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Selectivity and potency of 2-alkyl analogues of the α_2-adrenoceptor antagonist idazoxan (RX 781094) in peripheral systems

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1 The profiles of four analogues of idazoxan have been examined at α-adrenoceptors and the results compared to those obtained with idazoxan and yohimbine. The compounds possessed either a methyl (RX 801079), ethyl (RX 811033), *n*-propyl (RX 811054) or isopropenyl (RX 811005) group at the two position of idazoxan.

2 The rank order of antagonist potency against UK-14,304 at prejunctional α_2-adrenoceptors of the rat isolated vas deferens was RX 811054 > RX 811033 > idazoxan > RX 811005 > yohimbine = RX 801079. All compounds were competitive antagonists.

3 The rank order of antagonist potency against noradrenaline at postjunctional α_1-adrenoceptors of the rat isolated anococcygeus muscle was RX 811054 = RX 811033 = idazoxan = yohimbine > RX 811005 = RX801079. All compounds were competitive antagonists. The rank order of α-adrenoceptor selectivity (α_2/α_1) was RX 811005 > RX 801079 > RX 811054 > RX 811033 > idazoxan > yohimbine.

4 In pithed rats, intravenous administration of all compounds fully reversed the prejunctional α_2-adrenoceptor agonist effects of clonidine and guanabenz on electrically-induced contractions of the vas deferens and anococcygeus muscle respectively.

5 In pithed rats the rank order of antagonist potency against UK-14,304 at cardiac prejunctional α_2-adrenoceptors was RX 811054 > RX 811033 > idazoxan > yohimbine > RX 811005 > RX 801079. In contrast, the rank order of antagonist potency against cirazoline pressor effects (vascular postjunctional α_1-adrenoceptors) was RX 811054 > RX 811033 > yohimbine > idazoxan > RX 811005 > RX 801079. The rank order of α_2-adrenoceptor selectivity was RX 811033 = RX 801079 = RX 801005 > RX 811054 > idazoxan > yohimbine.

6 Although idazoxan produced contractions of the anococcygeus muscle and increased blood pressure in pithed rats, three of the analogues (RX 811005, RX 801079 and RX 811033) were inactive.

7 In conclusion, alkyl substitution in the 2-position of idazoxan can enhance either α_2-adrenoceptor antagonist potency or selectivity or both and furthermore, the weak partial α_1-adrenoceptor agonist properties of idazoxan can be removed.

Introduction

Idazoxan (RX 781094) is a potent and selective α_2-adrenoceptor antagonist both in the periphery (Doxey *et al.*, 1983a) and in the central nervous system (Dettmar *et al.*, 1983). Recently however, idazoxan has been reported to evoke dose-related increases in the resting blood pressure of pithed rats which are inhibited by the selective α_1-adrenoceptor antagonist, prazosin (Paciorek & Shepperson, 1983; Roach *et al.*, 1983). These workers concluded that idazoxan is a partial agonist at α_1-adrenoceptors. In the present studies the selectivity profiles of four analogues of idazoxan are described; all four compounds possessed alkyl substituents at the 2-position of idazoxan (see Table 1). The selectivity and potency of the analogues were examined in isolated tissues and pithed rats. The effects of selected analogues on either the resting blood pressure or anococcygeus muscle tension were also studied in pithed rats in

order to assess possible partial-agonist effects at α_1-adrenoceptors. Preliminary findings have been presented to the British Pharmacological Society (Doxey *et al.*, 1983b).

Methods

In vitro *experiments*

All experiments were carried out using 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 experiments using the vas deferens and at 37°C for those using the rat anococcygeus muscle. The tissues were bathed in Krebs solution containing cocaine (0.9 μM), corticosterone (40 μM) and propranolol (0.1 μM). Contractions of the isolated tissues were measured isometrically with a Statham Gold Cell (UC 3) transducer and displayed on Smith Servoscribe pen recorders. Tissues were obtained from male albino rats (Sprague-Dawley, 200–250g).

α_2-Adrenoceptor antagonist potency: Rat vas deferens The prostatic half of the vas deferens was cleared of connective tissue and suspended under an initial tension of 0.5g in an organ bath of 8–10 ml capacity. The bath fluid was renewed continuously with a peristaltic pump which delivered 3–5 ml min^{-1}. The intramural nerves of the vas deferens were stimulated by rectangular pulses of 3 ms duration, 40 V at a frequency of 0.1 Hz (2.5 mamp; S.R.I. stimulator) (Doxey *et al.*, 1983a). The selective α_2-adrenoceptor agonist UK-14,304 (Cambridge, 1981) produces an inhibition of the stimulation-evoked twitch response of the vas deferens. Cumulative UK-14,304 concentration-response curves were constructed in the absence and presence of the antagonist and prejunctional α_2-adrenoceptor antagonist potency was expressed as a pA_2 value. pA_2 values for the antagonists were calculated from Arunlakshana & Schild (1959) plots constructed of log (dose-ratio −1) against log (antagonist concentration).

