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SELECTIVITY OF BLOCKING AGENTS FOR PRE- AND POSTSYNAPTIC α-ADRENOCEPTORS

J.C. DOXEY, C.F.C. SMITH & JULIE M. WALKER
Reckitt and Colman, Pharmaceutical Division, Dansom Lane, Hull, HU8 7DS

1 Low frequency (0.1 Hz) electrical stimulation of the rat isolated vas deferens produced regular contractions that were inhibited by low concentrations of clonidine.

2 The inhibition of the vas deferens produced by clonidine was presynaptic in origin and involved α-adrenoceptors.

3 Presynaptic α-adrenoceptor antagonist activity was assessed by studying the effects of increasing concentrations of the antagonists on cumulative clonidine dose-response curves on the stimulated vas deferens.

4 Postsynaptic α-adrenoceptor antagonist activity was assessed by comparison of control cumulative noradrenaline dose-response curves with those in the presence of increasing concentrations of antagonists in the rat anococcygeus muscle.

5 The results indicate that yohimbine and phentolamine are more potent in blocking presynaptic than postsynaptic α-adrenoceptors. Phenoxybenzamine and prazosin block postsynaptic α-adrenoceptors preferentially.

6 The findings support the view that presynaptic and postsynaptic α-adrenoceptors differ in their sensitivity to α-adrenoceptor antagonists.

Introduction

There is considerable evidence to support the concept that sympathetic nerve terminals possess α-adrenoceptors. Activation of these presynaptic α-adrenoceptors inhibits, and their blockade potentiates, the release of noradrenaline by nerve impulses (Langer, 1974). There is also evidence that presynaptic and postsynaptic α-adrenoceptors differ in their sensitivity to both agonists (Starke, 1972; Starke, Montel, Gayk & Merker, 1974; Starke, Endo & Taube, 1975b), and antagonists (Dubocovich & Langer, 1974; Thoenen, Hurlimann & Haefely, 1964; Starke, Borowski & Endo, 1975a).

The effects of several α-adrenoceptor antagonists have been studied to assess their relative potencies at presynaptic and postsynaptic α-adrenoceptors. Presynaptic α-adrenoceptor activity was assessed on the isolated vas deferens of the rat, stimulated at low frequency. Postsynaptic α-adrenoceptor activity was assessed on the isolated anococcygeus muscle of the rat.

Methods

Rat vas deferens

Male albino rats in the weight range 80–110 g were killed by cervical fracture. The vas deferens was dissected free from the surrounding connective tissue and suspended in an organ bath of 2 ml capacity. The tissue was bathed in Krebs solution of the following composition (mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 0.6, $NaHCO_3$ 25.0 and dextrose 11.1 which was maintained at 27°C and gassed with 95% O_2 and 5% CO_2. The bath fluid was renewed continuously with a peristaltic pump which delivered 3 ml/minute. Longitudinal contractions of the tissue were recorded isometrically with a Statham Gold cell transducer (model UC3) linked to a Smiths Servoscribe recorder. The intramural nerves of the vas deferens were stimulated by rectangular pulses of 3 ms duration 10–30 V at a frequency of 0.1 hertz.

Rat anococcygeus muscle

Male albino rats in the weight range 200–250 g were killed by cervical fracture. The anococcygeus muscle (Gillespie, 1971) was exposed, dissected free from the surrounding tissues and suspended in a 5 ml organ bath. The tissue was bathed in Krebs solution which was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. A Statham Gold cell transducer was used to measure contractions.

Quantitative analysis of α-adrenoceptor antagonist activity

Corticosterone 40 μM, desmethylimipramine 10 nM and propranolol 0.1 μM were present in the Krebs solution throughout the experiments outlined below.

Presynaptic activity

Contractions of the stimulated vas deferens were inhibited by clonidine. By exposing the tissue to increasing concentrations of clonidine it was possible to construct cumulative dose-response curves. Following the production of two successive and comparable cumulative dose-response curves to clonidine, the effects of increasing concentrations of antagonists were studied. Antagonists were allowed a contact time of 15 min before initial challenge with clonidine.

Postsynaptic activity

Noradrenaline stimulates postsynaptic α-adrenoceptors of the rat anococcygeus muscle to produce a contraction. Noradrenaline (dissolved in Krebs solution) was added to the bath in 0.05 ml volumes to obtain cumulative dose-response curves. Antagonists were added to the bath only after two successive and comparable dose-response curves to noradrenaline had been obtained. Antagonists (maximum volume 0.2 ml) were allowed a contact time of 10 min before initial challenge with noradrenaline.

