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Studies on RX 781094: a selective, potent and specific antagonist of α_2-adrenoceptors

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1 The selectivity and specificity of RX 781094 [2-(2-(1,4 benzodioxanyl))2-imidazoline HCl] for α-adrenoceptors have been examined in peripheral tissues.

2 In isolated tissue experiments RX 781094 was a competitive antagonist at prejunctional α_2-adrenoceptors situated on the sympathetic nerve terminals of the rat ($pA_2 = 8.56$) and mouse ($pA_2 = 7.93$) vas deferens and on the parasympathetic nerve terminals of the guinea-pig ileum ($pA_2 = 8.55$).

3 Although RX 781094 was also a competitive antagonist at the postjunctional α_1-adrenoceptors of the rat anococcygeus muscle ($pA_2 = 6.10$) its affinity for these receptors was markedly less than that displayed for prejunctional sites. From pA_2 values obtained in the rat vas deferens and anococcygeus muscle the calculated α_2/α_1-adrenoceptor selectivity ratio for RX 781094 was 288.

4 The rank order of α_2/α_1-adrenoceptor selectivities for the antagonists studied was RX 781094 > RS 21361 > yohimbine > piperoxan > phentolamine > WB 4101 > prazosin.

5 RX 781094 had extremely low affinity for β-adrenoceptors, histamine receptors, cholinoceptors, 5-hydroxytryptamine and opiate receptors *in vitro*.

6 In pithed rats, intravenous administration of RX 781094 antagonized the prejunctional α_2-adrenoceptor agonist effects of clonidine and guanabenz on electrically-induced contractions of the vas deferens and anococcygeus muscle respectively.

7 In the vas deferens the rank order of α_2-adrenoceptor antagonist potencies was RX 781094 > phentolamine > piperoxan > yohimbine > RS 21361 > WB 4101. Only RX 781094, yohimbine and RS 21361 were active against guanabenz in the anococcygeus muscle.

8 In the pithed rat, RX 781094 preferentially antagonized the pressor responses evoked by postjunctional α_2-adrenoceptor activation by UK 14,304 although higher doses also inhibited the effects of phenylephrine and cirazoline at postjunctional α_1-adrenoceptors.

9 RX 781094 had little effect on the cardiovascular responses to 5-hydroxytryptramine, angiotensin II, histamine, acetylcholine and isoprenaline in pithed rats and rats anaesthetized with pentobarbitone.

10 These results demonstrate that RX 781094 is a potent and selective α_2-adrenoceptor antagonist with a high degree of specificity for these receptors.

Introduction

Clinical applications for selective α_1-adrenoceptor agonists (e.g. phenylephrine), α_1-adrenoceptor antagonists (e.g. prazosin) and α_2-adrenoceptor agonists (e.g. clonidine) have been established (Starke & Docherty, 1981) although the designation of these selectivity profiles was retrospective. In contrast, the clinical applications of selective α_2-adrenoceptor antagonists are relatively unexplored due to the lack of suitable drugs. Although yohimbine has been shown to act preferentially at α_2-adrenoceptors (Starke, Borowski & Endo, 1975) its selectivity is markedly less than that of prazosin for α_1-adrenoceptors (Chapleo, Doxey, Myers & Roach, 1981) and, moreover, it lacks receptor specificity (for references see Scatton, Zivkovich & Dedek, 1980). Selective α_2-adrenoceptor antagonist properties have been described recently in two series of benzodioxans (Mouillé, Dabiré, Andréjak & Schmitt, 1980; Michel & Whiting, 1981; Chapleo *et al.*, 1981) and in a series of substituted benzoquinolizines (Lattimer, Rhodes, Ward, Waterfall & White, 1982).

This paper describes the α_2-adrenoceptor selectivi-

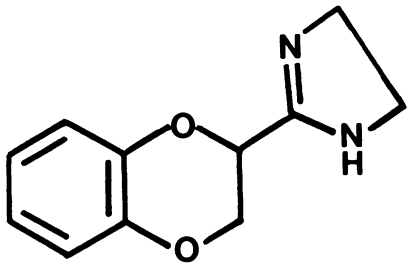

Figure 1 Chemical structure of RX 781094.

ty and specificity of RX 781094 [2-(2-(1,4 benzodioxanyl))-2-imidazoline HCl, Figure 1] in peripheral tissues. A comparison is made between the profiles of RX 781094 and several classical α-adrenoceptor antagonists as well as the recently reported antagonist RS 21361 (Michel & Whiting, 1981). Preliminary findings have been presented to the British Pharmacological Society (Chapleo *et al.*, 1981).

Methods

In vitro *experiments*

Except where stipulated, all experiments were carried out in Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 0.6, $NaHCO_3$ 25 and dextrose 11.1. The Krebs solution was gassed with 95% O_2 and 5% CO_2 and maintained at 30°C in all experiments except for those using the rat anococcygeus muscle which were carried out at 37°C. Contractions of the isolated tissues were measured isometrically with Statham Gold cell (UC 3) transducers (except where stated in the methods) and displayed on Smith Servoscribe pen recorders. Tissues obtained from male rats (Sprague-Dawley, 200–250 g), adult mice (MFI, > 30 g) and male guinea-pigs (Dunkin-Hartley 0.4–0.7 kg) were used in isolated tissue experiments.

α_2-Adrenoceptor antagonist potency

Rat vas deferens Prejunctional α_2-adrenoceptor antagonist potencies were determined against clonidine using the prostatic section of the rat vas deferens (Doxey, Smith & Walker, 1977).

Mouse vas deferens Vasa deferentia were set up in a 50 ml organ bath containing magnesium-free Krebs solution. The preparations were field stimulated between platinum electrodes at 0.1 Hz with rectilinear pulses of 3.0 ms width. The voltage (100–140 V) was adjusted to give a twitch response of approximately 100 mg. Repeated cumulative concentration-response curves to the inhibitory effects of clonidine were constructed until consistent ED_{50} values were obtained. A fixed concentration of RX 781094 was then included in the Krebs solution and concentration-response curves to clonidine constructed until consistent ED_{50} values were again obtained.

Guinea-pig ileum Sections of the terminal ileum (2–3 cm in length) were suspended in a 50 ml organ bath under an initial tension of 1 g. The tissues were stimulated transmurally at a frequency of 0.1 Hz using a pulse width of 3.0 ms at supramaximal voltage. Propranolol (1 μM) and prazosin (70 nM) were present throughout the experiments. The effects of increasing concentrations of RX 781094 on noradrenaline inhibitory effects were studied; competitive antagonist potency was expressed as a pA_2 value.

α_1-Adrenoceptor antagonist potency in rat anococcygeus muscle Postjunctional α_1-adrenoceptor antagonist potencies were determined against noradrenaline in the rat anococcygeus muscle (Doxey *et al.*, 1977).

Presynaptic opiate receptors in mouse vas deferens The mouse vas deferens was set up and used as described above except that clonidine was replaced by normorphine.

Nicotinic and 5-hydroxytryptamine responses in the guinea-pig ileum Sections of the ileum were connected to isotonic transducers (Sangamo type DC/DC) and superfused (3 ml/min) with Krebs solution. Submaximal contractions of the tissue to dimethylphenylpiperazinium iodide (DMPP, 0.5 μg) and 5-hydroxytryptamine (5-HT) (1.8–3.7 μg) were elicited by injecting these agonists into the flow of Krebs at 3 min intervals. When the responses of the two agonists had become constant the effects of RX 781094 (12 or 40 μM) on these responses were studied. A contact time of 30 min was allowed for each concentration of RX 781094 to reach equilibrium. The results were expressed as the percentage inhibitions of control responses. Three separate experiments were performed and the results expressed as the mean together with the s.e.mean.

Muscarinic responses in the guinea-pig ileum Sections of guinea-pig ileum were suspended in a 50 ml static organ bath and connected to an isotonic transducer. All experiments were performed in the presence of hexamethonium (73 μM). Cumulative concentration-response curves were constructed to acetylcholine and antagonism of these responses was used to determine a pA_2 value for RX 781094 at

muscarinic receptors. A further series of experiments was also carried out in which neostigmine (100 nM) was also included in the Krebs solution.

Histamine responses in guinea-pig ileum Antagonism of histamine-induced contractions of the guinea-pig ileum was used to determine a pA_2 for RX 781094 at histamine (H_1)-receptors. The experimental conditions were identical to those described for muscarinic receptors except that the bath-fluid was normal Krebs solution.

β-Adrenoceptor and histamine (H_2)-responses in guinea-pig atria Right and left atria were placed under an initial resting tension of 0.5 g. The right atrium was allowed to beat spontaneously while the left atrium was paced at a frequency of 1.0 Hz using continuous field stimulation between two platinum electrodes (30 V, 1.0 ms pulse width, SRI stimulator). The effects of RX 781094 (100 μM) on isoprenaline responses were studied in both right and left atria whereas histamine responses were only studied in right atria. After a 60 min stabilization period, cumulative concentration-response curves to the agonists were constructed. The preparations were then washed several times and the agonist concentration-response curves repeated after incubation with RX 781094 (100 μM) for 20 min. It was established that cimetidine (3 μM) inhibited the effects of histamine and was without effect on isoprenaline; conversely, propranolol (1 μM) antagonized only the isoprenaline responses.