α_1-Adrenoceptor antagonist potency: rat anococcygeus muscle This was obtained from the same animals that were used for the vas deferens experiments. The muscle was exposed via an incision in the scrotal sac, dissected free from surrounding connective tissue and suspended in a 5 ml organ bath at an initial tension of 0.5g. Cumulative noradrenaline concentration-contractile response curves were constructed in the absence and presence of the test antagonist. Reproducible concentration-response curves to noradrenaline were established before introduction of the antagonist. Postjunctional α_1-adrenoceptor antagonist potency was expressed as a pA_2 value which was determined by the method of Arunlakshana & Schild (1959).

The selectivities of the antagonists for α_2-adrenoceptors were determined from 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 275–350g) 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}; 1 ml $100g^{-1}$ 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) connected to a preamplifier (H.P. 8805 A or D) and recorded on a 4 channel pen recorder (H.P. 7754 B). All animals were bivagotomised and the uncannulated carotid artery ligated. Atropine (1 mg kg^{-1}, i.v.) was injected before all pharmacological investigations. In studies in which the sympathetic outflow was stimulated electrically the animals also received tubocurarine (3.0 mg kg^{-1}, i.v.). In experiments in which pressor responses were studied the animals were pretreated with propranolol (1 mg kg^{-1}, i.v.).

Vas Deferens and anococcygeus muscle The vas deferens or anococcygeus muscle was dissected free from connective tissue and attached to an isometric transducer (Statham Gold Cell, UC3) which was in turn connected to a preamplifier (H.P. 8805 B). Both tissues were initially placed under a tension of 0.5g. 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 40V, 50 μs pulse width, 6 Hz for 2 s every 30 s and for the anococcygeus they were 40V, 500 μs pulse width, 1 Hz for 20 s every 3 min. Contractions of the tissues were recorded on a 4 channel pen recorder.

α_2-Adrenoceptor antagonist potency–reversal experiments Stimulation-induced contractions of the vas deferens and anococcygeus muscle were completely inhibited by clonidine (100 μg kg^{-1}, i.v.) and guanabenz (30 μg^{-1}, i.v.), respectively. In control experiments clonidine and guanabenz caused a prolonged

(> 30 min) inhibition of the effects of nerve stimulation in the respective experiments. Five to seven minutes after the administration of the agonists, increasing intravenous doses of the test antagonist were injected. 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 agonists. 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 dose-response curves were plotted and the antagonist potency was assessed by determining the cumulative dose which produced a 50% reversal (AD_{50}) of the response to either clonidine or guanabenz. The mean AD_{50} values (nmol kg^{-1}, i.v.) ± s.e.mean were determined from the individual log dose-response curves.

Determination of α_1- and α_2-adrenoceptor antagonist potencies and selectivities Prejunctional cardiac α_2-adrenoceptor antagonist potency was determined in pithed rats in which the resting heart rate (HR) was elevated by 90–110 beats min^{-1} by continuously stimulating (0.1–0.3 Hz, 60V, 0.5 ms duration) the thoracic (T1–T5) spinal cord. Separate groups of rats ($N = 6–8$) were used to determine the cumulative intravenous dose of the selective α_2-adrenoceptor agonist UK-14,304 required to reduce the tachycardia by 50% (ED_{50}) following either saline or a selected dose of an antagonist. Postjunctional vascular α_1-adrenoceptor antagonist activity was assessed by constructing dose-pressor response curves to the selective α_1-adrenoceptor agonist cirazoline (Van Meel *et al.*, 1981; Cavero *et al.*, 1982). The doses of cirazoline required to elevate diastolic blood pressure (DBP) by 50 mmHg (ED_{50}) were determined in saline and drug-treated rats. In all experiments, saline or antagonist doses were administered 10 min before constructing a dose-response curve to either UK-14,304 or cirazoline. If the slopes of the linear portions of the agonist dose-response curves in saline and antagonist-treated animals were found to be parallel then the agonist dose-ratio (DR) shift produced by the antagonist against UK-14,304 or cirazoline were calculated from the ED_{50} values. The intravenous antagonist doses (μmol kg^{-1}) required to produce a two fold shift (DR2) of the agonist dose-response curve were then determined. The selectivity value of an antagonist was determined by dividing the DR2 at α_1-adrenoceptors by the DR2 value calculated at α_2-adrenoceptors.

α_1-Adrenoceptor agonist activity Pithed rats were prepared for measurement of arterial blood pressure and contractions of the anococcygeus muscle, as described previously. Before a test compound was evaluated for α_1-agonist properties consistent increases in diastolic blood pressure to noradrenaline (0.3 μg kg^{-1}, i.v.) and regular contractions of the anococcygeus muscle to electrical stimulation of the spinal cord were obtained. Increasing doses of idazoxan or its analogues were administered intravenously (at approximately 5 min intervals) and maximal changes in diastolic blood pressure and resting tension of the anococcygeus muscle recorded.

