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Prejunctional dopamine receptors modulate twitch responses to parasympathetic nerve stimulation in the rabbit isolated rectococcygeus muscle

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1 Field stimulation of the parasympathetic nerves supplying the rabbit isolated rectococcygeus muscle produced individual twitch responses that were inhibited by dopamine (1×10^{-8}–1×10^{-5} M).

2 The twitch-inhibitory effect of dopamine was reversed by haloperidol or sulpiride (1×10^{-8} – 1×10^{-5} M for either) but not by phentolamine or yohimbine (up to 1×10^{-4} M). Haloperidol and sulpiride were potent, specific, competitive antagonists of the twitch-inhibitory effect of dopamine; their pA_2 values were 8.39 and 7.75, respectively. In contrast, *cis* α-flupenthixol, fluphenazine, bulbocapnine and thioridazine were weak or inactive against dopamine.

3 Concentrations of dopamine that inhibited the twitch response to parasympathetic nerve stimulation had little or no effect on contractions elicited by carbachol or by direct muscle stimulation after abolition of neuronal conduction by tetrodotoxin. Thus, the effects of dopamine on responses elicited by parasympathetic nerve stimulation seem to be exerted at a prejunctional level rather than directly on the smooth muscle.

4 The twitch-inhibitory effect of dopamine was mimicked by epinine, N,N-diethyldopamine, N,N-di-*n*-propyldopamine, 5,6-ADTN, N,N-di-*n*-propyl 5,6-ADTN, 6,7-ADTN, apomorphine and Sandoz 27-403. Sulpiride reversed the effects of all these agonists.

5 N,N-di-*n*-propyl-6,7-ADTN and SK & F 82526 also inhibited the twitch response but their effects were not reversed by sulpiride.

6 SK & F 38393 and DPI had little effect on the twitch response.

7 The pharmacological characteristics of the presynaptic dopamine receptors in the rabbit rectococcygeus muscle show that they resemble those in the cat heart and rabbit ear artery in some respects but there are differences that suggest that the presynaptic dopamine receptors in the rabbit rectococcygeus muscle constitute a specific subgroup of receptors.

Introduction

Several isolated tissue preparations contain presynaptic dopamine receptors, stimulation of which inhibits neurotransmission. These include the cat spleen (Langer, 1973), cat atrium (Long *et al.*, 1975), cat nictitating membrane (Enero & Langer, 1975), rat and rabbit mesentery (Anwar & Mason, 1981), rabbit ear artery (Rand *et al.*, 1975; Steinsland & Hieble, 1978; Brown & O'Connor, 1981; Brown *et al.*, 1983), rabbit heart (Fuder & Muscholl, 1978) and bovine renal arteries (Kalsner & Chan, 1980). Each of these preparations is innervated by sympathetic nerves; the tissue responses to nerve stimulation are mediated via α- or β-adrenoceptors. Dopamine, and many related compounds, stimulate these α- and β-adrenoceptors as well as the presynaptic dopamine receptors; the effects of adrenoceptor stimulation may obscure the consequences of prejunctional dopamine receptor stimulation, making it difficult to assess, accurately, the effects of the drugs interacting with the presynaptic dopamine receptors. It is often difficult to prevent the adrenoceptor stimulant effects of dopamine receptor agonists without also interfering with the end-organ responses to nerve stimulation; furthermore many of the dopamine receptor antagonists are potent α-adrenoceptor antagonists (Peroutka *et al.*, 1977) and this limits the concentrations that can be used to block the presynaptic dopamine receptors in tissues in which the end-organ response to nerve stimulation is mediated via α-adrenoceptors.

In order to overcome the problems outlined above, a preparation was sought in which prejunctional

dopamine receptors were present on the terminals of postganglionic, non-adrenergic motor nerves: in such a preparation it ought to be possible to antagonize specifically any unwanted adrenoceptor stimulant effects of dopamine and related compounds, without impairing neuroeffector transmission or the actions of drugs at the prejunctional dopamine receptors. Ambache *et al.* (1974) showed that the rabbit rectococcygeus muscle provided a robust, ganglion-free preparation in which the twitch contractions to transmural stimulation, at low pulse widths, were mediated by acetylcholine, acting at postjunctional muscarinic receptors, after its release from the cholinergic nerve component of the parasympathetic nerve supply to the tissue. Preliminary experiments, in these laboratories, revealed that dopamine inhibited the twitch responses to field nerve stimulation of the rabbit rectococcygeus muscle, an effect mediated apparently via presynaptic dopamine receptors. This paper describes the pharmacological characteristics of these receptors. A preliminary account of this work was presented to the British Pharmacological Society (Drew & Hilditch, 1983).