β_2-Adrenoreceptor responses in guinea-pig tracheal chain The trachea was removed and tracheal chains prepared as in the method of Foster (1960). The chain was suspended in a 30 ml organ bath and subjected to an initial tension of 0.5 g. Following a stabilization period of 60 min the tracheal muscle was contracted with a submaximal concentration of carbachol (0.3 μM). After a sustained contraction had been obtained, a cumulative concentration-response curve to the relaxant effects of isoprenaline was constructed. After a suitable recovery period, a second response to carbachol was established. RX 781094 (100 μM) was then added to the bathing fluid and left in contact with the tissue for 20 min. Invariably RX 781094 caused a relaxation of the preparation due to an inhibitory action against carbachol. The tone of the preparation was restored by increasing the concentration of carbachol to 1–2 μM and a cumulative concentration-response curve to isoprenaline was constructed in the presence of RX 781094. The relaxant responses to isoprenaline before and after RX 781094 were expressed as a percentage of the control maximum isoprenaline response.

Presentation of in vitro *results* Agonist potencies were expressed as pD_2 values and antagonist potencies as either pA_2 or pD_2' values (Van Rossum, 1963). Schild plots of log. (concentration ratio – 1) against negative log. (molar concentration of the antagonist) were also constructed (Arunlakshana & Schild, 1959) and the slopes of the plot and the correlation coefficients calculated. Results from individual preparations were measured and shown with their respective standard error.

The selectivity ratio of an antagonist at α_2- and α_1-adrenoceptors was calculated fom the antilogarithm of the difference between the pA_2 values obtained at α_2- and α_1-adrenoceptors.

In vivo *experiments*

Pithed rats Male rats (Sprague-Dawley, in the weight range 275–350 g) were pithed during a brief period of halothane (4% v/v in room air) anaesthesia. The animals were subsequently artificially respired (100 strokes/min; 1 ml/100 g body weight; Palmer Small Animal Respirator) with room air.

The left common carotid artery and a femoral vein were cannulated for blood pressure measurement and intravenous drug administration respectively. The arterial blood pressure was measured with a Hewlett Packard pressure transducer (H.P. 1280) and recorded on a 4 channel pen recorder (H.P. 7754 B). In experiments in which heart rate was measured the arterial pressure pulse was used to trigger a ratemeter. All animals were bivagotomised and the uncannulated carotid artery ligated.

Vas deferens and anococcygeus muscle The vas deferens or anococcygeus muscle were dissected free from connective tissue and attached to an isometric transducer (Statham Gold Cell, UC3). Both tissues were initially placed under a tension of 0.5 g. Contractions of the vas deferens and the anococcygeus muscle were evoked by electrical stimulation of the sympathetic outflow (Digitimer Stimulator DS9 or S.R.I. stimulator) via the pithing rod. The stimulation parameters for the vas deferens were 40 V, 50 μs pulse width, 6 Hz for 2 s every 30 s and for the anococcygeus they were 40 V, 500 μs pulse width, 1 Hz for 20 s every 3 min. Contractions of the tissues were recorded on a 4 channel pen recorder (H.P. 7754B). All rats were pretreated with atropine (1.0 mg/kg, i.v.) and (+)-tubocurarine (1.0 mg/kg, i.v.); exceptions are described in the methodology.

Series 1: Stimulation-induced contractions of the vas deferens and anococcygeus muscle were completely inhibited by clonidine (100 μg/kg, i.v.) and guanabenz (30 μg/kg, i.v.), respectively. Increasing intravenous doses of the antagonist were injected

5–7 min after the administration of the agonists. Antagonist doses were initially given at 3 min (vas deferens) and 6 min (anococcygeus muscle) intervals until reversal effects were observed against the inhibition produced by the antagonists. Subsequent antagonist doses were administered when the effect of the previous dose level had attained a plateau. The effects of the cumulative doses of the antagonists were expressed as percentage reversals of the inhibitory response to clonidine and guanabenz. Cumulative antagonist-response curves were plotted and the antagonist potency was assessed by determining the cumulative dose which produced a 50% reversal of the response to either clonidine or guanabenz. The mean AD_{50} values (mg/kg, i.v.) ± s.e.mean were determined from the individual log. dose-response curves. The antagonists used to reverse the effects of the α_2-adrenoceptor agonists in the vas deferens and anococcygeus muscle were RX 781094, yohimbine, piperoxan, phentolamine, RS 21361, WB 4101 and prazosin.

Series 2: Experiments using the stimulated anococcygeus muscle were performed as above with the exception that guanabenz was replaced with either the adrenergic neurone blocker, guanethidine (1.0 mg/kg, i.v.) or the ganglion blocker, mecamylamine (1.0 mg/kg, i.v.). After inhibition of the stimulation-induced contractions by the above treatments, increasing doses of RX 781094 (0.001–1.0 mg/kg, i.v.) were injected. In the experiments using guanethidine, amphetamine (0.1–0.3 mg/kg, i.v.) was injected 5 min after the 1 mg/kg, i.v. dose of RX 781094.

Series 3: Separate groups of pithed rats ($n = 6–7$) were prepared for the measurement of stimulation-induced contractions of the vas deferens. After the contractions had stabilized, the rats were given either saline (1.0 ml/kg, i.v.), yohimbine (1.0 or 3.0 mg/kg, i.v.) or RX 781094 (0.3 or 1.0 mg/kg, i.v.) and 5 min later a cumulative dose-inhibitory response curve to clonidine was constructed. The cumulative doses of clonidine inhibiting the stimulation-evoked twitch response of the vas deferens by 50% (ED_{50} μg/kg) were calculated from the individual log dose-response curves. Mean clonidine ED_{50} values ± s.e.mean for each treatment were calculated. Diastolic blood pressure responses to clonidine were also measured in these experiments. The clonidine pressure-response curves were plotted non-cumulatively since the pressor responses were of shorter duration than the prejunctional effects on the vas deferens.

Table 1 α-Adrenoceptor antagonist properties of RX 781094 and standard agents

	α_2-Antagonism (rat vas deferens)		*α_1-Antagonism (rat anococcygeus)*		
Compound	pA_2 vs clonidine	Slope Schild plot (Corr. coefficient)	pA_2 vs noradrenaline	Slope Schild plot (Corr. coefficient)	α_2/α_1 ratio
RX 781094	8.56 ± 0.05	0.85 ± 0.04 (0.98)	6.10 ± 0.05	0.83 ± 0.09 (0.92)	288
RS 21361	6.98 ± 0.02	1.1 ± 0.03 (0.99)	4.85 ± 0.07 $pD'_2 = 3.17 \pm 0.01$	0.75 ± 0.15 (0.84)	135
Yohimbine	8.14 ± 0.05	0.92 ± 0.10 (0.97)	6.49 ± 0.06	0.84 ± 0.07 (0.96)	45
Piperoxan	7.72 ± 0.03	0.96 ± 0.04 (0.98)	6.61 ± 0.08	0.85 ± 0.09 (0.89)	13
Phentolamine	8.38 ± 0.09	0.81 ± 0.16 (0.93)	7.70 ± 0.17	1.00 ± 0.36 (0.81)	5
WB 4101	6.89 ± 0.08	1.25 ± 0.08 (0.97)	8.34 ± 0.12	0.87 ± 0.13 (0.89)	0.04
Prazosin	5.94 ± 0.10	—	8.18 ± 0.11	0.73 ± 0.13 (0.91)	0.006

The results for pA_2 values are the mean of a minimum of 6 determinations ± s.e.mean. The pD_2 values for clonidine ranged from 8.7–8.9 and for noradrenaline from 6.4–6.6.

Pressor responses to various constrictor agents The effects of saline, RX 781094 and yohimbine on the pressor responses (increases in diastolic blood pressure of between 50–70 mmHg) to single intravenous doses of several vasoconstrictor agents were studied in pithed rats pretreated with atropine (1.0 mg/kg, i.v.) and propranolol (1.0 mg/kg, i.v.). Since the number of pressor agents was too numerous to inject consecutively in the same experiment three groups of experiments were performed. The pressor agents studied were (group 1) noradrenaline (0.3 μg/kg, i.v.), phenylephrine (5 μg/kg, i.v.), (group 2) cirazoline (1 μg/kg, i.v.), 5-HT (0.1 mg/kg, i.v.), (group 3), UK 14,304 (10 μg/kg, i.v.), tyramine (0.3 mg/kg, i.v.) and angiotensin II (0.3 μg/kg, i.v.). After obtaining consistent pressor responses to noradrenaline (0.3 μg/kg, i.v., normally 2–3 challenges being sufficient) in all experiments, control responses to the agonists were established and then repeated 5 min after two subsequent injections of saline (1.0 ml/kg, i.v.), RX 781094 (0.3 and 1.0 mg/kg, i.v.) or yohimbine (1.0 and 3.0 mg/kg, i.v.).