Drugs

The following drugs were used in the present studies: atropine sulphate (Burroughs Wellcome), cirazoline hydrochloride (Synthélabo), clonidine hydrochloride (Bonapace), cocaine hydrochloride (MacFarlane Smith), corticosterone (Sigma), guanabenz acetate (Wyeth), (±)-idazoxan hydrochloride (RX 781094, Reckitt and Colman), (−)-noradrenaline bitartrate (Koch Light), (±)-propranolol hydrochloride (ICI), (+)-tubocurarine chloride (Burroughs Wellcome), UK-14,304 tartrate (5-bromo-6-[2-imidazolin-2-ylamino-quinoxaline Pfizer) and yohimbine hydrochloride (Sigma). The analogues of idazoxan were RX 801079 (2-(2-(2-methyl-1,4 benzodioxanyl))2-imidazoline hydrochloride), RX 811033 (2-(2-(2-ethyl-1,4 benzodioxanyl))2-imidazoline, this compound is hygroscopic and contains 1/4 molecule H_2O), RX 811054 (2-(2-(2-propyl-1,4

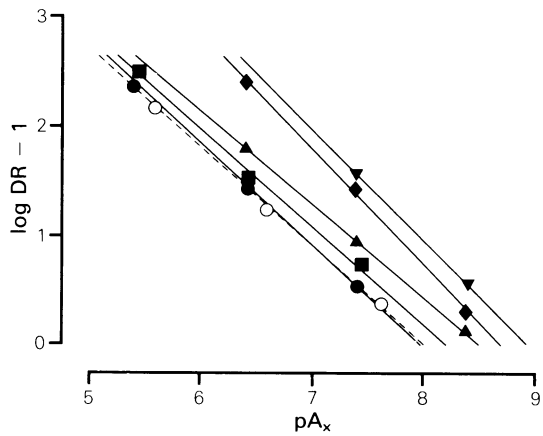


Figure 1 Schild plots for the interaction between either analogues of idazoxan or yohimbine and UK-14,304 on the electrically-stimulated (0.1 Hz) rat isolated vas deferens. Compounds studied: idazoxan (▲), RX 801079 (●), RX 811033 (◆), RX 811054 (▼), RX 811005 (■) and yohimbine (○). The lines were fitted by linear regression analysis of the data. Each point is the mean of a minimum of three determinations. pA_2 values determined from these plots are shown in Table 1.

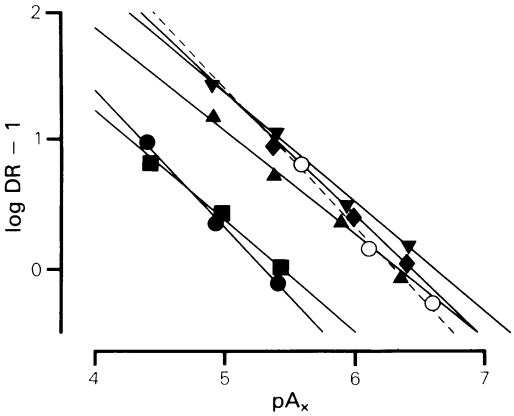


Figure 2 Schild plots for the interaction between either analogues of idazoxan or yohimbine and noradrenaline on the rat isolated anococcygeus muscle. Compounds studied: idazoxan (▲), RX 801079 (●), RX 811033 (◆), RX 811054 (▼), RX 811005 (■) and yohimbine (○). The lines were fitted by linear regression analysis of the data. Each point is the mean of a minimum of three determinations. pA_2 values determined from these plots are shown in Table 1.

benzodioxanyl))2-imidazoline), RX 811005 (2-(2-(2-isopropenyl-1,4 benzodioxanyl))2-imidazoline hydrochloride, Reckitt and Colman); all compounds being the (±)-racemic mixture.

All drug solutions were made up in either distilled water (*in vitro* experiments) 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 compounds (as bases or salts) as shown above. Calculated AD_{50} and DR2 values of the antagonists have been converted to molar doses in order to compare potencies of the compounds.

Results

In vitro *experiments*

Determination of prejunctional α_2-adrenoceptor antagonist potency and selectivity Prejunctional α_2-adrenoceptor antagonism was assessed by determining pA_2 values against the inhibitory effects of the prejunctional agonist UK-14,304 on the rat vas deferens stimulated at a frequency of 0.1 Hz. All of the