Methods

Rectococcygeus muscles were removed from male New Zealand white rabbits (2–3 kg) as described by Ambache *et al.* (1974). They were divided in half, longitudinally, and each part was suspended between platinum electrodes, approximately 10 mm apart, under an initial tension of 1 g in Krebs-Henseleit solution of the following composition (mM): Na^+ 143.4, K^+ 5.9, Mg^{2+} 0.6, Ca^{2+} 1.3, Cl^- 124.4, $H_2PO_4^-$ 1.2, SO_4^{2-} 0.6, HCO_3^- 25.0 and glucose 11.1. In addition, indomethacin (5×10^{-6} M) and ascorbic acid (1×10^{-4} M) were added to the solution to inhibit production of endogenous prostanoids and to inhibit catecholamine oxidation, respectively. Except in preliminary experiments, cocaine (3×10^{-5} M) and corticosterone (4×10^{-5} M) were also added to the Krebs solution; early experiments showed that these catecholamine uptake inhibitors increased the twitch-inhibitory potency of dopamine 9 fold. Furthermore, despite earlier reports of its weak activity against isoprenaline in this preparation (Ambache *et al.*, 1974; King & Muir, 1981; Drew & Hilditch, unpublished observations), propranolol (1×10^{-6} M) was also included in the bathing fluid in an attempt to block β-adrenoceptors and to make the experimental conditions similar to those used previously for isolated preparations (e.g. Hilditch & Drew, 1981). The Krebs solution was gassed continuously with 95% O_2 and 5% CO_2 and maintained at 32°C; this temperature was chosen to delay the onset of spontaneous activity (Ambache *et al.*, 1974). In most preparations, intramural nerves were stimulated using field pulses, 0.4 ms in duration, at supramaximal voltage delivered using a Devices DS9A stimulator coupled to an Amcron power amplifier. Changes in isometric tension were measured using Statham Gold Cells (UC3) and displayed on a Devices MX6 chart recorder. The stimulation frequency was 0.1 Hz; under these conditions, field stimulation produced individual twitch responses that recovered fully before the next response.

The effects of dopamine and noradrenaline on the twitch response to field stimulation

When twitch responses to field stimulation had become constant, dopamine (1×10^{-7}–1×10^{-4} M) was added to the bathing fluid cumulatively. Enough time (3–5 min) was allowed to elapse between successive doses to allow the twitch-inhibitory effect of each to become fully established. When dopamine had abolished the twitch response to field stimulation, 1×10^{-8}–1×10^{-5} M of yohimbine, phentolamine, sulpiride or haloperidol was added to the bathing fluid, cumulatively, in an attempt to reverse the twitch-inhibitory effect of dopamine. Similar experiments with phentolamine or sulpiride were carried out when noradrenaline (1×10^{-9}–1×10^{-6} M) was used in place of dopamine; noradrenaline has been shown previously to inhibit twitch responses to field stimulation in this preparation (Ambache *et al.*, 1974; King & Muir, 1981).

The sites of action of dopamine and noradrenaline

In order to establish whether the twitch-inhibitory effects of dopamine and noradrenaline were attributable to direct smooth muscle relaxation or to an action at nerve terminals the following series of experiments were carried out.

In the first, preparations were subjected to field stimulation and a concentration-effect curve to dopamine or noradrenaline was obtained as previously described. This was repeated to show that responses were reproducible and stimulation was then stopped. Carbachol (3×10^{-6} M) was added to the bathing fluid to induce muscle tone and, when the contraction had stabilized, dopamine or noradrenaline was added, again, to the bathing fluid and its effect on the carbachol-induced tone was measured.

In the second series of experiments, one end of each preparation was clamped in contact with two punctate electrodes, of the type described by Blinks (1965), and the other end was connected to a strain gauge. The preparation was then suspended in Krebs solution between two platinum field electrodes as previously described. Twitch responses were initially elicited by field stimulation in the usual manner and

the inhibitory effects of dopamine or noradrenaline were determined. The preparation was washed in drug-free Krebs solution and tetrodotoxin (1×10^{-7} M) was administered to the bathing fluid to abolish the twitch response to field stimulation. The preparation was then stimulated *directly*, at 0.1 Hz, via the punctate electrodes at supramaximal voltage and a pulse width (20–80 ms) sufficient to produce twitch responses of a similar magnitude to those previously obtained to field stimulation. The effects of dopamine or noradrenaline on these responses were then established.

Attempts were made to determine the effects of dopamine, alone, on the relaxant responses elicited by stimulation of the non-adrenergic non-cholinergic nerves supplying this tissue. Initially, preparations were subjected to field stimulation at 0.1 Hz, 0.4 ms pulse width at supramaximal voltage, to elicit twitch responses; stimulation was then stopped and carbachol (6×10^{-5} M) was administered to contract the preparation, as described by King & Muir (1981). When the contraction had stabilized the preparation was again stimulated, first at 0.1 Hz (0.4 ms, at the same voltage as before) and then at increasing frequencies up to 32 Hz. Stimulation at any particular frequency was applied for about 1 min or until any relaxant response, so produced, reached its peak, after which the next highest frequency was selected without waiting for recovery of the previous response. Stimulation was then stopped and the carbachol-induced tone was allowed to recover. When this had happened the preparation was stimulated continuously at 0.1 Hz and dopamine (1×10^{-8}–1×10^{-4} M) was administered to the bathing fluid during field stimulation.

Further evaluation of the effects of dopamine receptor and α-adrenoceptor agonists on the twitch response to field stimulation

Preparations were electrically stimulated with 0.4 ms duration pulses until the twitch responses became constant. Dopamine was added to the tissue as previously described and after two reproducible concentration-effect curves had been obtained the test agonist was added to the bathing fluid in the same way. In each experiment, the concentrations of dopamine and the test agonist required to reduce the twitch response by 50% (EC_{50}) were determined and their equipotent concentration-ratios were calculated by division from these values. Only one test agonist and dopamine were compared in any individual preparation. Each test agonist was examined in at least five preparations from different rabbits. In preparations in which the test agonist inhibited the twitch response, sulpiride (1×10^{-5} M) was added to the bathing fluid after the highest concentration of the test agonist.

Determination of antagonist potency

Dopamine was used as the agonist in all experiments. Pairs of preparations were dosed until two reproducible concentration-effect curves had been obtained in each. The antagonist was added to one of the pair of preparations whilst the vehicle, alone, was added to the other. Thirty minutes later a concentration-effect curve to dopamine was obtained in the presence of the antagonist or vehicle. The antagonist-induced shift in the dopamine concentration-effect curve was determined at the EC_{50} level and the dopamine concentration-ratio determined in this way was corrected for the spontaneous or vehicle-induced change in the corresponding control preparation. Only one concentration of antagonist was examined in each experiment but several separate determinations were made and for most antagonists, the effects of a range of concentrations were examined.