Anaesthetized rats Male Sprague-Dawley rats (weighing 300–350 g) were anaesthetized with sodium pentobarbitone (60 mg/kg, i.p.). The rats were bilaterally vagotomized and prepared for the measurement of arterial blood pressure and heart rate as well as the intravenous administration of drugs as previously described for the pithed rat. The experimental design was identical to that described above with the exception that the vasodepressor responses to acetylcholine (1 μg/kg, i.v.), isoprenaline (0.1 μg/kg, i.v.) and histamine (3 μg/kg, i.v.) were studied. The tachycardic responses to isoprenaline were also monitored.

Analysis of in vivo *data* Analysis of variance was used to investigate the significance of the effects of various antagonists (and saline) on the pressor and depressor responses in pithed and anaesthetized rats, respectively. In series 3 an unpaired *t* test was used to compare the clonidine ED_{50} values after the antagonists to that obtained after saline. $P < 0.05$ was regarded as the level of significance in all cases.

Drugs

RX 781094 exists as two optical isomers; the racemic mixture was used in the present studies. Other drugs used were: acetylcholine chloride (Sigma), (+)-amphetamine sulphate (Sigma), angiotensin II (CIBA), atropine sulphate (Burroughs Wellcome), carbachol (B.D.H.), cimetidine (Tagamet injection S.K. and F.), cirazoline hydrochloride (Synthélabo), clonidine hydrochloride (Boehringer Ingelheim and Bonapace), corticosterone (Sigma), desmethylimipramine hydrochloride (Geigy), dimethylphenylpiperazinium iodide (Fluka A.G.), guanabenz acetate (Wyeth), guanethidine sulphate (CIBA), hexamethonium chloride (Koch Light), histamine acid phosphate (B.D.H.), 5-hydroxytryptamine creatinine acid phosphate (Sigma), isoprenaline sulphate (Burroughs Wellcome), mecamylamine hydrochloride (Merck, Sharp and Dohme), neostigmine bromide (Sigma), (−)-noradrenaline bitartrate (Koch Light), normorphine (synthesized in Medicinal Chemistry Department, Reckitt and Colman), phenylephrine hydrochloride (Koch-Light), piperoxan hydrochloride (synthesized in the Medicinal Chemistry Department, Reckitt and Colman), prazosin hydrochloride (Pfizer), (±)-propranolol hydrochloride (I.C.I.), RS 21361 ([2-(1-ethyl-2-imidazolylmethyl)-1,4-benzodioxan hydrochloride], Syntex), (+)-tubocurarine chloride (Burroughs Wellcome), tyramine hydrochloride (Sigma), UK 14,304 tartrate (Pfizer), WB 4101 (Ward Blenkinsop) and yohimbine hydrochloride (Sigma).

All drugs solutions were made up in either distilled water or 0.9% w/v sodium chloride solution (saline). Corticosterone was dissolved in propylene glycol. All doses in the text are in terms of the respective salts.

Results

In vitro *experiments*

Determination of prejunctional α_2-adrenoceptor antagonist potency

Rat vas deferens The rat isolated vas deferens, bathed in Krebs solution containing corticosterone (40 μM), propranolol (100 nM) and desipramine (10 nM) and stimulated at a frequency of 0.1 Hz, was used to study the effects of RX 781094 and several other antagonists on cumulative concentration-response curves to clonidine. All of the compounds were competitive antagonists and their potencies were expressed as pA_2 values (Table 1). RX 781094 produced parallel, concentration-dependent shifts to the right of the clonidine concentration-response curve (Figure 2a) the calculated pA_2 value was 8.56 ± 0.05. A plot of log (concentration-ratio − 1) against log (antagonist concentration) gave a linear regression with a slope of 0.85 ± 0.06 (Table 1). RX 781094 was more potent than both yohimbine and RS 21361 which had pA_2 values of 8.14 ± 0.05 and 6.98 ± 0.02 respectively. A Schild plot could not be constructed for prazosin since the effects of this compound could only be studied at a single concentration (2.4 μM). Lower concentrations of prazosin

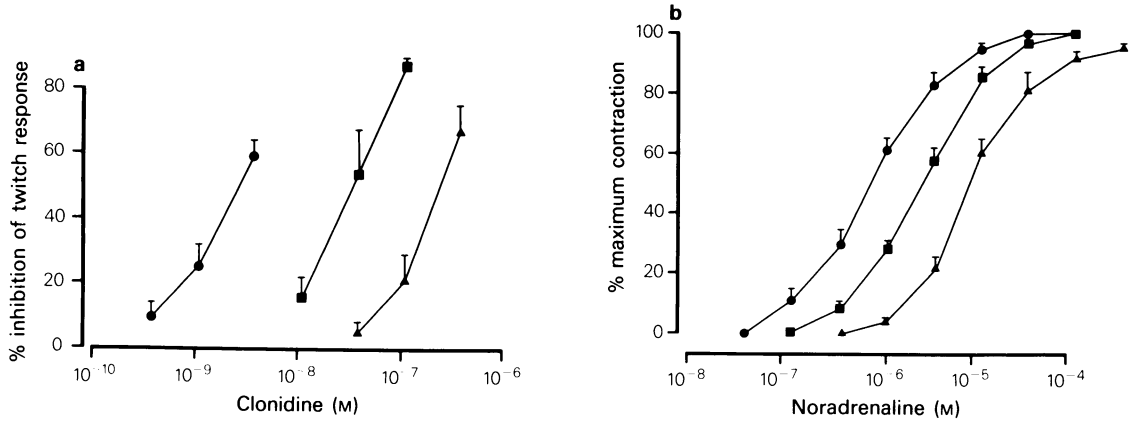

Figure 2 (a) Antagonism by RX 781094 of the inhibitory effects of clonidine in the stimulated (0.1 Hz) rat vas deferens. The results are the mean of 5 experiments for clonidine alone (●) and clonidine in the presence of 42 nM (■) and 420 nM (▲) RX 781094; vertical lines show s.e.mean. (b) Antagonism by RX 781094 of the contractile effects of noradrenaline on the rat anococcygeus muscle. (●) Noradrenaline alone and in the presence of 1.26 μM (■) and 12.6 μM (▲) RX 781094. The results are the mean of 4 experiments; vertical lines show s.e.mean. The stimulation parameters for the vas deferens were 40 V, 0.1 Hz, 3 ms. The Krebs solution contained corticosterone (40 μM), propranolol (100 nM) and desipramine (10 nM).

were ineffective whereas higher concentrations caused marked inhibition of the twitch response. One series of experiments was performed in Krebs solution which did not contain desipramine, propranolol or corticosterone. In these experiments RX 781094 had a pA_2 value of 8.44 ± 0.08 ($n = 6$). In the concentrations used none of the antagonists affected baseline tone of the vas deferens.

Mouse vas deferens The concentration-response curves to the inhibitory effects of clonidine were also displaced to the right by RX 781094 in the mouse vas deferens; the calculated pA_2 value being 7.93 ± 0.15 ($n = 3$).

Table 2 The effects of RX 781094 on a number of isolated receptor systems

Receptor subtype	*Preparation*	*Agonist*	*Antagonist effect*
	Rat vas deferens	Clonidine	pA_2 8.56 ± 0.05
α_2	Mouse vas deferens	Clonidine	pA_2 7.93 ± 0.15
	Guinea-pig ileum	Noradrenaline	pA_2 8.55 ± 0.08
α_1	Rat anococcygeus	Noradrenaline	pA_2 6.10 ± 0.05
Presynaptic opiate	Mouse vas deferens	Normorphine	> 100 μM
H_1	Guinea-pig ileum	Histamine	pD'_2 4.81 ± 0.04
H_2	Guinea-pig atria	Histamine chronotropism	pD'_2 3.73 ± 0.04
Muscarinic	Guinea-pig ileum	Acetylcholine	pA_2 4.84 ± 0.04
Nicotinic	Guinea-pig ileum	DMPP	12 μM 31 ± 12% inhibition 40 μM 70 ± 11% inhibition
Neuronal 5-HT	Guinea-pig ileum	5-HT	12 μM 45 ± 12% inhibition 40 μM 66 ± 6% inhibition
β_1	Guinea-pig atria	Isoprenaline Chronotropism Inotropism	 pD'_2 3.41 ± 0.07 > 100 μM
β_2	Guinea-pig trachea	Isoprenaline	> 100 μM

Guinea-pig ileum The effects of RX 781094 on prejunctional α_2-adrenoceptors located on cholinergic nerves were studied in the transmurally stimulated guinea-pig ileum. In these experiments the Krebs solution contained propranolol (1 μM) and prazosin (70 nM). Noradrenaline produced concentration-dependent inhibitions of the contractile responses of the ileum, the calculated ED_{40} value being 0.15 ± 0.03 μM ($n = 5$). RX 781094 competively antagonized the inhibitory effects of noradrenaline and its mean pA_2 value in 5 preparations was 8.55 ± 0.08.