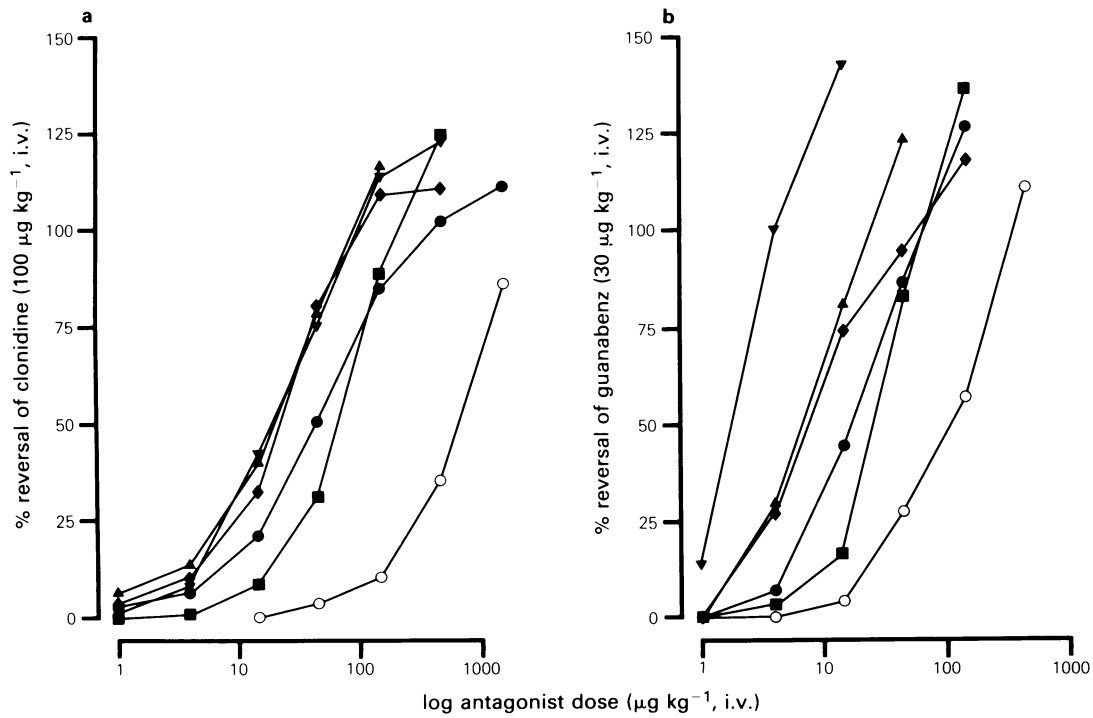
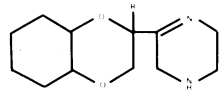


Figure 3 Antagonism of the inhibitory effects of clonidine (100 μg kg^{-1}, i.v.) and guanabenz (30 μg kg^{-1}, i.v.) by either idazoxan analogues or yohimbine on the electrically stimulated vas deferens (a) and anococcygeus muscle (b) of the pithed rat, respectively. The compounds studied were idazoxan (▲), RX 801079 (●), RX 811033 (◆), RX 811054 (▼), RX 811005 (■) and yohimbine (○). 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 1 α-Adrenoceptor antagonist properties of idazoxan, its four analogues and of yohimbine in isolated tissue experiments: pA_2 values (plus 95% confidence limits) were determined from Arunlakshana & Schild plots (1959).

Compound	*R 2-substitution* 	*α_2-Antagonism Rat vas deferens* pA_2 vs UK-14,304	*α_1-Antagonism Rat anococcygeus* pA_2 vs noradrenaline	*Ratio* α_2/α_1
Idazoxan	H	8.50(8.38–8.64)	6.32(6.14–6.57)	151
RX 801079	methyl	7.96(7.80–8.14)*	5.30(5.19–5.44)*	457
RX 811033	ethyl	8.69(8.54–8.87)	6.40(6.35–6.44)	195
RX 811054	*n*-propyl	8.92(8.74–9.17)*	6.56(6.35–6.88)	229
RX 811005	isopropenyl	8.21(8.00–8.47)*	5.42(5.37–5.48)*	617
Yohimbine	–	8.00(7.92–8.10)*	6.30(6.26–6.35)	50

The results are the mean of a minimum of 9 determinations. * indicates pA_2 values which are significantly different to those of idazoxan ($P<0.05$; unpaired *t* test).

compounds studied tended to increase the twitch response of the vas deferens with no inhibitory effects being evident, the resting tension of the tissue was unaffected by the antagonists. Each antagonist produced a parallel, concentration-dependent shift to the right of the UK-14,304 concentration-response curve. Plots of log (DR−1) against log (pAα) gave linear regressions with slopes (0.85–1.05) close to unity (Figure 1). The pA_2 values derived from these Schild plots are shown in Table 1. The rank order of α_2-adrenoceptor antagonist potency was RX 811054 > RX 811033 > idazoxan > RX 811005 > yohimbine = RX 801079 (Figure 1, Table 1.).

Postjunctional α_1-adrenoceptor antagonist potency was expressed as a pA_2 value against noradrenaline on the rat anococcygeus muscle. None of the antagonists increased the resting tension of the anococcygeus muscle. The log concentration-response curves for noradrenaline were competitively antagonized by all antagonists studied. Linear Schild plots were obtained with all of the compounds and the slopes (0.80–1.10) were close to unity (Figure 2). The pA_2 values derived from these plots are shown in Table 1. The α_1-adrenoceptor antagonist potencies of yohimbine, idazoxan, RX 811033 and RX 811054 were relatively similar; RX 801079 and RX 811005 were approximately an order of magnitude less potent than the other compounds studied (Figure 2, Table 1).