Results for each antagonist were pooled and subsequently plotted according to the method of Arunlakshana & Schild (1959). A regression line was fitted by computer using the method of least squares and the pA_2 and slope of the regression obtained.

In a further series of experiments, antagonist specificity was determined using noradrenaline in place of dopamine. In these experiments only the highest concentrations of the antagonists used in the above study were investigated.

Drugs and solutions

The following drugs were used: apomorphine hydrochloride (Macfarlan Smith), ascorbic acid (BDH), atropine sulphate (BDH), (+)-bulbocapnine (Research Biochemicals), carbachol chloride (BDH), *cis* α-flupenthixol dihydrochloride (Lundbeck), clonidine hydrochloride (Boehringer), 2-[(3,4-dihydroxyphenyl) amino]-2-imidazoline (DPI; Boehringer), cocaine hydrochloride (Macfarlan-Smith), corticosterone (Sigma), dopamine hydrochloride (Sigma), fluphenazine hydrochloride (Squibb and Sons), hexamethonium bromide (Koch Light), haloperidol (Searle), indomethacin (Sigma), noradrenaline bitartrate (Koch-Light), N-methyldopamine hydrochloride (epinine; Sigma), papaverine hydrochloride (Sigma), phentolamine mesylate (Ciba), propranolol hydrochloride (ICI), N,N -bis[6-(3,4-dihydroxyphenethylamino) hexyl]-hexamethylenediamine tetrahydrobromide (Sandoz 27-403; Sandoz), 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine hydrochloride (SK & F 38393; Smith, Kline and French), (±)-sulpiride (Chemitechna), tetrodotoxin (citrated; Sigma), thioridazine hydrochloride (Sandoz) and yohimbine hydrochloride (Sigma).

The hydrobromide salts of the following compounds were synthesized by members of the Chemistry Research Department of Glaxo Group Research: N,N-diethyldopamine, N,N-di-*n*-propyldopamine, 2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene (5,6-ADTN), N,N-di-*n*-propyl 5,6-ADTN, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (6,7-ADTN), N,N-di-*n*-propyl 6,7-ADTN and 6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1H-3-benzazepine-7,8-diol (SK & F 82526).

Corticosterone (69 mg) was dissolved in a minimum quantity of absolute ethanol (0.2 ml) prior to adding to 5 l Krebs solution. Haloperidol was obtained in solution (Serenace ampoules). Bulbocapnine and sulpiride were dissolved in a minimum quantity of 2 N HCl. Other compounds were dissolved in 0.9% w/v NaCl solution (saline) and further dilution of all compounds was made with saline. All drug concentrations mentioned in the text refer to the free base.

Results

Stimulation of the rabbit isolated rectococcygeus muscle at 0.1 Hz produced individual twitch responses. The size of the twitch varied between 0.2–1.5 g in different preparations but, in any individual preparation, was well sustained (± < 5%) for at least 1 h. With more prolonged periods of stimulation, changes in basal tension and spontaneous spike contractions were seen and the twitch height declined progressively. These effects could be prevented if periods of rest were allowed between successive periods of stimulation. For this reason, stimulation was discontinued for periods of 30–45 min in all those experiments in which more than one agonist concentration-effect curve on the response to field stimulation, was carried out.

Atropine (1×10^{-8} – 3×10^{-7} M) or tetrodotoxin (1×10^{-9} – 1×10^{-7} M) reduced and eventually abolished the twitch response, but hexamethonium (up to 1×10^{-4} M) had no effect.