Determination of postjunctional α_1-adrenoceptor antagonist potency and selectivity ratios The effects of the same series of antagonists on postjunctional α_1-adrenoceptors were studied in the rat anococcygeus muscle using noradrenaline as the agonist. The tissue was bathed in Krebs solution which contained corticosterone (40 μM), propranolol (100 nM) and desipramine (10 nM). The log concentration-response curves to noradrenaline showed a parallel displacement to the right with all of the antagonists. However the interaction between noradrenaline and high concentrations (36–110 μM) of RS 21361 appeared to be non-competitive in nature since the maximum response attainable was reduced (approx. 15% at 110 μM). The influence of RX 781094 on noradrenaline contractile responses is illustrated in Figure 2b. The pA_2 value for RX 781094 was 6.10 ± 0.05; the pA_2 values for the other antagonists at postjunctional α_1-adrenoceptors are shown in Table 1. None of the antagonists tested affected baseline tone of the anococcygeus muscle.

From the results obtained in this tissue and the rat vas deferens it was possible to compare the selectivity of the individual antagonists for α_2-adrenoceptors (Table 1). RX 781094 was the most selective antagonist in the present studies and has a ratio (α_2/α_1) of 288. Although RS 21361 had low potency at prejunctional α_2-adrenoceptors it was more selective for these receptors than yohimbine (Table 1). Piperoxan and phentolamine showed only a marginal selectivity towards prejunctional α_2-adrenoceptors and WB 4101 and prazosin were selective α_1-adrenoceptor antagonists. RX 781094 in concentrations up to 42 μM failed to affect the resting tension of the anococcygeus muscle.

Other receptors RX 781094 only produced effects on the other receptor systems studied at concentrations which were 3–4 orders of magnitude greater than those acting at prejunctional α_2-adrenoceptors; the results are summarized in Table 2.

In vivo *experiments*

Antagonism of α_2-adrenoceptor agonist effects in the stimulated vas deferens and anococcygeus muscle of pithed rats

Vas deferens In control experiments clonidine

Table 3 Relative potencies of RX 781094 and six standard α-adrenoceptor antagonists at prejunctional α_2-adrenoceptors in the pithed rat

	α_2-Adrenoceptor antagonist potencies AD_{50} (μg/kg, i.v.)			
Antagonists	Vas deferens 50% reversal of clonidine (100 μg/kg)		Anococcygeus muscle 50% reversal of guanabenz (30 μg/kg)	
RX 781094	18 ± 2	(15)	5 ± 1	(6)
Yohimbine	775 ± 149	(11)	109 ± 33	(6)
RS 21361	5,661 ± 1,512	(5)	660 ± 109	(6)
Phentolamine	131 ± 71	(5)	No reversal (1 mg/kg)	(4)
Piperoxan	543 ± 106	(4)	No reversal (10 mg/kg)	(3)
WB 4101	9,050 ± 2,786	(3)	No reversal (3 mg/kg)	(4)
Prazosin	No reversal (3 mg/kg)	(5)	No reversal (1 mg/kg)	(4)

Cumulative i.v. doses (μg/kg) of the antagonists reversing the inhibitory effects of clonidine and guanabenz on stimulation responses by 50% (AD_{50}) in the vas deferens and anococcygeus muscle respectively, are shown. The values in parentheses represent the group numbers. The stimulation parameters were 40 V, 6 Hz, 50 μs for 2 s every 30 s in the vas deferens and 40 V, 1 Hz, 500 μs for 20 s every 3 min in the anococcygeus muscle.

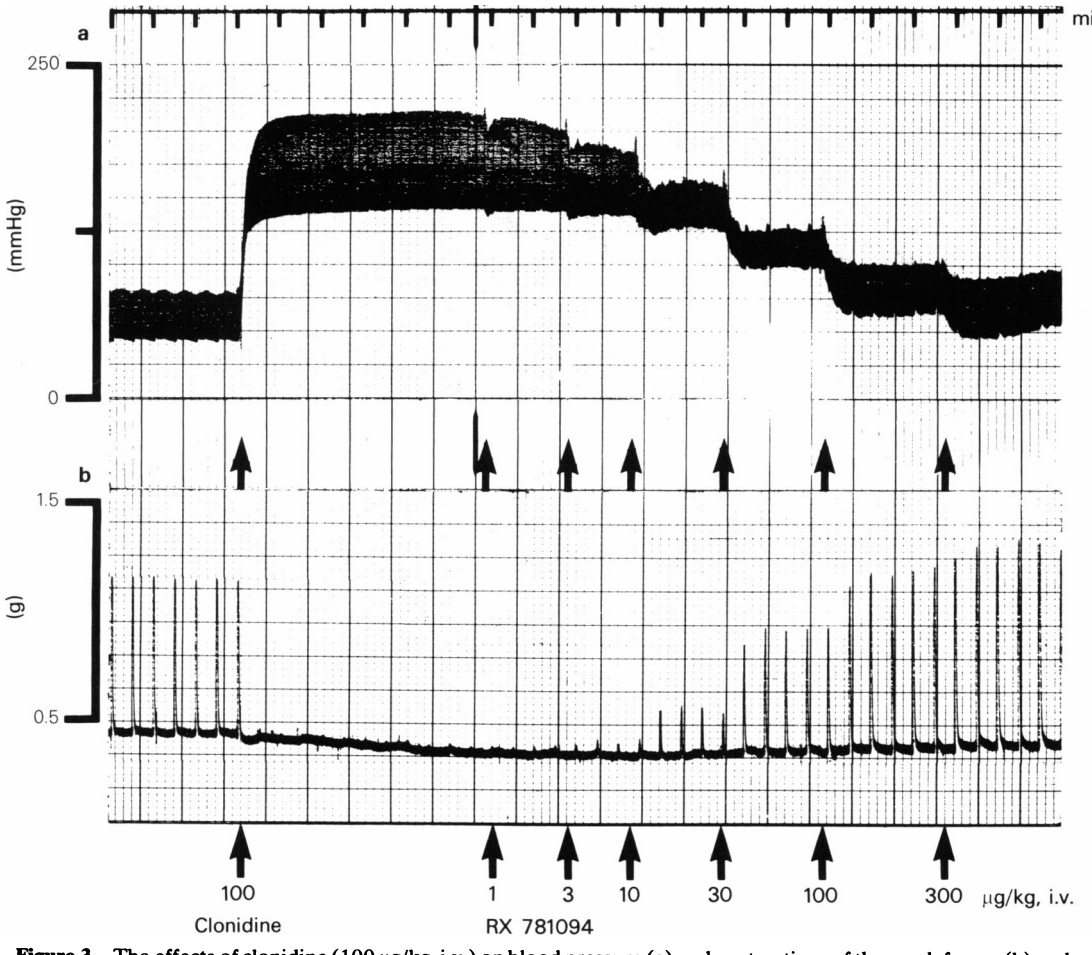

Figure 3 The effects of clonidine (100 μg/kg, i.v.) on blood pressure (a) and contractions of the vas deferens (b) and its reversal by increasing intravenous doses of RX 781094 in pithed rat. The hypogastric nerves were stimulated via the pithing rod using stimulation parameters of 6 Hz, 50 μs and 40 V for 2 s every 30 s.

(100 μg/kg, i.v.) produced a complete and prolonged (> 30 min) inhibition of stimulation-evoked twitch responses of the rat vas deferens. All of the antagonists tested, with the exception of prazosin, reversed the inhibitory effects of clonidine (Table 3). RX 781094 was the most potent compound in this respect being approximately 43 times more potent than yohimbine (Table 3). A typical example of the efficacy of RX 781094 in reversing clonidine can be seen in Figure 3. A comparison of the reversal effects of RX 781094 and yohimbine on the vas deferens is shown in Figure 4a. The rank order of antagonist potencies producing 50% reversal of clonidine's prejunctional α_2-adrenoceptor stimulant effect was RX 781094 > phentolamine > piperoxan > yohimbine > RS 21361 > WB 4101 (Table 3). Amongst these compounds, only WB 4101 failed to produce complete reversal of the inhibitory effects of clonidine; at a cumulative intravenous dose of 14.4 mg/kg, WB 4101 produced 71% reversal.