In vivo *experiments*

Prejunctional α_2-adrenoceptor antagonist properties in pithed rats The α_2-adrenoceptor antagonist properties of idazoxan and its analogues as well as yohimbine were assessed *in vivo* by testing their ability to reverse the inhibitory effects of either clonidine on the vas deferens or guanabenz on the anococcygeus muscle of pithed rats. All of the compounds tested produced a dose-related and complete reversal of the

Table 2 Antagonist potencies of idazoxan and four 2-substituted analogues, as well as yohimbine, at prejunctional α_2-adrenceptors in pithed rats: peripheral α_2-adrenoceptor antagonist activities were obtained from the reversal of the α_2-agonist effects of clonidine ($100\,\mu g\,kg^{-1}$, i.v.) and guanabenz ($30\,\mu g\,kg^{-1}$ i.v.) on the electrically-stimulated contractions of the vas deferens and anococcygeus muscle

Compound	*Rat vas deferens* AD_{50} ($nmol\,kg^{-1}$, i.v.) Antagonist dose producing 50% reversal of clonidine	*Rat anococcygeus muscle* AD_{50} ($nmol\,kg^{-1}$, i.v.) Antagonist dose producing 50% reversal of guanabenz
Idazoxan	82± 9	37± 10
RX 810079	203± 51	95± 39
RX 811033	115± 29	47± 25
RX 811054	96± 23	7± 1
RX 811005	292± 57	121± 23
Yohimbine	1762± 341	278± 84

Values represent mean AD_{50} values ± s.e.mean obtained from separate groups of rats ($n = 4–7$). Asterisks indicate that the AD_{50} values were significantly different to those of idazoxan (unpaired *t* test; $P<0.05$).

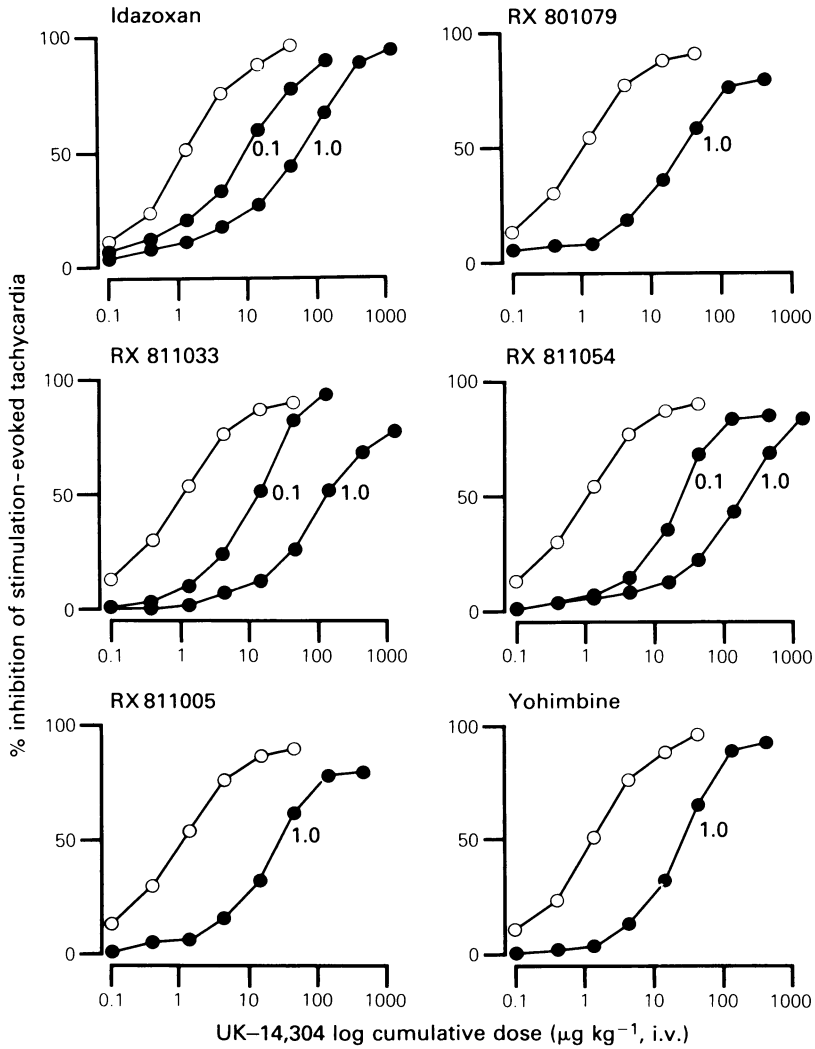


Figure 4 Effects of either saline (○) or test compound (●) on the reduction in heart rate produced by UK-14,304 in pithed rats. Heart rate was elevated by continuous stimulation of the cardiac sympathetic nerves (0.1–0.3 Hz, 60 V, 500 μs) prior to administration of UK-14,304. Each point is the mean of 6–8 determinations. The antagonist doses shown on the curves are expressed as mg kg^{-1}, i.v.

effects of clonidine and guanabenz (Figure 3). The cumulative intravenous doses (nmol kg^{-1}) required to produce 50% reversal (AD_{50}) are shown in Table 2. None of the antagonists altered the resting tension of either the vas deferens or anococcygeus muscle.