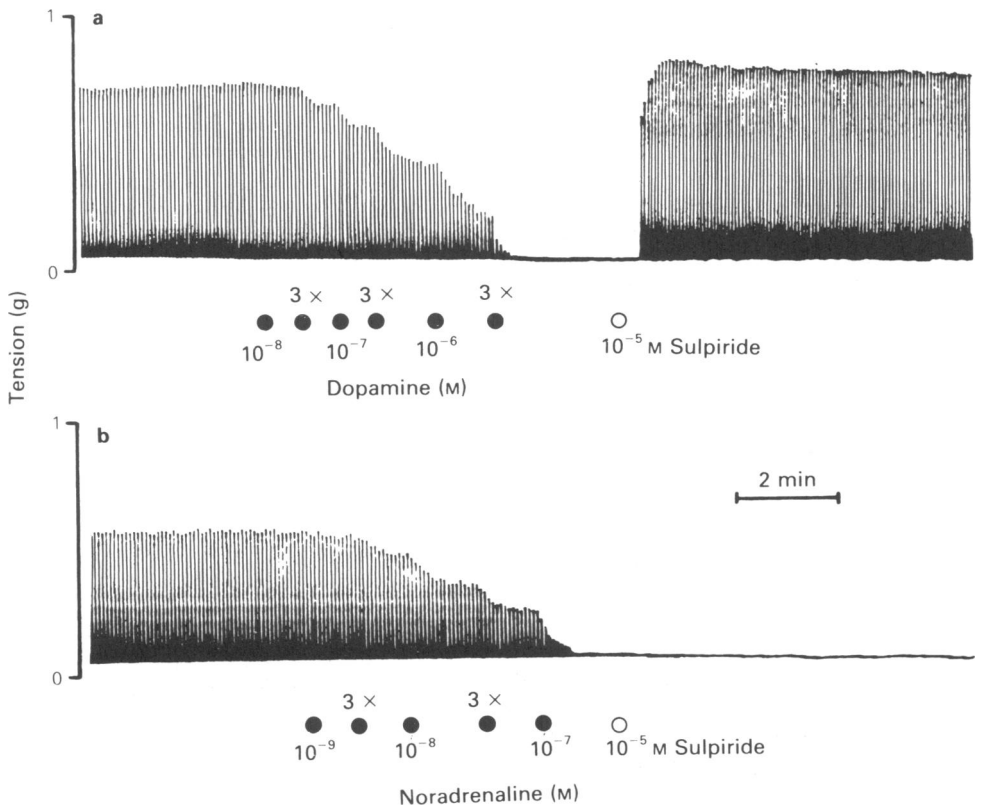

Figure 1 The effects of dopamine (a) and noradrenaline (b) on the twitch response of the rabbit isolated rectococcygeus muscle to electrical field stimulation (0.1 Hz, 0.4 ms, supramaximal voltage) and the effect of sulpiride on the inhibitory effects of dopamine and noradrenaline.

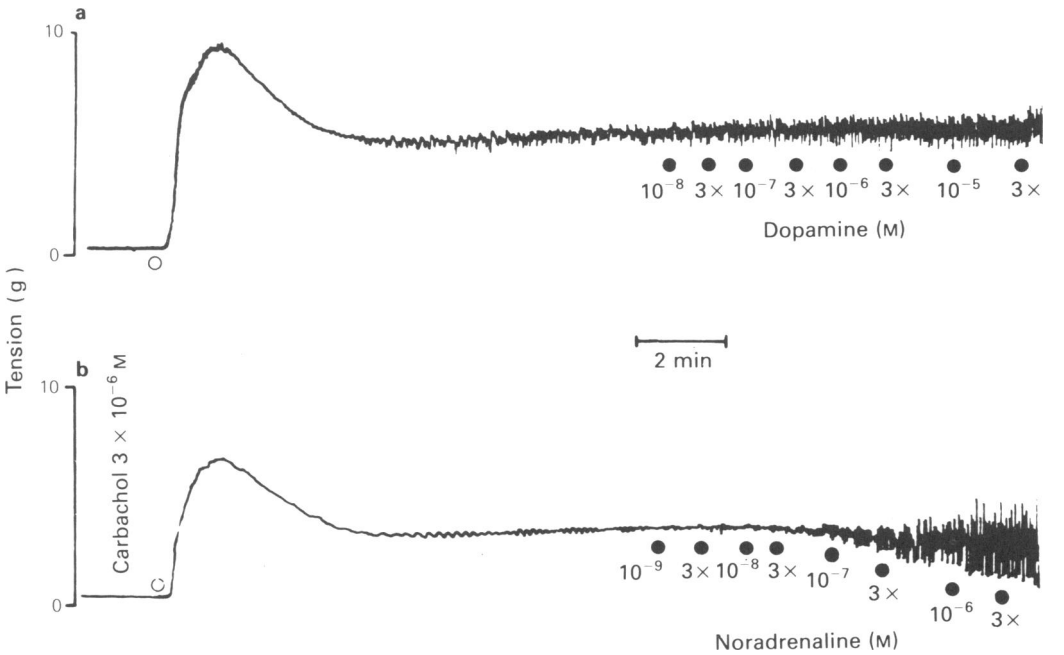

Figure 2 The effects of dopamine (a) and noradrenaline (b) on carbachol-induced tone in the rabbit isolated rectococcygeus muscles.

The effects of dopamine and noradrenaline on the twitch response to field stimulation

Dopamine ($1 \times 10^{-8} - 1 \times 10^{-5}$ M) inhibited the twitch response to field stimulation in approximately half of the preparations in which it was examined (Figure 1). At the highest concentrations used dopamine abolished the twitch response. The subsequent addition of haloperidol or sulpiride ($1 \times 10^{-8} - 1 \times 10^{-5}$ M) to the bathing fluid caused a rapid, concentration-dependent restoration of the twitch response. In untreated preparations the same concentrations of haloperidol and sulpiride altered the twitch responses to field stimulation by less than ±16% ($n = 3-4$). In contrast, neither phentolamine nor yohimbine ($1 \times 10^{-8} - 1 \times 10^{-4}$ M) reversed the effects of dopamine.

Noradrenaline ($1 \times 10^{-9} - 1 \times 10^{-6}$ M) inhibited the twitch response in all preparations examined and was approximately 30 times more potent than dopamine in this respect (Figure 1). Neither sulpiride nor phentolamine, in concentrations up to 1×10^{-4} M reversed the inhibitory effect of noradrenaline on the twitch response.

In the remainder of the preparations to which it was administered, dopamine either did not inhibit the twitch response or was much less potent than in the experiments described above. In the latter cases, sulpiride failed to reverse the twitch-inhibitory effect of dopamine. Noradrenaline-insensitive preparations were not encountered. The effects of noradrenaline were examined in some dopamine-insensitive preparations; it inhibited the twitch response over the usual concentration range. Preparations subsequently encountered that did not respond to dopamine over the lower concentration range were rejected without further experimentation.

The sites of action of dopamine and noradrenaline

Concentrations of dopamine ($1 \times 10^{-8} - 1 \times 10^{-5}$ M) or noradrenaline ($1 \times 10^{-9} - 1 \times 10^{-6}$ M) that inhibited the twitch responses to field stimulation had little or no effect on the contraction elicited by carbachol (3×10^{-6} M). Higher concentrations of dopamine (up to 1×10^{-4} M) also had little effect on carbachol-induced tone, although higher concentrations of noradrenaline ($1 \times 10^{-6} - 1 \times 10^{-5}$ M) caused some relaxation (Figure 2).