In both presynaptic and postsynaptic studies the methods of Van Rossum (1963) were used to calculate pA_2, pD'_2 and pD_2 values.

Drugs

The following drugs were used in this study: bretylium tosylate (Burroughs Wellcome); clonidine (Boehringer Ingelheim); corticosterone (Sigma), desmethylimipramine hydrochloride (Geigy); guanethidine sulphate (CIBA); noradrenaline bitartrate (Koch Light Laboratories Ltd.); papaverine sulphate (Macfarlane Smith); phenoxybenzamine hydrochloride (SKF); phentolamine mesylate (CIBA); prazosin hydrochloride (Pfizer); propranolol hydrochloride (ICI) and yohimbine hydrochloride (Sigma). With the exception of prazosin, which was made up at a concentration of 1 mg/ml in warm 0.9% w/v NaCl solution (saline) containing 30% ethanol and corticosterone which was dissolved in propylene glycol, all drugs were dissolved in saline.

Results

The rat vas deferens as a model for presynaptic α-adrenoceptor activity

Stimulation of the intramural nerves of the rat vas deferens at low frequency (0.1 Hz), 10–30 V, and 3 ms produced regular contractions of the tissue. The preparation was very stable with no spontaneous changes in resting tension and the contractions remained constant for at least 4–5 hours. The electrically induced contractions of the vas deferens were inhibited by low concentrations of clonidine hydrochloride (Figure 1). There was little variation in the sensitivity of different preparations to clonidine and clonidine dose-response curves could be constructed repeatedly, again with little change in sensitivity, in individual experiments. The contractions of the vas deferens were also inhibited by guanethidine sulphate (3 μg/ml), bretylium tosylate (10 μg/ml) and papaverine sulphate (100 μg/ml).

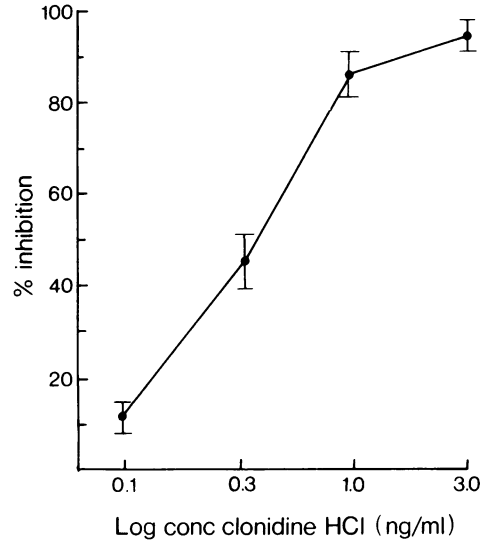

Figure 1 The effect of clonidine on electrically induced contractions of the rat vas deferens. The results are the mean of 5 experiments. Vertical lines show s.e. means.

In order to determine whether clonidine was blocking sympathetic nerves or decreasing the sensitivity of the vas deferens itself, a second experiment was performed in which the tissue was stimulated directly. The stimulus parameters used were 100 V, 100 ms pulse width and a frequency of 0.1 hertz. Under these conditions clonidine, bretylium and guanethidine did not inhibit the contractions of the vas deferens whilst papaverine still caused inhibition of the 'twitch' response.

That clonidine, like bretylium and guanethidine, was interfering with nervous transmission and having little or no effect on the sensitivity of the vas deferens itself was confirmed in an experiment in which the vas deferens was contracted alternately by electrical stimulation (3 ms, 10–30 V, 0.1 Hz) and noradrenaline (1 μg injected into the Krebs stream). Clonidine hydrochloride (3 ng/ml) markedly antagonized the stimulation-induced contractions (Figure 2) but had little or no inhibitory effect on contractions evoked by noradrenaline.

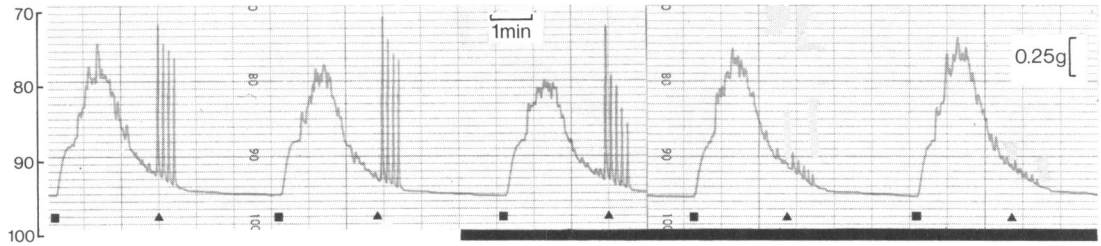

Figure 2 The effect of clonidine on contractions of the rat vas deferens induced by noradrenaline and field stimulation: (■) noradrenaline 1 μg; (▲) electrical stimulation 0.1 Hz, 10–30 V, 3 ms; bar indicates presence of clonidine HCl 3 ng/ml.

