects of adrenaline (Fig. 3B). Concentration-effect curves of methacholine, used as controls, were not shifted by timolol (not shown), indicating that the effect of timolol was specific for adrenaline.

A slight autoinhibitory effect was observed for methacholine, noradrenaline and adrenaline control curves (Figs 2A, 2B, 3B). In addition, the maximum effects were slightly depressed when higher doses of agonist were used in the presence of competitive antagonists. The reasons for these results not yet have been investigated.

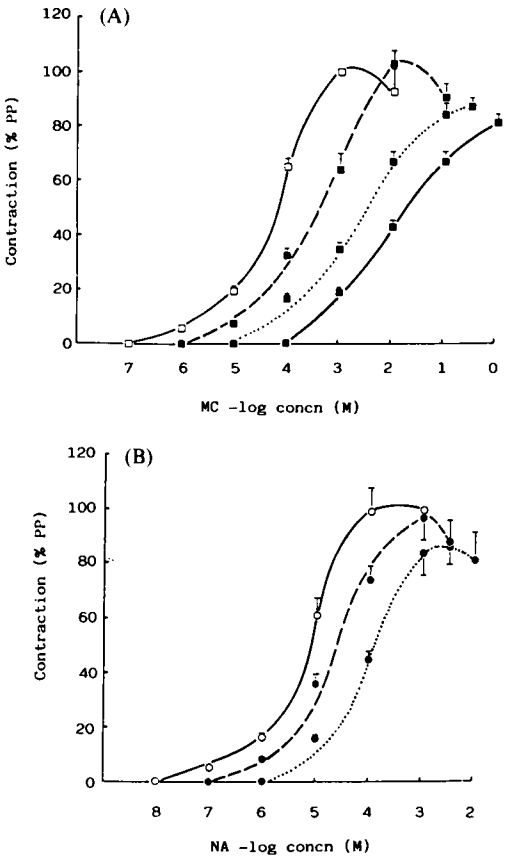

FIG. 2. (A) Mean concentration-effect curves on the circular smooth muscle of the rat vas deferens, after internal perfusion of methacholine (MC), before (□) and in the presence of the competitive antagonist atropine (■) at the concentrations of 10^{-8} M (broken line), 3×10^{-8} M (dotted line) and 10^{-7} M (full line). Effects (PP = perfusion pressure) are mean percent values of the maximal effect shown in the first curve of methacholine. (B) Mean concentration-effect curves for noradrenaline, before (○) and in the presence of the competitive antagonist indoramin (●) at the concentrations of 3×10^{-8} M (broken line) and 3×10^{-7} M (dotted line). Effects are mean percent values of the maximal effect shown in the first curve of noradrenaline. Each point represents the mean of 6 experiments. Vertical lines show the s.e.m.

Table 1. Values of pD_2 determined respectively for the circular and longitudinal smooth muscle layers of the rat vas deferens, for methacholine (MCA), noradrenaline (N) and isoprenaline (IPNA), perfused through the lumen or externally. Data represent mean ± s.e.m.

		Circular Muscle		Longitudinal Muscle	
	n	Internal Drug	External Drug	Internal Drug	External Drug
MCA	7	4·13 ± 0·07	3·27 ± 0·23*	3·49 ± 0·12	5·27 ± 0·18‡‡
N	16	5·00 ± 0·08	4·28 ± 0·09*	4·17 ± 0·12†	5·48 ± 0·12‡‡
IPNA	7	5·22 ± 0·25	5·85 ± 0·49	5·04 ± 0·25	5·98 ± 0·07‡

*Significantly different from the respective value for internally perfused drug for circular muscle ($P < 0·001$).

†Significantly different from the respective value for the circular muscle layer ($P < 0·02$).

‡,‡‡Significantly different from the values for the circular layer, for the respective agonist perfused internally ($P < 0·01$ and $P < 0·001$, respectively).

The action of uptake blockers

Concentration-effect curves of noradrenaline were made in the absence and presence of cocaine or corticosterone. All the drugs were perfused by the internal route. In this case, the results obtained in the circular layer were also compared with those obtained simultaneously in the longitudinal muscle (Table 3).

Considering the circular muscle (Fig. 4A), the dose-response curve of the agonist was shifted to the left by the uptake blockers, resulting in an 8·9 and 3·8 fold increase in sensitivity, respectively (Table 3). However, the potentiation obtained in the longitudinal muscle after cocaine was more evident than in the circular layer (Table 3). In addition, the neuronal uptake blocker potentiated the effects of noradrenaline more than corticosterone, indicating that the extra-