Dopamine (up to 1×10^{-4} M) did not reduce the twitch response to direct electrical stimulation of the rectococcygeus muscle after neural conduction had

been prevented by tetrodotoxin. Subsequent administration of papaverine (2×10^{-4} M) markedly decreased the twitch response to direct muscle stimulation (Figure 3). In contrast to dopamine, noradrenaline (1×10^{-9} – 1×10^{-6} M) reduced the twitch responses to direct muscle stimulation, but by a maximum of only about 40%. Higher concentrations produced no further effect.

In four preparations, carbachol (6×10^{-5} M) initially caused a marked increase in muscle tone, but this subsided and eventually stabilised at 1.5–2.8 g (mean = 2.2 g); this compared with a tension of 0.4–1.1 g (mean 0.8 g) that was developed during field stimulation of the same preparations before carbachol administration. In the presence of carbachol, field stimulation at 0.1 Hz did not elicit contractile responses; nor did it cause relaxation of the tissue. Relaxation was seen only at stimulation frequencies of 0.4 Hz and above. At 0.4 Hz, carbachol-induced tone was decreased by a mean of 5%, and at 32 Hz it was reduced by 34%. Tension recovered quickly after cessation of stimulation. Subsequent continuous stimulation at 0.1 Hz again failed to relax the tissue and dopamine ($1 \times 10^{-8} - 1 \times 10^{-4}$ M) failed to bring about any relaxation in response to nerve stimulation; instead there was a slight increase in tone (11% of carbachol-induced tone, after dopamine 1×10^{-4} M). Further attempts were made to reveal relaxant responses to field stimulation at 0.1 Hz by reducing the concentration of carbachol to 1×10^{-6} M; however, at this concentration, carbachol elicited marked rhythmical contractions of the tissue, making it difficult to assess the responses to field stimulation. Even so, there was no indication that

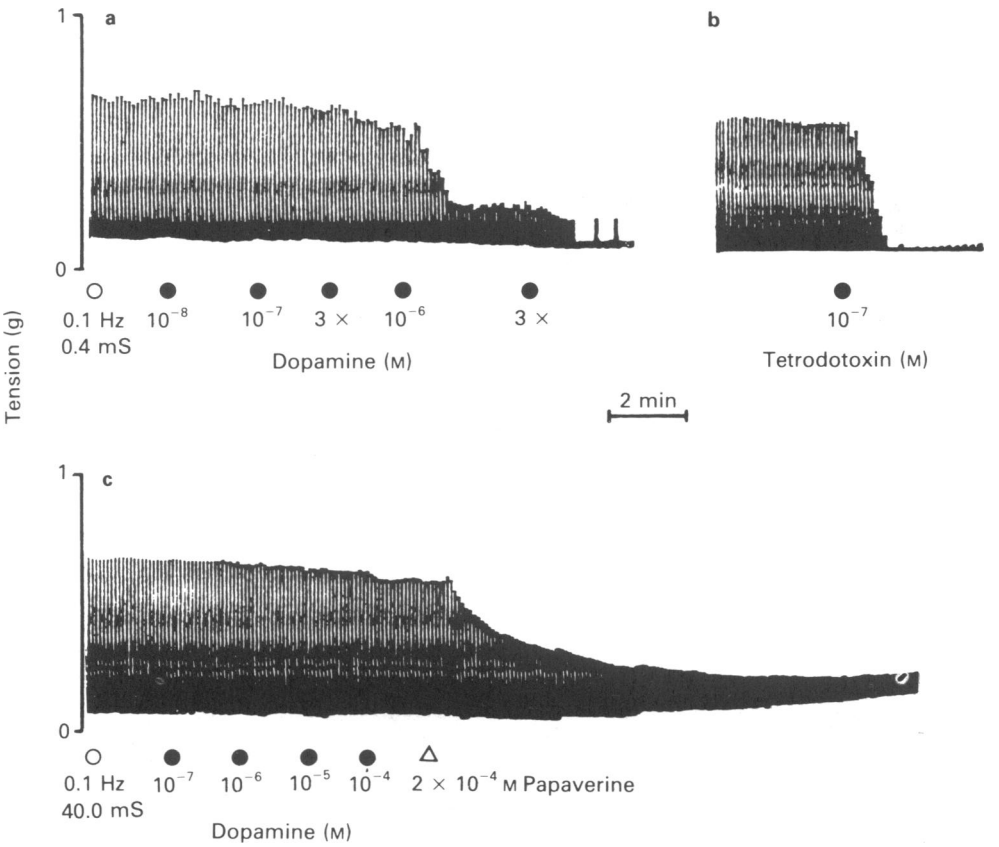

Figure 3 The effect of dopamine on the twitch response of a rabbit isolated rectococcygeus muscle preparation to field stimulation (0.1 Hz, 0.4 ms, supramaximal voltage; a) and to direct muscle stimulation (0.1 Hz, 40 ms, supramaximal voltage, c) after neuronal transmission had been abolished by administration of tetrodotoxin (b). Despite the inability of dopamine to inhibit the twitch response to direct muscle stimulation, in concentrations that inhibited responses to field stimulation, the subsequent administration of papaverine almost abolished the response to direct stimulation.

field stimulation elicited any relaxation until the stimulation frequency was increased to 0.4 Hz or above.