Anococcygeus muscle RX 781094, yohimbine and RS 21361 were the only antagonists that produced full reversal of the inhibitory effects of guanabenz (30 μg/kg, i.v.) on the stimulation-induced contractions of the anococcygeus muscle (Table 3). RX 781094 was approximately 22 times more potent than yohimbine which was in turn 6 times more potent than RS 21361 against guanabenz. A comparison of the reversal effects of RX 781094 and yohimbine in the anococcygeus muscle is shown in Figure 4b. The remaining compounds failed to produce any antagonism of guanabenz. In contrast to its effects on guanabenz, RX 781094 (1–1000 μg/kg)

failed to influence the blockade of the stimulation-evoked contractions of the anococcygeus muscle produced by the ganglion blocking agent, mecamylamine (1.0 mg/kg) or the adrenergic neurone blocker, guanethidine (1.0 mg/kg). Amphetamine (0.1–0.3 mg/kg, i.v.) readily reversed the blockade produced by guanethidine.

Comparison of the effects of RX 781094 and yohimbine at prejunctional α_2-adrenoceptors in the vas deferens and various receptors in the cardiovascular system of the rat.

Prejunctional α_2-adrenoceptors in vas deferens of the pithed rat RX 781094 (0.3 and 1.0 mg/kg) and yohimbine (1.0 and 3.0 mg/kg) potentiated the stimulation-evoked twitch response of the vas deferens (Table 4). Saline was without effect. Yohimbine produced dose-related increases in the tension developed by the vas deferens, whereas the 0.3 mg/kg dose of RX 781094 produced a greater potentiation than 1.0 mg/kg, RX 781094 (Table 4).

Both RX 781094 and yohimbine produced a dose-related competitive antagonism of the inhibitory effects of clonidine in the rat vas deferens (Figure 5a). The clonidine ED_{50} values (cumulative i.v. doses of clonidine producing 50% inhibition of the stimulation-induced twitch response) after saline and the two antagonists are listed in Table 4. RX 781094 (0.3 mg/kg) produced a significantly greater displacement of the clonidine dose-response curve than was observed with yohimbine (1.0 mg/kg). However, the effects of RX 781094 (1.0 mg/kg) and yohimbine (3.0 mg/kg) against clonidine were not significantly different. Under these experimental conditions, RX 781094 was approximately 3.5–6.5 times more potent than yohimbine in antagonising the clonidine dose-response curve.

Vasoconstrictor responses in pithed rats From the vas deferens experiments described above the effects of RX 781094 and yohimbine on resting blood pressure

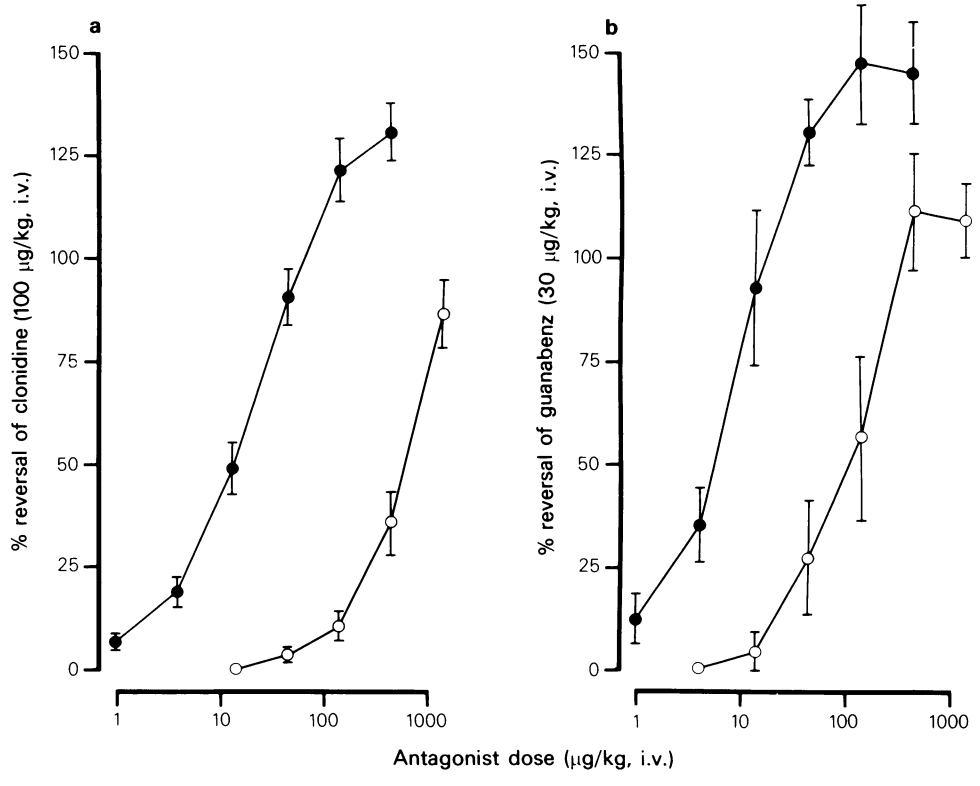

Figure 4 Antagonism of the inhibitory effects of clonidine (100 μg/kg, i.v.) and guanabenz (30 μg/kg, i.v.) by RX 781094 (●) and yohimbine (○) in the electrically stimulated vas deferens (a) and anococcygeus muscle (b) of the pithed rat, respectively. The results are the mean of groups of 5–6 rats, vertical lines show s.e.mean. The stimulation parameters used were 6 Hz, 50 μs, 40 V for 2 s every 30 s for the vas deferens and 1 Hz, 500 μs, 40 V for 20 s every 3 min for the anococcygeus muscle.

Table 4 Pithed rat preparation: the effects of saline, RX 781094 (0.3 and 1.0 mg/kg) and yohimbine (1.0 and 3.0 mg/kg) on the contractions of the vas deferens produced by electrical stimulation (40 V, 50 μs pulse width, 6 Hz for 2 s every 30 s) of the spinal cord

			Tension (mg)				
Treatment	*Dose* (ml/kg or mg/kg)	n	*Initial tension developed*	*Max. tension developed after antagonist*	*Increase produced by antagonist*	*Tension 5 min after treatment antagonist alone*	*Clonidine* ED_{50} (μg/kg, i.v.) (cumulative dose producing 50% inhibition of twitch)
Control (saline)	1.0	6	477 ±56	—	—	467 ±49	6 ±1
RX 781094	0.3	6	469 ±58	895 ±91	383 ±90	491 ±78	74 ±1
	1.0	6	461 ±36	663 ±43	201 ±15	551 ±36	169 ±27
Yohimbine	1.0	7	458 ±35	651 ±60	194 ±32	631 ±59	37 ±6
	3.0	7	492 ±65	790 ±69	297 ±25	729 ±68	146 ±21

Cumulative dose-response curves to clonidine were constructed 5 min after administration of saline, RX 781094 or yohimbine. The cumulative i.v. doses of clonidine inhibiting the contractions of the vas deferens by 50% (ED_{50}) after the respective treatments are shown.

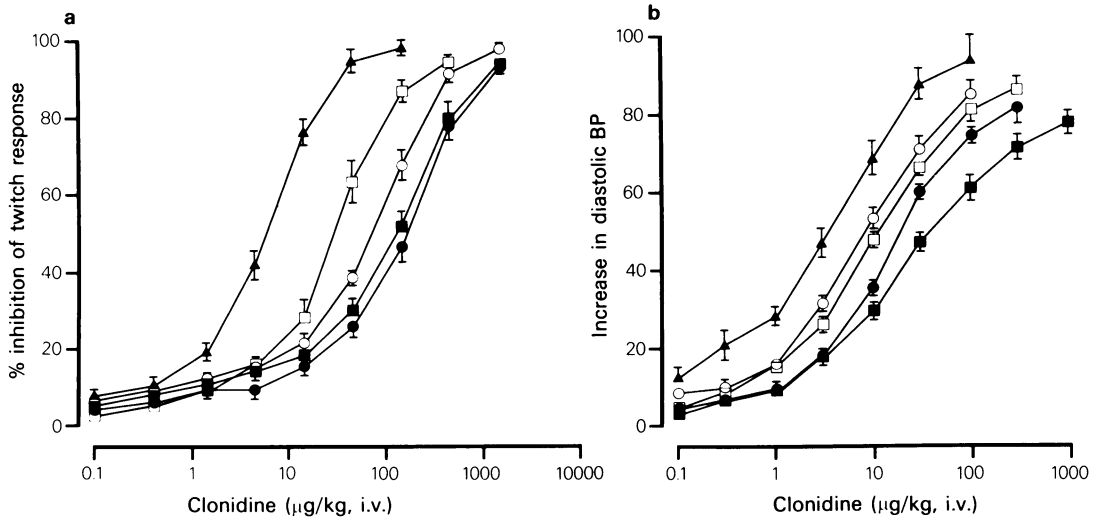

Figure 5 Clonidine dose-response curves in pithed rats pretreated with saline (1.0 ml/kg, ▲), RX 781094 (0.3 mg/kg, ○ or 1.0 mg/kg, ●) or yohimbine (1.0 mg/kg, □ or 3.0 mg/kg ■). The inhibitory effects of clonidine on the contractions of the vas deferens induced by electrical stimulation (6 Hz, 50 μs duration, 40 V for 2 s every 30 s) of the spinal cord (a) and diastolic blood pressure increases produced by clonidine in the same experiments (b) are shown. The effects of clonidine on the vas deferens are plotted as cumulative dose-response curves whereas the blood pressure-response curves are plotted non-cumulatively. Individual points on the dose-response curves represent means values obtained from groups containing 5–7 rats; vertical lines show s.e.mean.