Determination of post α_1- and prejunctional α_2-adrenoceptor antagonist potency and selectivity in pithed rats The antagonism produced by idazoxan and its four analogues of the responses to UK-14,304 at prejunctional cardiac α_2-adrenoceptors and of cirazoline at postjunctional vascular α_1-adrenoceptors are shown in Figures 4 and 5, respectively. The intravenous doses (μmol kg^{-1}) of the antagonists producing 2 fold shifts (DR2) in the UK-14,304 and cirazoline dose-response curves were calculated from the displacements of the curves induced by the five compounds shown in Figure 4 and 5. These values, which are shown in Table 3, represent the *in vivo* antagonist potencies of the compounds at α_2- and α_1-adrenoceptors. At prejunctional cardiac α_2-adrenoceptors, the rank order of antagon-

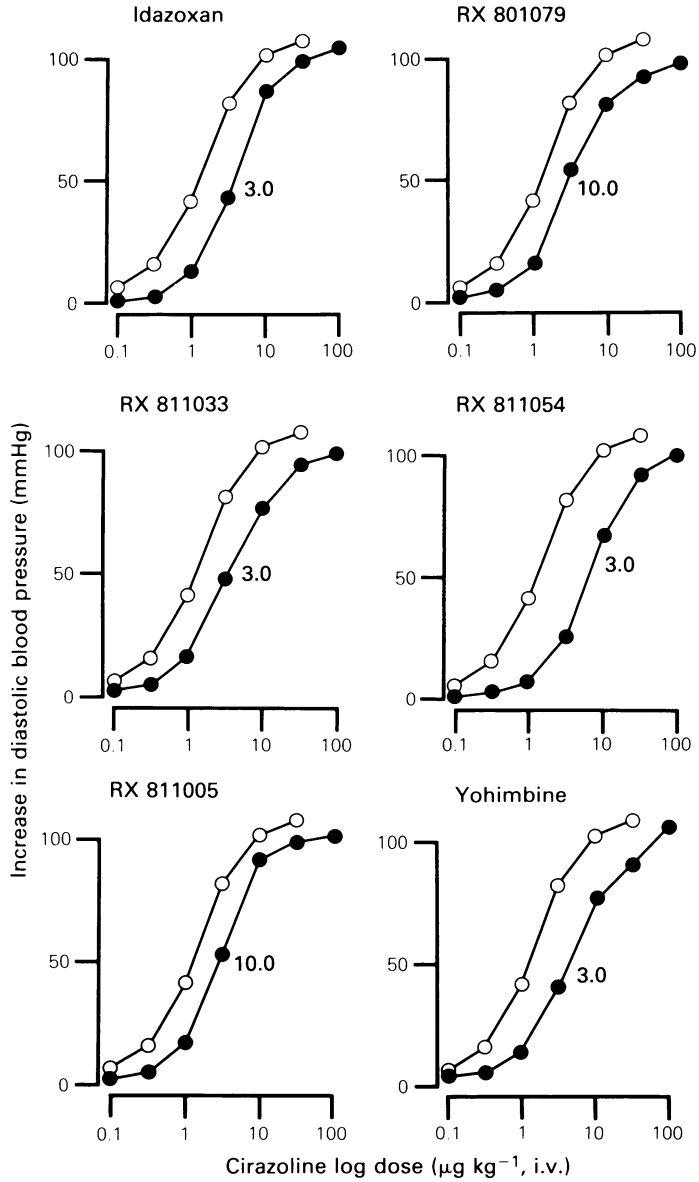


Figure 5 Effects of either saline (○) or test compound (●) on the increases in diastolic blood pressure produced by the selective α_1-adrenoceptor agonist cirazoline in pithed rats. Each point is the mean of 6–8 determinations. The antagonist doses shown on the curves are expressed as mg kg^{-1}, i.v.

ist potencies was RX 811054 > RX 811033 > idazoxan > yohimbine > RX 811005 > RX 801079. The antagonist potencies of idazoxan, RX 811033, RX 811054 and yohimbine were not significantly different at postjunctional vascular α_1-adrenoceptors; RX 811005 and RX 801079 were significantly less potent than the former compounds. All the analogues of idazoxan were found to be more selective than the parent compound and yohimbine was the least selective antagonist for α_2-adrenoceptors (Table 3).

Table 3 α-Adrenoceptor antagonist properties of idazoxan, its four analogues as well as yohimbine in pithed rats

Compound	*Cardiac pre* α_2*-receptors* Antagonist dose (μmol kg^{-1}) to produce DR2 against UK-14,304	*Vascular post* α_1*-receptors* Antagonist dose (μmol kg^{-1}) to produce DR2 against Cirazoline	*Ratio* α_2/α_1
Idazoxan	0.13 ± 0.02	7.53 ± 2.64	58
RX 801079	0.31 ± 0.09	39.5 ± 7.43*	127
RX 811033	0.07 ± 0.01*	9.18 ± 1.83	131
RX 811054	0.04 ± 0.01*	3.59 ± 0.31	90
RX 811005	0.26 ± 0.04*	31.1 ± 3.00*	120
Yohimbine	0.19 ± 0.04	4.26 ± 1.08	22

The results are the mean of a minimum of 6 experiments ± s.e.mean.