Further evaluation of the effects of dopamine receptor and α-adrenoceptor agonists on the twitch response to field stimulation

In four preparations in which two reproducible concentration-effect curves to dopamine had been obtained, a third concentration-effect curve to dopamine was obtained. This was almost superimposable on the previous one (concentration ratio at $EC_{50} = 0.79$, range 0.53–1.20) showing that the effect of dopamine did not alter appreciably over the time course of the experiments.

In other preparations the inhibitory effect of dopamine was mimicked by epinine, N,N-diethyldopamine, N,N-di-*n*-propyldopamine, 5,6-ADTN, N,N-di-*n*-propyl 5,6-ADTN, 6,7-ADTN and Sandoz 27-403. Over the concentration-ranges tested, the concentration-effect curves for these agonists were approximately parallel to those of dopamine, and the maximum effect of each agonist was similar to that of dopamine in each preparation (Figure 4). Furthermore, the twitch inhibitory effect of each agonist was reversed by sulpiride (1×10^{-5} M), suggesting a common site of action. The EC_{50} values of the compounds and their equipotent concentrations, relative to dopamine, are shown in Table 1.

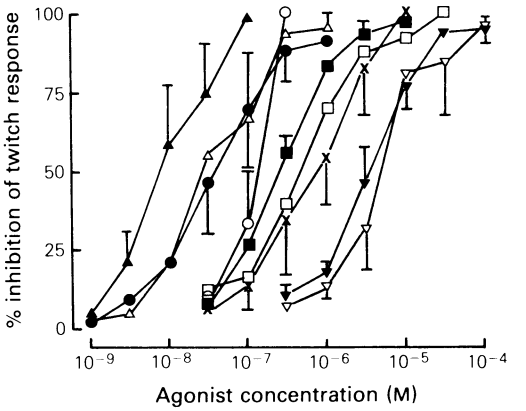

Figure 4 The inhibitory effects of dopamine (■), 6,7-ADTN (▲), N,N-di-*n*-propyl 5,6-ADTN (△), Sandoz 27-403 (●), epinine (○), 5,6-ADTN (×), apomorphine (□), N,N-di-*n*-propyldopamine (▼) and N,N-diethyldopamine (▽) on the twitch response of the rabbit rectococcygeus muscle preparation elicited by field stimulation (0.1 Hz, 0.4 ms, supramaximal voltage). Values shown are group means ($n = 40$ for dopamine and 5 for other agonists). Standard errors of the mean have been omitted in places for clarity.

Table 1 The potencies of agonists at inhibiting the twitch response of the rabbit rectococcygeus muscle to nerve stimulation

Agonist	EC_{50} (μM) (geometric means and 95% confidence interval)	*Equipotent concentration-ratio* (geometric means and 95% confidence interval)
Dopamine	0.28 (0.19–0.37)	1.00
6,7-ADTN	0.01 (0.003–0.049)	0.04 (0.01–0.14)
N,N-di-*n*-propyl 5,6-ADTN	0.06 (0.007–0.51)	0.09 (0.05–0.15)
Sandoz 27-403	0.07 (0.007–0.61)	0.25 (0.15–0.43)
Epinine	0.12 (0.06–0.24)	0.46 (0.22–0.95)
5,6-ADTN	0.63 (0.07–5.83)	2.88 (1.1–7.4)
Apomorphine	0.56 (0.034–9.09)	3.10 (1.1–8.8)
N,N-diethyl dopamine	4.59 (1.99–10.61)	11.83 (7.7–18.2)
N,N-di-*n*-propyldopamine	6.12 (1.33–28.20)	26.35 (11.7–59.5)

EC_{50} values and relative potencies, compared to dopamine were obtained from individual experiments. The EC_{50} for dopamine was obtained from the concentration-effect curve obtained immediately prior to that of the test agonist ($n = 40$ for dopamine and 5 for each other agonist).

Apomorphine also inhibited the twitch response to field stimulation in 7 preparations; however, its effect was reversed by sulpiride in only 5 of these preparations. The mean EC_{50} values for apomorphine in the sulpiride-sensitive and insensitive preparations were 1.3 and 3.5 μM respectively. The EC_{50} values for dopamine in the same groups of preparations were 0.3 and 1.0 μM respectively. The results obtained with apomorphine in sulpiride-sensitive preparations, only, are also shown in Figure 4 and Table 1. N,N-di-*n*-propyl-6,7-ADTN (1×10^{-9}– 3×10^{-6} M) and SK & F 82526 (1×10^{-6} – 3×10^{-5} M) also inhibited the twitch response. They were respectively 1.2 and 36 times less potent than dopamine but sulpiride did not reverse their effects in any preparation. Clonidine (1×10^{-6} – 3×10^{-4} M) inhibited the twitch response in two preparations and was approximately equipotent with dopamine but its effects were not reversed by sulpiride. In four other preparations clonidine, in concentrations up to 1×10^{-4} M, did not have any effect. SK & F 38393 and DPI, in concentrations up to 1×10^{-4} M, inhibited the twitch response by less than 20%.

The effects of neuroleptics on the inhibitory effect of dopamine

The effects of bulbocapnine (1×10^{-5} M), *cis* α-flupenthixol (3×10^{-8} – 1×10^{-5} M), fluphenazine (1×10^{-6} – 1×10^{-5} M), haloperidol (1×10^{-8} – 1×10^{-6} M), sulpiride (3×10^{-8} – 3×10^{-6} M) and thioridazine (1×10^{-5} M) on the twitch-inhibitory effect of dopamine were determined.