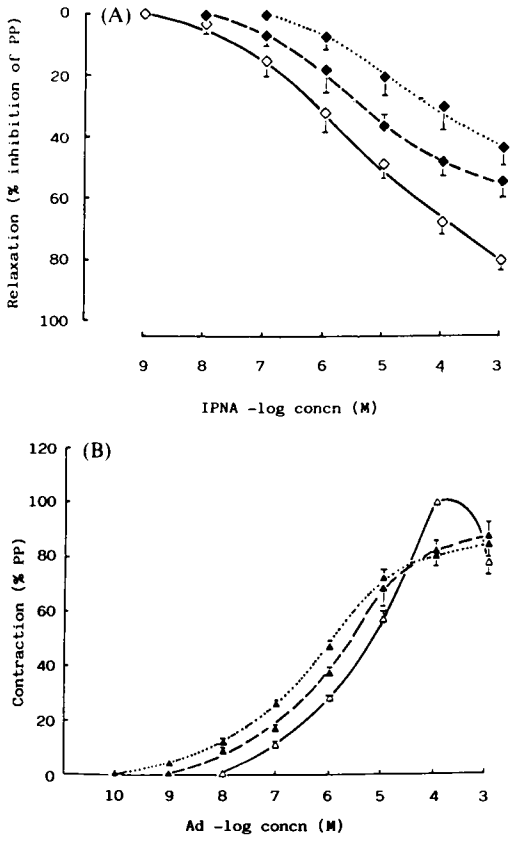

FIG. 3. (A) Relaxation induced by isoprenaline in the circular smooth muscle of the rat vas deferens, contracted by barium chloride (3×10^{-3} M), in the absence (◊) and presence of the competitive antagonist timolol (♦), at the concentrations of 3×10^{-9} M (broken line) and 10^{-8} M (dotted line). (B) Contraction induced by adrenaline in the absence (△) and presence of the same doses of timolol (▲) indicated above (broken and dotted lines, respectively). Effects (PP = perfusion pressure) are mean percent values of the maximal effect shown in the first curve of adrenaline. All the drugs were administered through the lumen. Each point represents the mean of at least 6 experiments.

Table 2. Values of pA_2 obtained for the antagonists used in the experiments shown in Figs 2 and 3A. All the drugs were perfused through the lumen of the preparation. Data are means ± s.e.m.

	Circular muscle	Longitudinal muscle
Atropine	8·53 ± 0·14	8·68 ± 0·26
Indoramin	7·78 ± 0·18	7·83 ± 0·13
Timolol	8·68 ± 0·07	8·73 ± 0·08

neuronal uptake is less significant, in both muscle layers, as expected from previous experiments performed in the longitudinal layer (Langeloh & Jurkiewicz 1982).

The influence of denervation

Fig. 4B shows the results obtained in the circular smooth muscle after denervation. The dose-response curve of noradrenaline was shifted to the left when compared with that obtained in the normal contralateral vas deferens. This shift resulted in a 2·6 fold increase in sensitivity of the circular layer to noradrenaline (Table 4). However, this potentiation was less evident than in the longitudinal layer (8·3 fold).

Table 3. Values of $pD_2 \pm$ s.e.m. for noradrenaline (NA) and potentiation induced by 5×10^{-5} M corticosterone (CORT) or 10^{-5} M cocaine (COC) in the circular and longitudinal muscle layers of the vas deferens, obtained from the experiments shown in Fig. 4A. Drugs were perfused through the lumen.

	Circular muscle			Longitudinal muscle		
	pD_2	log DR	DR†	pD_2	log DR	DR†
NA (control)	5·16 ± 0·07	0·0	1·0	3·97 ± 0·05	0·0	1·0
CORT + NA	5·74 ± 0·02**	0·58	3·8	4·35 ± 0·09*	0·38	2·4
COC + NA	6·11 ± 0·15***	0·95	8·9	5·17 ± 0·17***	1·20	15·8

†The dose-ratio (DR) was calculated from log DR, which is the difference between the pD_2 values for the corticosterone-treated or cocaine-treated group and the corresponding control.

*, **, *** Significantly different from the corresponding control ($P < 0·05$, $P < 0·01$ and $P < 0·001$, respectively).

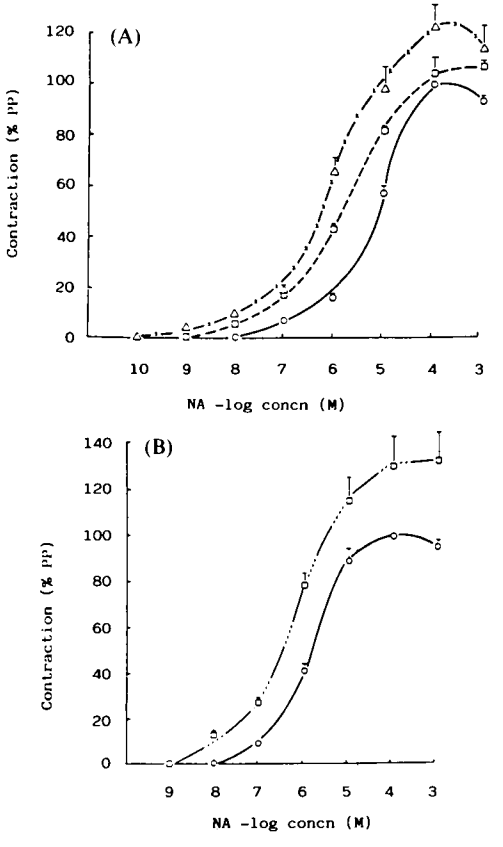

FIG. 4. (A) Mean concentration-effect curves on the circular smooth muscle of the rat vas deferens for noradrenaline in the absence (○) and presence of 5×10^{-5} M cocaine (△) or 10^{-5} M corticosterone (□). Effects (PP = perfusion pressure) are mean percent values of the maximal effect shown in the first curve of noradrenaline. The drugs were perfused by the internal route and each point represents the mean of 7 experiments. Vertical lines show the s.e.m. (B) Mean concentration-effect curves for noradrenaline in control (○) and denervated (□) vas deferens. The drugs were perfused by the internal route. Effects are mean percent values of the maximal effect shown in the control curve. Each point represents the mean of 7 experiments and vertical lines show the s.e.m.