Agonist effects at postjunctional α_1*-adrenoceptors*
The effects of idazoxan and 3 of its analogues (RX 801079, RX 811005 and RX 81033) on diastolic blood pressure and resting tension of the anococcygeus muscle were examined in pithed rats. Although intravenously administered idazoxan produced dose-related increases in both the diastolic blood pressure and resting tension of the anococcygeus muscle, the analogues were inactive (Figure 6).

Discussion

We have previously described the profile of idazoxan and shown it to be a potent, selective α_2-adrenoceptor antagonist with a high degree of specificity for α-adrenoceptors (Doxey *et al.*, 1983a). Idazoxan is more selective for α_2-adrenoceptors than long standing reference antagonists such as yohimbine and rauwolscine (Doxey *et al.*, 1983a; 1984). Although idazoxan is a valuable tool for investigating the physiological and pharmacological roles of α_2-adrenoceptors, under certain experimental conditions it demonstrates weak α_1-adrenoceptor agonist properties (Paciorek & Shepperson, 1983; Roach *et al.*, 1983; this study). This action could complicate the interpretation of experimental observations particularly in functional studies using tissues in which the postjunctional receptor has characteristics of an α_1-adrenoceptor.

The structure of idazoxan has been modified with the aim of producing compounds which have enhanced potency and selectivity for α_2-adrenoceptors and are devoid of α_1-agonist properties. This paper describes studies with four analogues of idazoxan which possess either methyl (RX 801079), ethyl (RX 811033), *n*-propyl (RX 811054) or isopropenyl

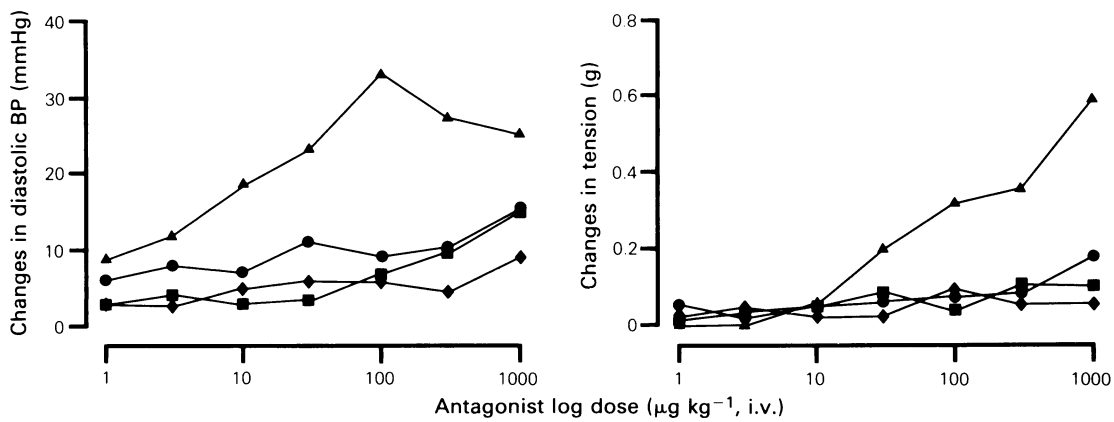


Figure 6 The effect of increasing intravenous doses of idazoxan (▲), RX 801079 (●), RX 811033 (■) and RX 811005 (◆) on the diastolic blood pressure and resting tension of the anococcygeus muscle of pithed rats. Each point is the mean of 5–6 rat experiments.

(RX 811005) substituents in the 2 position of the parent compound see (Table 1). Yohimbine has been included for comparative purposes.

In isolated tissue experiments, all of the compounds were competitive antagonists at α-adrenoceptors; the alkyl analogues were more selective for α_2-adrenoceptors than idazoxan. In the case of the 2-ethyl and 2-*n*-propyl derivatives this increased selectivity was primarily the result of improved α_2-adrenoceptor antagonist potency. In contrast, the improved selectivity of the 2-methyl and 2-isopropenyl derivatives was a reflection of reduced α_1-adrenoceptor antagonist potency. Yohimbine was the least selective antagonist at α_2-adrenoceptors tested in this study. RX 801079 (the 2-methyl analogue) was equipotent to yohimbine as an α_2-adrenoceptor antagonist but was 10 times less potent than the latter at α_1-adrenoceptors.