Haloperidol and sulpiride produced parallel, concentration-dependent shifts to the right of the dopamine concentration-effect curve, but did not reduce the maximum effect of dopamine. Schild analysis yielded pA_2 values for haloperidol and sulpiride of 8.39 (95% confidence intervals = 8.07 –8.90) and 7.75 (7.48–8.14) respectively; the slopes of the Schild plots from which these values were derived were 1.00 (0.74–1.25) and 1.12 (0.90–1.41) respectively. Thus haloperidol and sulpiride were potent, competitive antagonists of dopamine. Furthermore, at the highest concentrations used to antagonize dopamine, haloperidol (1×10^{-6} M) and sulpiride (3×10^{-6} M) caused less than a 2 fold shift to the right in concentration-effect curves for noradrenaline ($n = 5$ for each).

In contrast to these compounds, *cis* α-flupenthixol and fluphenazine produced weak and inconsistent antagonism of the twitch inhibitory effects of dopamine. The effects of haloperidol, sulpiride, *cis* α-flupenthixol and fluphenazine against dopamine are shown in Figure 5.

When tested at a concentration of 1×10^{-5} M, neither bulbocapnine nor thiordazine exerted any appreciable antagonism against dopamine.

Discussion

Preliminary experiments confirmed that the rabbit rectococcygeus muscle was a ganglion-free, parasympathetically innervated preparation as reported by Ambache *et al.* (1974) and that low frequency field stimulation produced individual, well maintained twitch responses.

The twitch response was reduced, in a concentration-dependent manner, by dopamine and noradrenaline. The inhibitory effect of dopamine was reversed by sulpiride and by haloperidol, at concentrations that block prejunctional (Steinsland & Hieble, 1978; Brown & O'Connor, 1981) but not postjunctional dopamine receptors (Hilditch & Drew, 1981); it was not, however, reversed by the α-adrenoceptor antagonists, phentolamine and yohimbine. Interestingly, the inhibitory effect of noradrenaline was not affected by either sulpiride or phentolamine. Ambache *et al.* (1974) and King & Muir (1981) have previously reported that the inhibitory effect of noradrenaline on motor transmission in this tissue is not blocked by phentolamine or by propranolol. Thus, although the receptor type through which noradrenaline inhibits the twitch response has not been identified, it is clearly different from that which mediates the effects of dopamine. The ability of haloperidol and sulpiride to reverse the effects of dopamine strongly suggests that dopamine acts through dopamine receptors.

Concentrations of dopamine greater than those required to inhibit responses to field stimulation had no effect on carbachol-induced tone. Noradrenaline also had little effect although the higher concentrations used caused some relaxation of carbachol-induced tone. However, these concentrations were much higher than those required to abolish the twitch response to field stimulation. One drawback to these experiments was that the tone induced by carbachol was much greater than that produced at the peak of the twitch response, and so the difference in the effects of dopamine and noradrenaline on the carbachol-induced and nerve stimulation-induced responses may merely have been attributable to the different levels of tension that were developed. However, lower concentrations of carbachol could not be used because they promoted only spontaneous activity instead of a sustained contracture. For this reason, the effects of dopamine and noradrenaline on twitch responses of a similar magnitude to those obtained to field stimulation, but produced by direct muscle stimulation instead, were determined. When used in concentrations that inhibited the twitch response to

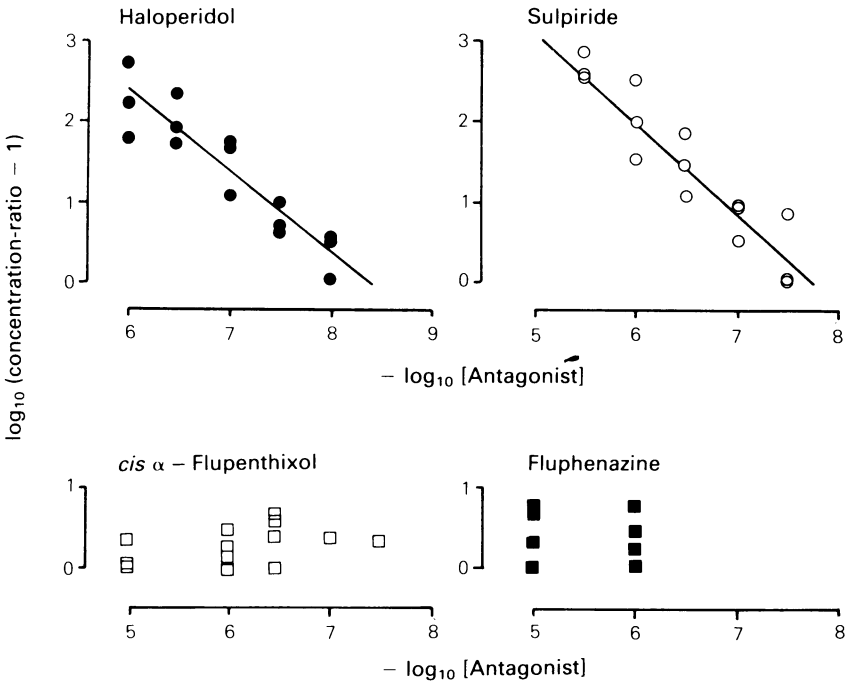

Figure 5 The effects of haloperidol, sulpiride, *cis* α-flupenthixol and fluphenazine on the inhibitory effect of dopamine on twitch responses elicited by field stimulation (0.1 Hz, 0.4 ms, supramaximal voltage) of the rabbit isolated rectococcygeus muscle. Each point shows the result obtained from a single experiment.