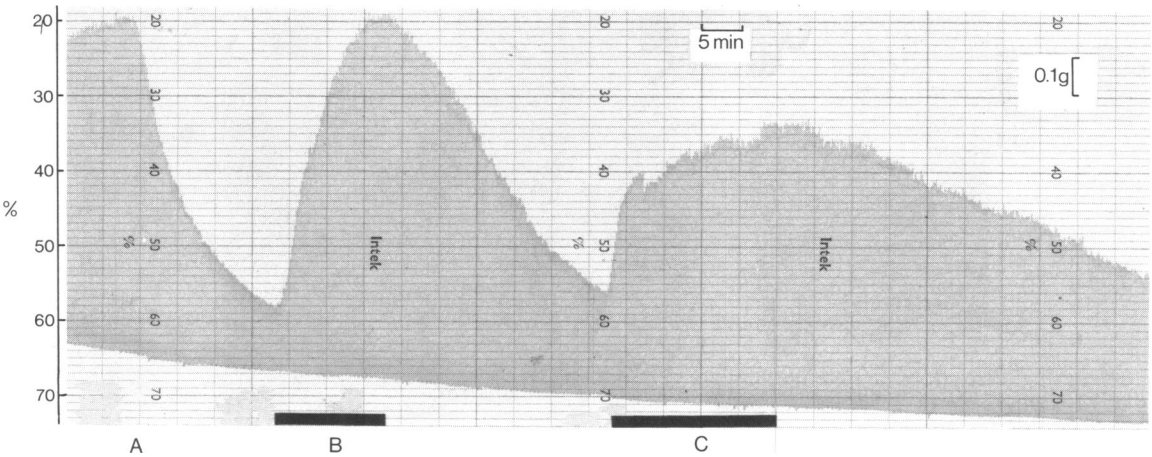

Figure 3 The effect of antagonists on the inhibition produced by clonidine on the rat vas deferens. The vas deferens was stimulated at 10–30 V, 3 ms and 0.1 Hz; clonidine HCl was continuously present from point (A) at 3 ng/ml; (B) yohimbine HCl 100 ng/ml; (C) phentolamine mesylate 100 ng/ml.

Having established that clonidine affected nervous transmission, its mechanism of action was studied in the vas deferens stimulated at 10–30 V, 3 ms and 0.1 hertz. The inhibitory effects of clonidine could be antagonized competitively by phentolamine and yohimbine (Figure 3). The inhibition produced by bretylium, guanethidine or papaverine was not affected by phentolamine or yohimbine.

Determination of presynaptic α-adrenoceptor antagonist activity

The rat vas deferens, stimulated at 0.1 Hz was used to construct cumulative dose-response curves to clonidine and the effects of the α-adrenoceptor antagonists yohimbine, phentolamine, phenoxybenzamine and prazosin upon them were examined.

Table 1 Drug antagonism at the presynaptic α-adrenoceptor of the rat vas deferens

Agonist	*Antagonist*	*Drug parameter*	n
Clonidine		$pD_2 = 8.81 \pm 0.06$	11
	Yohimbine	$pA_2 = 8.18 \pm 0.11$	6
	Phentolamine	$pA_2 = 8.38 \pm 0.09$	6
	Prazosin	$pA_2 < 6.62$	3
	Phenoxybenzamine	$pA_2 < 7.54$	6

The Krebs solution contained corticosterone 40 μM, desmethylimipramine 10 nM and propranolol 0.1 μM. The results are expressed as the mean ± s.e. mean.