were monitored together with their effects on the dose-related pressor responses induced by intravenous clonidine. Saline did not produce any effect on the baseline blood pressure of the pithed rat. RX 781094 (0.3 and 1.0 mg/kg) produced transient (5–10 min) pressor responses of 28 ± 4 and 36 ± 5 mmHg, respectively. The peak increases in blood pressure were obtained between 0.3 and 1.0 min after RX 781094 administration. Yohimbine (1.0 and 3.0 mg/kg) on the other hand produced transient dose-related falls in blood pressure of -5 ± 1 and -10 ± 2 mmHg, respectively. In contrast to the results obtained with the antagonists against clonidine in the vas deferens, yohimbine produced a greater antagonism of the clonidine-induced pressor responses than RX 781094 (Figure 5b). The clonidine ED_{50} values (i.v. doses of clonidine producing a 50 mmHg increase in diastolic blood pressure), after saline, RX 781094 (0.3 and 1.0 mg/kg) and yohimbine (1.0 and 3.0 mg/kg) were 4 ± 1, 9 ± 1, 19 ± 1, 12 ± 1 and 44 ± 8 μg/kg, i.v. respectively.

In separate experiments the effects of yohimbine (1.0 and 3.0 mg/kg) and RX 781094 (0.3 and 1.0 mg/kg) on the pressor responses to single doses of UK 14,304, cirazoline, phenylephrine, noradrenaline, tyramine, angiotensin II and 5-HT were compared in β-adrenoceptor-blocked pithed rats. The results are shown in Figure 6. In the doses used, yohimbine and RX 781094 produced similar inhibitions of the pressor responses to UK 14,304 and tyramine. Yohimbine significantly antagonized the responses to cirazoline and phenylephrine after both doses of the antagonist (Figure 6). However, RX 781094 only produced significant antagonism of these α_1-agonists after the 1.0 mg/kg dose (Figure 6). Yohimbine inhibited the pressor response to noradrenaline to a significantly greater extent than RX 781094.

Yohimbine (1.0 and 3.0 mg/kg) produced small but significant inhibitions of the pressor response induced by angiotensin II (Figure 6). The angiotensin II pressor effect after RX 781094 (1.0 mg/kg) was not significantly different from its control response (in the same pretreatment group) although it was significantly smaller than the corresponding response in the saline treated group.

5-HT produced an initial transient increase in diastolic blood pressure followed by a consistent, more prolonged depressor effect. Neither antagonist greatly affected the 5-HT-induced pressor response although this effect after the 1.0 mg/kg dose of RX 781094 was significantly greater than its control response (Figure 6). With respect to the depressor phase of 5-HT, the responses were significantly smaller after yohimbine (3.0 mg/kg) and significantly greater after RX 781094 (0.3 mg/kg) than their respective control responses (Figure 6).

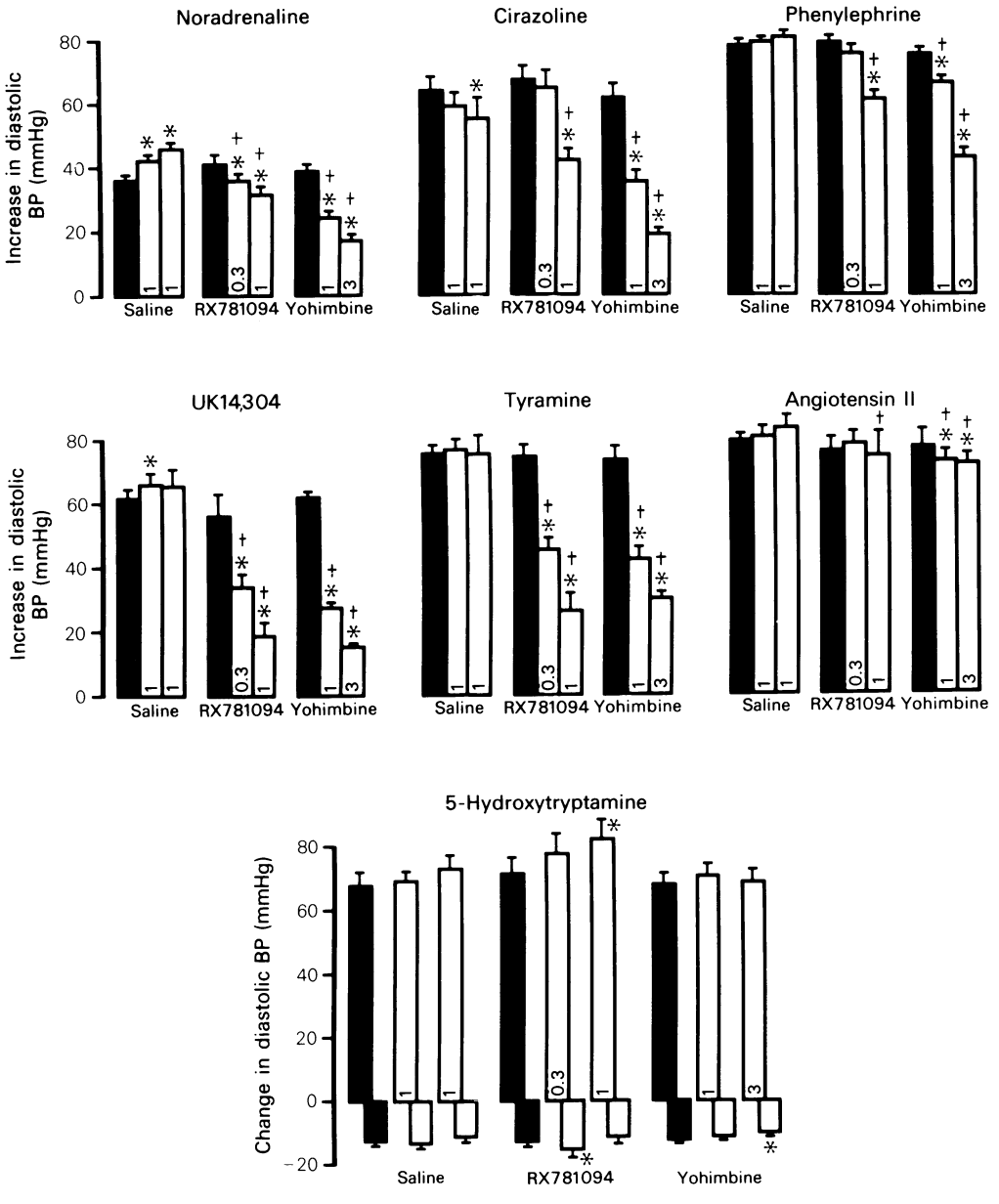

Figure 6 The effects of saline (two doses of 1.0 ml/kg), RX 781094 (0.3 and 1.0 mg/kg) and yohimbine (1.0 and 3.0 mg/kg) on the increases in diastolic blood pressure induced by noradrenaline (0.3 μg/kg), cirazoline (1.0 μg/kg), phenylephrine (5.0 μg/kg), UK 14304 (10.0 μg/kg), tyramine (0.3 mg/kg), angiotensin II (0.3 μg/kg) and 5-hydroxytryptamine (0.1 mg/kg) in pithed rats pretreated with (±)-propranolol (1.0 mg/kg). 5-Hydroxytryptamine produced a pressor response followed by a fall in diastolic blood pressure; these depressor effects are also shown. Details of the experimental design are described in the methods. The full columns represent control responses to the agonists before giving the antagonists or saline. Bars represent mean responses with their standard errors obtained from groups of 5–6 rats. An * designates a significant difference ($P < 0.05$) from the control response of the same treatment group and † illustrates a significant difference ($P < 0.05$) from the corresponding response in the saline treated group (analysis of variance).