field stimulation, dopamine had little or no effect on the twitch responses elicited by direct muscle stimulation at long pulse widths in the presence of tetrodotoxin. In contrast, noradrenaline reduced twitch responses to direct muscle stimulation but was much less effective than against responses elicited by field stimulation. These findings suggest that dopamine inhibits the twitch response to field stimulation by a prejunctional effect; noradrenaline also seems to act in this manner but a direct smooth muscle relaxant action may also contribute to its effect at higher concentrations. One possibility is that dopamine might inhibit the twitch response to field stimulation by enhancing the release of the inhibitory transmitter from the non-adrenergic, non-cholinergic nerves supplying the preparation (King *et al.*, 1977; King & Muir, 1981). This could lead to a greater relaxant response which would offset the postjunctional contractile effect of the cholinergic transmitter. However, no evidence for such a response elicited by field stimulation at 0.1 Hz in the presence of carbachol-induced tone could be found, despite the fact that Blakeley *et al.* (1979) have provided electrophysiological evidence that individual pulses can induce membrane hyperpolarization. Furthermore, dopamine, in concentrations that inhibited the twitch response to field stimulation at 0.1 Hz, did not bring out any relaxant response, under the same stimulation conditions, in the presence of carbachol-induced tone. Thus, although we cannot discount the possibility that the twitch-inhibitory effect of dopamine is in some way a consequence of its interaction with the non-adrenergic, non-cholinergic nerve supply to the tissue, the simplest explanation is that dopamine most probably inhibits the twitch response to low frequency field stimulation by inhibiting, directly, the release of acetylcholine from the cholinergic nerves that supply the rectococcygeus muscle.

The receptor at which dopamine exerted its effects was subsequently characterized using both agonists and antagonists. The inhibitory effect of dopamine on twitch response to field stimulation was mimicked by epinine, N,N-diethyldopamine, N,N-di-*n*-propyldopamine, 5,6-ADTN, N,N-di-*n*-propyl 5,6-ADTN, 6,7-ADTN, apomorphine and Sandoz 27-403. The maximum effects of all these agonists, like dopamine, were reversed by sulpiride suggesting a similar site of action for all compounds. N,N-di-*n*-propyl 6,7-ADTN and SK & F 82526 also inhibited the twitch response but, unlike the previous com-

pounds, were not reversed by sulpiride. SK & F 38393 was almost inactive. Both clonidine and DPI, relatively selective α_2-adrenoceptor agonists, were weakly active. The lack of effect of these compounds supports the view that the inhibitory effect of noradrenaline is not mediated through presynaptic α_2-adrenoceptors.

The results obtained with agonists show similarities to those obtained with these compounds at presynaptic dopamine receptors in the rabbit isolated ear artery (Brown *et al.*, 1983) and the cat heart *in vivo* (Drew *et al.*, 1982). However, the relative potencies of N,N-di-*n*-propyldopamine, and particularly N,N-di-*n*-propyl 6,7-ADTN are different from what would be expected if these receptors were the same. The equipotent molar concentration ratios are, however, markedly different from those obtained at 'postsynaptic' vascular dopamine receptors in the rabbit splenic artery and dog mesenteric vascular bed (Drew & Hilditch, 1981).

The twitch-inhibitory effect of dopamine in the rectococcygeus muscle was competitively and specifically inhibited by both haloperidol and sulpiride. The pA_2 values obtained for these antagonists are similar to those reported for haloperidol (8.85 *v* dopamine) and sulpiride (7.79 *v* 6,7-ADTN) by Steinsland & Hieble (1978) and Brown & O'Connor (1981), respectively, at presynaptic dopamine receptors in the rabbit ear artery. Thus the findings with some agonists and antagonists indicate that the receptor in the rabbit rectococcygeus muscle and the presynaptic dopamine receptor in the rabbit ear artery and cat heart are similar. However, neither *cis* α-flupenthixol nor fluphenazine antagonized the effects of dopamine in the rectococcygeus muscle. Neither compound has been evaluated in the rabbit ear artery but both compounds are potent antagonists against dopamine in the emetic centre of the dog and at presynaptic dopamine receptors in the cat heart (Drew *et al.*, 1982). In addition neither bulbocapnine nor thioridazine antagonized the effects of dopamine in the rabbit rectococcygeus. It seems likely therefore that the prejunctional dopamine receptors in the rabbit rectococcygeus have slightly different characteristics from those in other preparations. Interestingly, however, haloperidol and sulpiride, but not fluphenazine (Costall *et al.*, 1981) or *cis* α-flupenthixol (Naylor, personal communication), block the reduction of spontaneous locomotor activity caused by N,N-di-*n*-propyl 5,6-ADTN in mice. This effect is believed to be mediated via central presynaptic dopamine receptors. Furthermore, Dubocovich & Weiner (1981) have recently shown that S-sulpiride ($1 \times 10^{-8} - 1 \times 10^{-6}$ M) enhanced the release of radioactive dopamine from the amacrine neurones in the rabbit retina, presumably by blocking presynaptic dopamine receptors that mediate feedback-inhibition of dopamine release. In contrast, fluphenazine and *cis* α-flupenthixol ($1 \times 10^{-8} - 1 \times 10^{-5}$ M) were weak or ineffective suggesting that they have very low affinity for these presynaptic receptors.

In conclusion, dopamine inhibits the twitch response elicited by field stimulation of the rabbit rectococcygeus muscle by stimulating presynaptic dopamine receptors on cholinergic nerve terminals. These receptors are similar in many respects to presynaptic dopamine receptors in other tissues, such as the rabbit ear artery and cat heart, but also appear to differ in some respects. Thus the presynaptic dopamine receptors in the rabbit rectococcygeus muscle may constitute a specific sub-group of dopamine receptors.

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