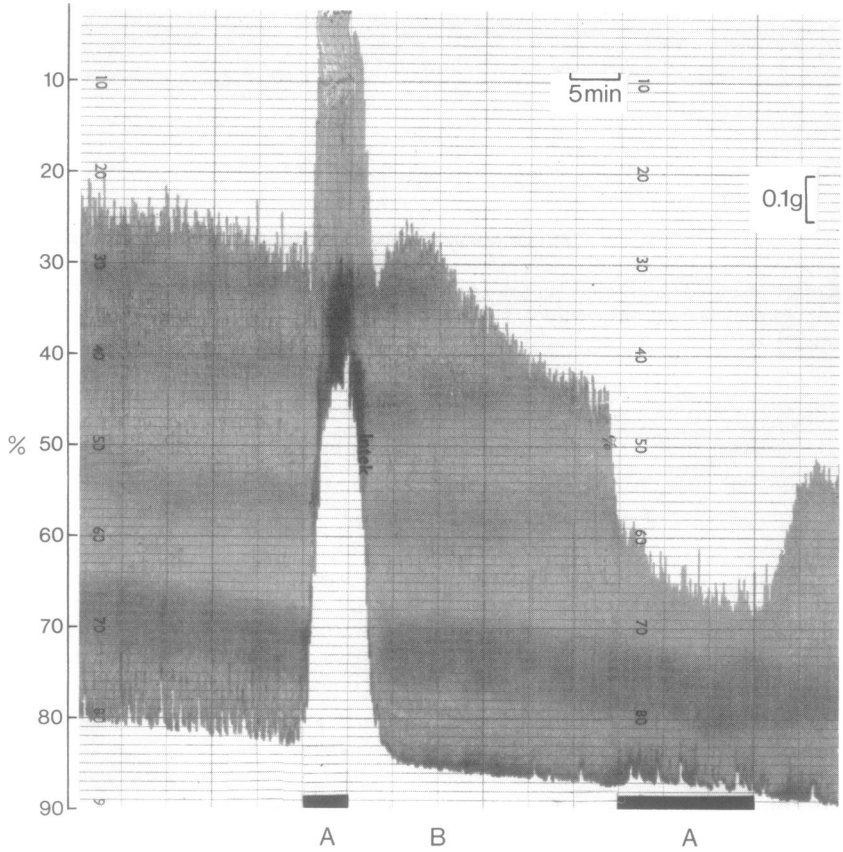

Figure 4 The effect of noradrenaline on contractions of the rat vas deferens. The tissue was stimulated at 10–30 V, 3 ms, at 0.1 Hz; noradrenaline, 1 μg/ml at (A); prazosin HCl, 10 ng/ml, continuously present from (B).

At the concentrations used, yohimbine and phentolamine had no effect on the contractions of the vas deferens but both competitively antagonized the inhibition produced by clonidine. The pA_2 values for the compounds are shown in Table 1. There was no significant difference ($P > 0.05$) between the pA_2 values of phentolamine and yohimbine. Prazosin and phenoxybenzamine did not antagonize the inhibition produced by clonidine at the concentrations studied. Higher concentrations of prazosin and phenoxybenzamine (>100 ng/ml and >10 ng/ml respectively) reduced the contraction height of the vas deferens and consequently the interaction of clonidine with higher concentrations of prazosin or phenoxybenzamine could not be studied.

Determination of postsynaptic α-adrenoceptor antagonist activity

The postsynaptic effects of the same antagonists in the rat anococcygeus muscle were studied using noradrenaline as the postsynaptic agonist. The log concentration-response curves for noradrenaline showed a parallel displacement to the right in the presence of phentolamine, yohimbine and prazosin. With phenoxybenzamine the maximum height attainable was reduced indicating that the antagonism was non-competitive at the concentrations studied. The pA_2 value (Table 2) of yohimbine was significantly different from that of phentolamine ($P < 0.001$) and prazosin ($P < 0.001$). The pA_2 value of phentolamine was significantly different from that of prazosin ($P < 0.05$).

From the experiments outlined in this and the previous section it was clear that prazosin had selectivity for postsynaptic α-adrenoceptors. This action was clearly demonstrated in a vas deferens preparation stimulated at 0.1 Hz, 20 V and 3 ms, in which an attempt was made to demonstrate the presynaptic agonist properties of noradrenaline. In this preparation noradrenaline produced no effect on the vas deferens at concentrations up to 100 ng/ml. At

1 μg/ml, noradrenaline produced a contraction of the vas deferens (Figure 4). When the effect of this concentration of noradrenaline was repeated in the presence of prazosin (10 ng/ml) the postsynaptic effect of noradrenaline was abolished and its presynaptic inhibitory effect unmasked.

Discussion

The majority of investigations into the presynaptic control of transmitter release from sympathetic nerve endings have employed methods which involve the measurement of transmitter overflow using [^{3}H]-noradrenaline (Langer, 1974). The rat vas deferens subjected to low frequency field stimulation provides an alternative method for studying the effects of agonists and antagonists at presynaptic α-adrenoceptors.