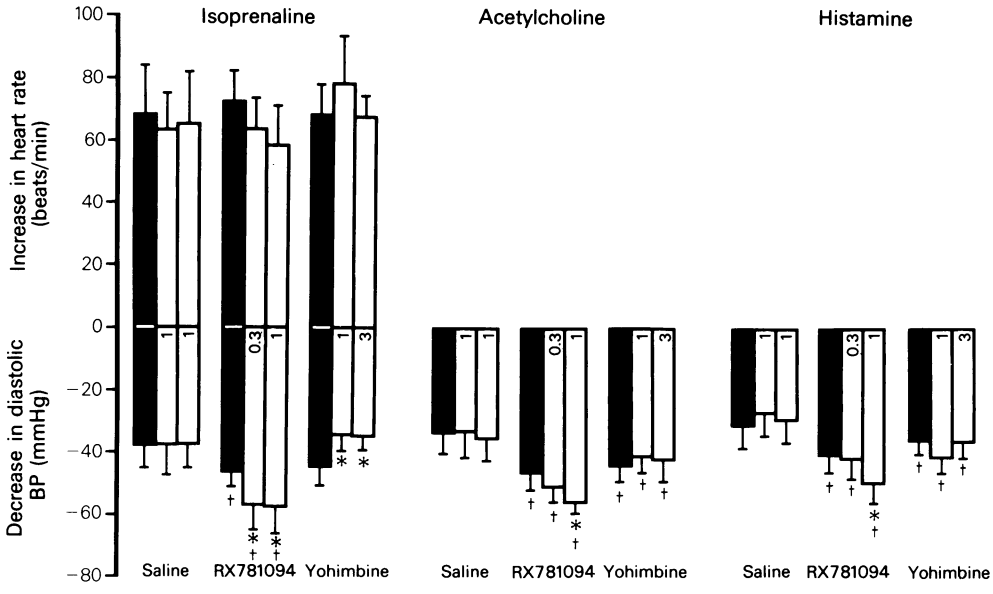

Figure 7 The effects of saline (two injections of 1.0 ml/kg), RX 781094 (0.3 and 1.0 mg/kg) and yohimbine (1.0 and 3.0 mg/kg) on the decreases in diastolic blood pressure induced by isoprenaline (0.1 μg/kg), acetylcholine (1.0 μg/kg) and histamine (3.0 μg/kg) as well as the heart rate increases produced by isoprenaline in pentobarbitone-anaesthetized, vagotomised rats. The full columns represent control responses to the agonists before giving the antagonists or saline. Bars represent mean responses with their standard errors obtained from groups of 6 rats. An * designates a significant difference ($P<0.05$) from the control response of the same treatment group and † illustrates a significant difference ($P<0.05$) from the corresponding response in the saline-treated group (analysis of variance).

Responses to acetylcholine, isoprenaline and histamine in anaesthetized rats In rats anaesthetized with pentobarbitone, yohimbine produced dose-related falls in diastolic blood pressure. The peak reductions in diastolic blood pressure produced by 1.0 and 3.0 mg/kg, i.v. doses of yohimbine were -33 ± 4 and -49 ± 8 mmHg; (the initial pressures were 83 ± 8 and 94 ± 5 mmHg respectively). Although the yohimbine-induced hypotensive effects were transient, the diastolic blood pressures prior to challenge with the first depressor agent (5 min post yohimbine) were below pretreatment values. Heart rate was also reduced by 28 ± 12 and 65 ± 7 beats/min following injections of 1.0 and 3.0 mg/kg, i.v. yohimbine respectively (initial heart rate values were 318 ± 16 and 338 ± 20 beats/min). In contrast to yohimbine, RX 781094 (0.3 mg/kg, i.v.) increased diastolic blood pressure by 14 ± 4 mmHg from an initial value of 80 ± 7 mmHg. This elevation was maintained until the next dose of RX 781094 was administered. RX 781094 (1.0 mg/kg, i.v.) lowered diastolic blood pressure by 12 ± 5 mmHg from a pre-dose value of 100 ± 8 mmHg.

Yohimbine and RX 781094 had little effect on the hypotensive effects of acetylcholine, isoprenaline and histamine (Figure 7); the changes that were seen probably resulted from the residual effects of the two antagonists on resting diastolic blood pressure. Similarly, the tachycardias produced by isoprenaline were unaffected by the antagonists (Figure 7).

Discussion

RX 781094 and standard α-adrenoceptor antagonists were compared at prejunctional α_2- and postjunctional α_1-adrenoceptors in the rat vas deferens and anococcygeus muscle respectively. Although RX 781094 was a competitive antagonist at both subclasses of α-adrenoceptor it displayed a much higher affinity for prejunctional α_2-adrenoceptors than for postjunctional α_1-adrenoceptors. Yohimbine has previously been shown to display higher affinity for α_2-adrenoceptors (Starke *et al.*, 1975; Doxey *et al.*, 1977); this selectivity profile was confirmed in the present studies. Yohimbine however, was less potent and less selective for prejunctional α_2-adrenoceptors than RX 781094, the respective selectivity ratios for yohimbine and RX 781094 being 45 and 288. Although RS 21361 displayed a

higher affinity for prejunctional α_2-adrenoceptors than for postjunctional α_1-adrenoceptors ($\alpha_2/\alpha_1 = 135$) its potency was lower than that of either RX 781094 or yohimbine. Piperoxan and phentolamine showed a marginal preference for prejunctional α_2-adrenoceptors whereas WB 4101 and prazosin were selective α_1-adrenoceptor antagonists; prazosin was the most selective α_1-adrenoceptor antagonist studied. These latter results support previous studies which have suggested that the selectivity of WB 4101 for α_1-adrenoceptors is less than that of prazosin (Langer, Massingham & Shepperson, 1980; Doxey, Howlett & Roach, 1981).

In the present studies, RS 21361 possessed α_1-adrenoceptor antagonist properties as judged by its inhibition of noradrenaline-induced contractions of the anococcygeus muscle. However, Michel & Whiting (1981) found that RS 21361 (100 μM) failed to antagonize amidephrine-induced contractions of the epididymal half of the rat vas deferens. These results emphasise the problems involved in comparing α-adrenoceptor antagonist potencies and selectivities determined under different experimental conditions. It is only pertinent to quote antagonist potencies, and hence selectivities, for particular tissues and defined experimental conditions. This is highlighted by the range of prejunctional α_2-adrenoceptor antagonist potencies obtained for RX 781094 in different tissues. Although RX 781094 had similar pA_2 values on noradrenergic neurones of the rat vas deferens and cholinergic nerves of the guinea-pig ileum, its potency was significantly lower in the mouse vas deferens. This latter observation is in agreement with published data on RX 781094 in the mouse vas deferens (Baker & Marshall, 1982). These observations could be a reflection of differences in either the prejunctional α_2-adrenoceptors of individual tissues or variations in the experimental conditions used.

Although RX 781094 displays improved α_2-adrenoceptor selectivity and potency over yohimbine, a major advantage in its overall pharmacological profile is that it has an extremely low affinity for other receptors. This is in marked contrast to yohimbine which has previously been reported to lack specificity for α-adrenoceptors (for refs. see Scatton *et al.*, 1980). In concentrations up to 40–100 μM, RX 781094 was essentially devoid of activity at β_1, β_2, histamine H_2 and presynaptic opiate receptors. In addition, effects of RX 781094 on histamine H_1, muscarinic and ganglionic nicotinic and 5-HT responses could only be demonstrated at concentrations which were more than two orders of magnitude greater than those affecting postjunctional α_1-adrenoceptors. Although RX 781094 was a weak antagonist at muscarinic receptors, this interaction was only competitive in the presence of an anticholinesterase. It is possible therefore that RX 781094 possesses weak anticholinesterase activity and muscarinic antagonist properties over the same concentration range. Furthermore, it is probable that these muscarinic antagonist properties result in the inhibitory effect of RX 781094 on 5-HT and DMPP responses in the guinea-pig ileum. The alternative explanation is that RX 781094 interacts with neuronal 5-HT and nicotinic receptors over the same concentration range. No functional pharmacological experiments have been performed with respect to the possible interaction between RX 781094 and dopamine receptors. However, from radioligand binding studies, RX 781094 in concentrations up to 100 μM did not significantly displace the dopamine ligand [^{3}H]-ADTN from striatal binding sites (Lane & Walter, personal communication). The fact that RX 781094 interacts with α_2-adrenoceptors at concentrations which are approximately 300 times less than those which affect α_1-adrenoceptors demonstrates the pronounced receptor specificity of the compound.

In the pithed rat, all antagonists with the exception of prazosin reversed the inhibitory effects of clonidine on the vas deferens although the reversal was incomplete with WB 4101. RX 781094 was the most potent compound studied it being approximately 43 times more potent than yohimbine under these experimental conditions. Using the vas deferens, therefore, it was possible to determine the α_2-adrenoceptor antagonist potency of non-selective antagonists such as phentolamine and even compounds which act preferentially at α_1-adrenoceptors e.g. WB 4101. However, when the α_1-adrenoceptor selectivity was marked, as with prazosin, prejunctional α_2-antagonist effects could not be detected. A second tissue, the anococcygeus muscle, was used to differentiate between selective and non-selective α_2-adrenoceptor antagonists. Reversal of the inhibitory effects of guanabenz on the anococcygeus muscle is a demanding test for α_2-adrenoceptor selectivity (Doxey & Easingwood, 1978). Only RX 781094, yohimbine and RS 21361 antagonized the inhibitory effects of guanabenz on the anococcygeus muscle thus confirming that these compounds are selective α_2-adrenoceptor antagonists. RX 781094 was 22 and 132 times more potent than yohimbine and RS 21361 respectively in these studies.