The *in vivo* α_2-adrenoceptor antagonist properties of idazoxan and its 2-alkyl analogues were assessed in pithed rats. Reversal of the inhibitory effects of either clonidine on stimulation-evoked twitch responses of the vas deferens or guanabenz on electrically-induced contractions of the anococcygeus muscle can be used as qualitative assessments of prejunctional α_2-adrenoceptor antagonist effects. All of the antagonists fully reversed the inhibitory effects of clonidine on electrically-induced contractions of the vas deferens. Since the twitch response of the vas deferens is resistant to α_1-adrenoceptor blockade it is possible to evaluate the prejunctional effects of non-selective α-adrenoceptor antagonists such as phentolamine (Doxey & Everitt, 1977). Consequently, it is not possible using this tissue to estimate the selectivity of such compounds. A second tissue, the anococcygeus muscle, can be used to differentiate between non-selective and selective α_2-adrenoceptor antagonists. Reversal of the inhibitory effects of the prejunctional α_2-adrenoceptor agonist guanabenz on electrically-induced contractions of the anococcygeus muscle can only be achieved with antagonists which possess a relatively high degree of selectivity for prejunctional α_2-adrenoceptors because the stimulation-evoked contractions are very sensitive to postjunctional α_1-adrenoceptor blockade (Doxey & Easingwood, 1978; Docherty & McGrath, 1979; Doxey *et al.*, 1984). Again all of the antagonists fully reversed the inhibitory effects of guanabenz indicating that the alkyl analogues of idazoxan, like the parent compound, interacted preferentially with prejunctional α_2-adrenoceptors. The rank order of antagonist potency in these reversal experiments was generally similar to that found for these compounds *in vitro* with the exception of yohimbine which was by far the least effective antagonist tested under these conditions. Reversal of the agonist effects of clonidine and guanabenz in the vas deferens and anococcygeus muscle by idazoxan and its analogues was extremely rapid. However in contrast, yohimbine was found to act more slowly than the other compounds. Yohimbine has slower receptor kinetics than idazoxan and its analogues. Therefore, the difference between the α_2-adrenoceptor antagonist potencies in these reversal experiments probably reflects the relative difficulty of yohimbine to displace the agonists from α_2-adrenoceptors.

Although experiments with the anococcygeus muscle in pithed rats accurately determines whether or not a compound is relatively selective for α_2-adrenoceptors, it does not provide a quantitative measure of α_2-selectivity. This was examined in pithed rats by comparing the antagonist potencies of the test compounds against the effects of the selective α_2-adrenoceptor agonist UK-14,304 at prejunctional cardiac α_2-adrenoceptors and the selective α_1-adrenoceptor agonist cirazoline at postjunctional vascular α_1-adrenoceptors. The *in vivo* antagonist potencies of the compounds tested at α_2- and α_1-adrenoceptors correlated well with those determined in isolated tissue experiments. Thus, at prejunctional cardiac α_2-adrenoceptors the 2-ethyl and 2-*n*-propyl analogues were more potent than idazoxan whereas the 2-methyl and 2-isopropenyl analogues were less potent. At postjunctional α_1- adrenoceptors, idazoxan and its 2-ethyl and 2-*n*-propyl analogues had similar potencies; the 2-methyl and 2-isopropenyl analogues were about 4–5 times less potent than the parent compound. All of the 2-alkyl analogues of idazoxan were more selective for α_2-adrenoceptors than the parent compound. Under these experimental conditions yohimbine was the least selective α_2-adrenoceptor antagonist, idazoxan being 2–3 times more selective than yohimbine. Although the potencies of yohimbine and idazoxan were not significantly different at each of the two receptor subtypes the difference in α_2-selectivity was due to the fact that idazoxan was slightly more potent than yohimbine at α_2-adrenoceptors and slightly less potent than yohimbine at α_1-adrenoceptors.

Finally, the abilities of the 2-ethyl, 2-methyl and 2-isopropenyl analogues to increase the resting tension of the anococcygeus muscle and to induce pressor responses were compared with idazoxan in naive pithed rats. In these studes the pithed rats were not subjected to additional procedures; in particular sympathetic outflow was not stimulated. Although idazoxan increased both diastolic blood pressure and the resting tension of the anococcygeus muscle the analogues studied were inactive. On the basis of these results these analogues do not appear to possess significant postjunctional α_1-adrenoceptor agonist properties in these systems. However, it should be stressed that the demonstration of contractile effects of idazoxan on the anococcygeus muscle of pithed

rats is dependent on the experimental conditions employed. In animals in which stimulation-evoked contractions of the anococcygeus muscle were inhibited by guanabenz, idazoxan had no effect on th resting tension of the tissue although it fully restored the contractile responses to electrical stimulation. This latter observation is consistent with previous studies which have demonstrated that idazoxan-induced pressor responses are difficult to demonstrate in pithed rats in which the sympathetic outflow is being regularly stimulated (Doxey *et al.*, 1983a).

In conclusion, the results obtained in the present studies demonstrate that alkyl substitution in the 2-position of idazoxan can produce compounds with either enhanced α_2-adrenoceptor antagonist potency or selectivity or both and which, furthermore, have minimal effects on the diastolic blood pressure and resting tension of the anococcygeus muscle of pithed rats. This latter observation suggests that the weak partial α_1- adrenoceptor agonist properties displayed by idazoxan in some tissues can be removed by appropriate alkyl substitution. The improved profiles of these compounds, with respect to idazoxan, have been confirmed in central studies (Gadie *et al.*, 1984).

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