Discussion

We have shown that the circular layer of the rat vas deferens responds quantitatively to the perfusion of methacholine, noradrenaline, isoprenaline, and their respective competitive

Table 4. Values of $pD_2 \pm$ s.e.m. for noradrenaline and potentiation induced by surgical denervation in the circular and longitudinal layers of the vas deferens, calculated from the experiments shown in Fig. 4B. Drugs were perfused through the lumen.

	Circular muscle			Longitudinal muscle		
	pD_2	log DR	DR†	pD_2	log DR	DR†
Control	$5{\cdot}65 \pm 0{\cdot}12$	0·0	1·0	$4{\cdot}51 \pm 0{\cdot}05$	0·0	1·0
Denervated	$6{\cdot}07 \pm 0{\cdot}17$*	0·42	2·6	$5{\cdot}43 \pm 0{\cdot}10$*	0·92	8·3

*Significantly different from the corresponding control value ($P < 0{\cdot}001$).

†The dose-ratio (DR) was calculated from the corresponding log DR, which is the difference between the pD_2 values obtained in denervated and control organs.

antagonists, characterizing the presence of muscarinic receptors, and of α- and β-adrenoceptors. Furthermore, the sensitivity to α-agonists was increased by cocaine or denervation, indicating the presence of neuronal uptake. In addition, the potentiation by corticosterone indicates the presence of extraneuronal uptake.

In some instances the values of pharmacological parameters were practically the same for both layers, as shown for instance in Table 2. This indicates that there are not striking differences between both layers in relation to the receptor systems here studied. The possible reasons for the small differences found, and for some discrepancies in relation to previous publications will be discussed below.

The circular layer contracted when a cholinergic agonist, methacholine, was perfused, contrary to the expectations derived from the publication of Anstey & Birmingham (1980), in which cholinergic agonists were reported to be inactive. This discrepancy can be due either to the different methods used, or to species-related sensitivity, since the latter authors used guinea-pig vasa deferentia. This would also explain why those authors could not obtain contractions of the circular layer when noradrenaline was added externally.

Regarding the adrenergic system, our main finding was related to the relaxation induced by isoprenaline and its antagonism by timolol, indicating the presence of β-adrenoceptors. As far as we know, this type of adrenoceptor has not been previously demonstrated in this layer. Further experiments are necessary, using other selective antagonists, to determine the subtype of β-receptor involved. Our results show that timolol shifts the curve of adrenaline to the left, indicating that this agonist interacts with both α- and β-receptors in the circular layer. The leftward shift would be due to the blockade of the negative component of adrenaline action, whereas the positive component represented by the interaction with α-adrenoceptors, would not be affected by the β-antagonist.

It was shown that neuronal uptake is slightly less effective in the circular than in the longitudinal layer (Table 3). This fact was corroborated by the results obtained in denervated preparations, whose sensitivity was increased more in the longitudinal than in the circular layer. In addition, extraneuronal uptake, was less conspicuous than neuronal uptake (Table 3), but was not significantly different between the two muscular layers. The potentiations induced by internally applied cocaine and by denervation were lower than that described for cocaine in a regular preparation of vas deferens, in which cocaine was applied externally (Jurkiewicz & Jurkiewicz 1976).

The circular layer was more sensitive than the longitudinal layer, provided that the drugs were added through the lumen (Table 1). However, this statement is not valid if the drug is an antagonist (Table 2) or a relaxation-inducing agonist, as isoprenaline (Table 1), in which case both layers were equireactive. These differences on pD_2 values between the two layers (Table 1) are unlikely to be due to the influence of neuronal uptake, since they were not eliminated by cocaine or denervation (Tables 3, 4). Extraneuronal uptake can also be ruled out, for similar reasons (Table 3). An alternative explanation is that diffusion of the agonist would generate a concentration gradient along the organ wall, resulting in lower pD_2 values if the muscle layer is more deeply located. However, in this case we would also expect differentiated values of pA_2 for antagonists, which was not observed (Table 2). The role played by diffusion barriers in relation to our system and the correlation of these barriers with the histological characteristics of the rat vas deferens has been discussed previously. The possibility has been advanced that drug diffusion through a muscle layer which is progressively contracting, as it occurs for agonists, can be different from a diffusion through a non-contracting layer, as it occurs for antagonists (Busatto & Jurkiewicz 1985).

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