The reversal of the inhibitory effects of clonidine and guanabenz by RX 781094 on the responses to nerve stimulation of the vas deferens and anococcygeus muscle of pithed rats was due to a specific antagonist action at prejunctional α_2-adrenoceptors since RX 781094 failed to influence the inhibition of nerve responses produced by ganglion blockade with mecamylamine or adrenergic neurone blockade with guanethidine. In contrast, amphetamine readily reversed the effect of guanethidine. Similar results

were reported by Natoff & Dodge (1980) who demonstrated that yohimbine reversed the inhibitory effects of clonidine but not bretylium on the pressor responses to sympathetic stimulation in pithed rats whereas amphetamine reversed bretylium but not clonidine. In addition, these results indicate that in the doses used, guanabenz reduced nerve stimulation responses of the anococcygeus muscle solely via activation of prejunctional α_2-adrenoceptors. Misu, Fujie & Kubo (1982) showed that guanabenz possessed α_2-adrenoceptor stimulant and adrenergic neurone blocking actions, *in vitro*.

In experiments in which antagonist potency was expressed as the dose required to reverse an established α_2-adrenoceptor agonist effect by 50%, RX 781094 was markedly more potent than yohimbine. However although RX 781094 was more potent than yohimbine in pretreatment experiments the separation was reduced. In reversal experiments, the antagonist effects of RX 781094 and yohimbine were recorded with respect to their maximal reversal effects irrespective of the time taken for this to be obtained, (yohimbine taking longer to reverse than RX 781094). In the pretreatment experiments construction of dose-response curves to clonidine commenced 5 min after either antagonist. Since yohimbine has a longer duration of action in the rat than RX 781094 (unpublished results; Tulloch, personal communication), one possible explanation for the difference in the relative antagonist potencies in the two methods could be that in the pretreatment experiments the antagonist effects of RX 781094 were assessed after the peak effect of RX 781094, thus reducing the dose-ratio for the antagonist. In these experiments the agonist dose-response curves were constructed between 5 and 20 min after administration of the antagonist and the peak tissue levels of RX 781094 do not decline sufficiently over this time to account for this difference. Therefore, it would appear that the different relative potencies of the antagonists obtained by the two experimental methods are unlikely to be the result of differences in duration of action.

From studies using selective α_1- and α_2-adrenoceptor agonists and antagonists in pithed rats, several groups of workers have concluded the existence of postjunctional α_1- and α_2-adrenoceptors located on vascular smooth muscle (see McGrath, 1982 for review). We investigated the effects of RX 781094 and yohimbine on the pressor responses to UK 14,304 (selective α_2-agonist; Cambridge, 1981), cirazoline and phenylephrine (two selective α_1-agonists; Roach, Lefèvre, & Cavero, 1978) as well as noradrenaline (a mixed α_1- and α_2-agonist; Langer, 1981) and tyramine (an indirectly acting sympathomimetic agent). Both antagonists at the doses used produced similar dose-related reductions in the pressor response mediated by UK 14,304. However, yohimbine at both dose levels significantly antagonized the vasoconstrictor responses to cirazoline and phenylephrine. In contrast, significant reductions of the responses to these α_1-adrenoceptor agonists were noted only after the highest dose of RX 781094 (cumulative dose of 1.3 mg/kg, i.v.). We have previously shown that the dose-pressor response curve to cirazoline was not significantly different after RX 781094 (1.0 mg/kg) and saline (Berridge, Doxey, Roach & Strachan, 1982). Therefore, from *in vivo* studies using pithed rats RX 781094 is both a more potent and selective α_2-adrenoceptor antagonist than yohimbine. As would be expected from an antagonist with activity at both receptors (i.e. a reduced selectivity), yohimbine produced a greater antagonism of the noradrenaline-induced pressor response than did RX 781094. This was also the case when clonidine was used as the α-adrenoceptor agonist. Yohimbine produced larger displacements of the clonidine dose-pressor response curve than RX 781094 indicating that the vasoconstrictor responses produced by clonidine are mediated via both postjunctional α_1- and α_2-adrenoceptors.

Yohimbine and RX 781094 (in the doses used) similarly antagonized the pressor responses to tyramine, the degrees of antagonism being similar to those observed with these compounds against UK 14,304. The similar effects of the two antagonists on tyramine are in contrast to their differential effects on noradrenaline pressor responses. Therefore, if one assumes that the majority of the tyramine pressor response was induced by endogenously released noradrenaline, then it would appear that the population and/or type of α-adrenoceptors responsible for mediating these two responses are different. Langer, Massingham & Shepperson (1981) reported that in the dog perfused hindquarters, prazosin was relatively more effective against nerve-induced than noradrenaline-induced pressor responses and concluded that α_1-adrenoceptors on vascular smooth muscle are predominantly intrasynaptic whereas α_2-adrenoceptors are mainly extrasynaptic. Although the noradrenaline released by tyramine and nerve impulses are from different stores and involves different mechanisms (see Smith, 1973), one might expect that the noradrenaline released by tyramine would preferentially act intrasynaptically and according to the theory of Langer and colleagues would be less susceptible to α_2-adrenoceptor antagonism. However, in the present study, RX 781094 produced equivalent antagonism of the responses to tyramine and the α_2-adrenoceptor agonist, UK 14,304. These results imply that endogenously released noradrenaline and possibly tyramine are capable of stimulating α_2-adrenoceptors. It is not possible to exclude completely the possibility that RX 781094

preferentially inhibits the pressor response to tyramine (compared to i.v. noradrenaline) by additionally blocking neuronal uptake. However, this explanation would appear extremely unlikely since *in vitro* experiments have failed to demonstrate significant noradrenaline uptake blocking properties for RX 781094 in brain synaptosomes (Dettmar, personal communication) and mouse vas deferens (Baker & Marshall, 1982). Additionally, the magnitude and duration of contractions of the anococcygeus muscle (a tissue containing mainly α_1-adrenoceptors, see above) to intra-arterial noradrenaline in pithed rats are not potentiated by RX 781094 (unpublished observations).

The antagonist effect of RX 781094 on the pressor responses to the α-adrenoceptor agonists was due to a specific action by the compound at postjunctional α-adrenoceptors because RX 781094 did not reduce the pressor responses to either angiotensin II or 5-HT. In fact RX 781094 significantly potentiated the vasoconstrictor response to 5-HT, the reason for this effect is unknown at present. An inhibitory action on 5-HT uptake is unlikely to account for the small potentiation seen after RX 781094 since *in vitro* RX 781094 does not significantly inhibit 5-HT uptake into brain synaptosomes (Dettmar, personal communication). A non-specific potentiation of the smooth muscle contraction process is also excluded since RX 781094 did not augment the pressor response to angiotensin II.

In addition to its characteristic pressor response (due to stimulation of D-type tryptamine receptors) 5-HT induces a hypotensive effect in pithed rats which is mediated via an uncharacterized tryptamine receptor (Cavero, Lefèvre-Borg & Roach, 1981). RX 781094 does not antagonize the effects of 5-HT at these receptors although yohimbine produced a small but significant inhibition. Furthermore RX 781094 did not inhibit the depressor responses to isoprenaline, acetylcholine and histamine and the isoprenaline-induced tachycardia in anaesthetized rats. Likewise, yohimbine did not influence these responses in the doses used in the present study.

In the present study, RX 781094 produced variable effects on blood pressure in anaesthetized and pithed rats. RX 781094 increased blood pressure in pithed rats used to study the effects of this antagonist on the pressor responses to various constrictor agents. In addition, RX 781094 (0.3 mg/kg, i.v.) increased diastolic blood pressure in rats anaesthetized with pentobarbitone, whereas the pressure fell after the 1.0 mg/kg dose. Pressor responses after RX 781094 in pithed rats are variable. In the experiments in which blood pressure and tension of the anococcygeus muscle following nerve stimulation were monitored in pithed rats RX 781094 given intra-arterially failed to influence either parameter (unpublished observations). Dabiré *et al.* (1981) and Mouillé, Dabiré, Fournier & Schmitt (1981) have also described studies with RX 781094 (their compound no. being 170150) in pithed rats and found no effect of the compound itself on blood pressure. In isolated tissue experiments, we have never observed effects attributable to α_1-adrenoceptor agonism (either indirectly or directly mediated) with RX 781094. Due to the variability of the pressor response seen with RX 781094 in rats, experiments to date have failed to resolve whether these effects are produced directly or indirectly by RX 781094 or a metabolite. From the present studies we have no evidence to suggest that RX 781094 is not a pure antagonist at both α_2- and α_1-adrenoceptors.

In conclusion, these results demonstrate that RX 781094 is a potent and selective antagonist of α_2-adrenoceptors with a high degree of specificity for α-adrenoceptors. In terms of its pharmacological profile, it is superior to antagonists such as yohimbine, RS 21361 and piperoxan and as such is an improved pharmacological tool with which to investigate and characterize α-adrenoceptors. The therapeutic potential of RX 781094 is now being assessed.

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