Contractions of the vas deferens, induced by low frequency stimulation of the intramural nerves, could be inhibited either presynaptically by agents such as guanethidine or bretylium or by agents which relax smooth muscle such as papaverine. The contractions were also inhibited by clonidine; yohimbine and phentolamine had no effect on the twitch whereas high concentrations of both phenoxybenzamine and prazosin caused inhibition. Clonidine, like guanethidine and bretylium, produced its action presynaptically since in vas deferens preparations where the smooth muscle was stimulated directly all three agents were without effect whereas papaverine caused inhibition of the contractions. The presynaptic action of clonidine was confirmed in an experiment where clonidine, in concentrations that completely blocked contractions induced by stimulation of the intramural nerves of the vas deferens, had no effect on noradrenaline contractions in the same tissue.

Although the inhibitory effects of guanethidine, bretylium and clonidine were presynaptic in origin the mechanisms by which the inhibition of the twitch was produced were different. The inhibition of the 'twitch' produced by clonidine was competitively antagonized by α-adrenoceptor blocking agents e.g. yohimbine and phentolamine. The inhibition of the 'twitch' produced by either guanethidine or bretylium was unaffected by these blocking agents.

It was concluded from these studies that clonidine inhibited contractions of the rat vas deferens by a presynaptic action which involved α-adrenoceptors. When the tissue was used in conjunction with the rat anococcygeus muscle it was possible to compare activity at presynaptic and postsynaptic α-adrenoceptors. The low sensitivity of the vas deferens to noradrenaline coupled with considerable animal variation (Ambache & Aboo Zar, 1971) precluded its use for postsynaptic studies.

In the studies where relative potencies of α-adrenoceptor blocking agents were compared at presynaptic and postsynaptic α-adrenoceptors it was clear that the structural requirements for each receptor were different. The pA_2 value for yohimbine against clonidine on the vas deferens was significantly ($P<0.001$) greater than its value against noradrenaline on the anococcygeus muscle. This result, although obtained by the use of a technique different from that used by Starke *et al.* (1975a), confirmed their finding that yohimbine was the first example of a preferential presynaptic α-adrenoceptor blocking agent. Phentolamine was also more active at presynaptic α-adrenoceptors than postsynaptic receptors, there being a significant difference ($P<0.01$) between its pA_2 values against clonidine and noradrenaline.

Prazosin acted preferentially at the postsynaptic α-adrenoceptor. At the concentrations that it was possible to use, prazosin was devoid of presynaptic activity. There was at least a hundred-fold difference in the activity of prazosin at presynaptic and postsynaptic α-adrenoceptors.

Prazosin, a drug which is thought to have a peripheral action involving direct relaxation of vascular smooth muscle and sympathetic blockade (Pfizer, 1970), was included in this study because Constantine, McShane, Scriabine & Hess (1973) and Wood, Phelan & Simpson (1975) found that prazosin interferes with α-adrenoceptors. The results described here are consistent with these findings since prazosin

Table 2 Drug antagonism at the postsynaptic α-adrenoceptor of the rat anococcygeus muscle

Agonist	*Antagonist*	*Drug parameter*	n
Noradrenaline		pD_2 = 6.60 ± 0.10	12
	Yohimbine	pA_2 = 6.4 ± 0.18	6
	Phentolamine	pA_2 = 7.7 ± 0.17	6
	Prazosin	pA_2 = 8.2 ± 0.11	6
	Phenoxybenzamine	pD'_2 8.3 ± 0.13	9

The Krebs solution contained corticosterone 40 μM, desmethylimipramine 10 nM and propranolol 0.1 μM. The results are expressed as the mean ± s.e. mean.

competitively antagonized noradrenaline contractions on the rat anococcygeus muscle over a wide concentration range. In preparations where the intramural nerves of the vas deferens were stimulated at low frequency, prazosin, at relatively low concentrations ($\simeq$100 ng/ml), caused inhibition of the contractions. It was considered that this action was due to direct relaxation of smooth muscle; however, in vas deferens preparations where the smooth muscle was stimulated directly, prazosin at concentrations up to 10 μg/ml produced no inhibition of the contraction. It would appear therefore that the inhibitory effects of prazosin on contractions of the vas deferens were due to the fact that it is a preferential postsynaptic α-adrenoceptor blocking agent.

Phenoxybenzamine was devoid of presynaptic activity at the concentrations that it was possible to use. Unlike the other antagonists studied, the interaction between noradrenaline and phenoxybenzamine at the postsynaptic α-adrenoceptor was of a non-competitive nature.

In conclusion the results endorse the view that presynaptic and postsynaptic α-adrenoceptors are different and hence it is possible for α-adrenoceptor blocking agents to have differential effects at these receptors.

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