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An investigation into the selectivity of a novel series of benzoquinolizines for α_2-adrenoceptors *in vivo*

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1 The potencies and selectivities of a novel series of benzoquinolizines for the α_2-adrenoceptor have been investigated in the rat in comparison with yohimbine and indoramin.

2 Peripheral postjunctional α_2- and α_1-adrenoceptor blockade was measured as the reversal of B-HT 933 and methoxamine-induced pressor responses, respectively, in the pithed rat.

3 Peripheral prejunctional α_2-adrenoceptor blockade was measured as the reversal of B-HT 933-induced inhibition of an electrically evoked tachycardia in the pithed rat.

4 Central α_2-adrenoceptor blockade was measured as a reversal of the hypotension induced in anaesthetized rats by central (i.c.v.) administration of clonidine.

5 Wy 25309, Wy 26392, Wy 26703 and yohimbine (0.3–3 mg kg^{-1} i.v.) evoked dose-dependent shifts to the right of the dose-response curves to B-HT 933 whilst having minimal effects on the methoxamine dose-response curve.

6 The selectivity for α_2-adrenoceptors increased with the dose of antagonist administered. In general, the order of selectivity was Wy 25309 > Wy 26392 > Wy 26703 > yohimbine.

7 Indoramin (1 mg kg^{-1} i.v.) shifted the methoxamine pressor dose-response curve to the right without affecting the B-HT 933 dose-response curves, confirming its selective α_1-antagonist activity.

8 Peripheral administration of all three benzoquinolizines (1–100 μg kg^{-1} i.v.) led to a dose-dependent reversal of the hypotension evoked by central administration of clonidine (500 ng i.c.v.). The reversal was incomplete, higher doses causing a further decrease in blood pressure. A similar degree of hypotension induced by the ganglion blocking agent chlorisondamine (1 mg kg^{-1} i.v.) was not reversed by the benzoquinolizines.

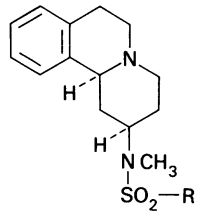
9 It is concluded that Wy 25309, Wy 26392 and Wy 26703 are selective α_2-adrenoceptor antagonists which readily penetrate the CNS.

Introduction

In recent years a great deal of evidence has accumulated in support of the concept that α-adrenoceptors can be divided into two subgroups using the commonly accepted terminology of α_1- and α_2-adrenoceptors (for reviews of the evidence see Langer, 1980; Gillespie, 1980; Starke, 1981). This classification is a pharmacological one, based upon the relative potencies of a series of α-adrenoceptor agonists and antagonists. (Langer, 1974; Berthelsen & Pettinger, 1977).

Since the recognition of these two α-adrenoceptor subtypes considerable chemical effort has been expended in the search for compounds with increasing selectivity for either one of these receptors. This goal was quickly achieved in the case of the α_1-adrenoceptor with the development of prazosin and indoramin which are highly selective α_1-adrenoceptor antagonists (Cambridge *et al.*, 1977; Rhodes & Waterfall, 1978). An antagonist with a comparable degree of selectivity for the α_2-adrenoceptor has yet to be developed. The aim of this paper is to present data from *in vivo* experiments on a novel series of benzoquinolizines which represent an advance towards this goal. These compounds Wy 25309, Wy 26392 and Wy 26703 (Figure 1), have been shown to be selective α_2-adrenoceptor antagonists *in vitro* (Lattimer *et al.*, 1982).

Anatomically, the α_2-adrenoceptor is found both pre- and post-junctionally in the peripheral noradrenergic neuro-effector junction. (Timmermans *et al.*, 1979; Kobinger & Pichler, 1981). The potencies of the α_2-adrenoceptor antagonists have been compared at each of these receptors in the pithed rat preparation. α_2-Adrenoceptors play a role in the



Wy 25309 R = CH_3
Wy 26392 R = ^nPr
Wy 26703 R = ^iBu

Figure 1 The chemical structures of the benzoquinolizines employed in this study.

central control of blood pressure via the sympathetic nervous system (Philippu, 1980). The α_2-adrenoceptor agonist clonidine, acting via these receptors, lowers blood pressure in a number of species following central administration (Kobinger, 1978). The potency of the benzoquinolizines as central α_2-adrenoceptor antagonists and their ability to cross the blood brain barrier has been investigated in an anaesthetized rat preparation.

A preliminary account of this work has been presented to the British Pharmacological Society. (Paciorek & Shepperson, 1983; Pierce & Shepperson, 1983).

Methods

Selectivity studies

Female Sprague-Dawley rats (230–270 g) were anaesthetized with halothane (5%) in O_2, and maintained in a state of surgical anaesthesia with halothane (2.5%) in O_2. The left carotid artery was cannulated and blood pressure recorded via a Statham P-231D pressure transducer on a Grass Model 7 polygraph. Heart rate was recorded from a tachograph triggered by the blood pressure signal. The left jugular vein was cannulated and used for drug administration. The trachea was intubated, and following pithing, rats were artificially ventilated at a rate of 55 min^{-1} and a stroke volume of 10 ml kg^{-1}. Deep body temperature was maintained at 37 ± 0.5°C using a heating blanket.

In a series of control experiments rats were adrenalectomized via a single midline incision which was subsequently closed. In a separate series of control experiments, rats were vagotomized by doubly ligating and then sectioning both the left and right vagi.

In another series of experiments, rats were pretreated with reserpine (5 mg kg^{-1} s.c.) 24 h before use. Noradrenaline levels were reduced over this time period by 96%, from 0.88 ± 0.11 $\mu g\,g^{-1}$ to 0.037 ± 0.003 $\mu g\,g^{-1}$ as measured in rat atria by the method of O'Hanlon *et al.* (1970) (Dr M. G. Wyllie, personal communication).

Evaluation of drug action at postjunctional α_1- and α_2-adrenoceptor sites

Rats were prepared as previously described, and pithed with a 16 gauge stainless steel rod. Following a period of equilibration, saline vehicle (1.0 ml kg^{-1}) or test agents were administered intravenously. Fifteen min later pressor dose-response curves were obtained by cumulative administration of the selective α_2-adrenoceptor agonist B-HT 933 or the selective α_1- agonist methoxamine hydrochloride. Shifts to the right in the pressor dose-response curves following drug administration were calculated to give a dose-ratio at approximately 50% maximal effect on the control curve (50 mmHg for B-HT 933 and 60 mmHg for methoxamine).

Evaluation of drug action at prejunctional α_2-adrenoceptor sites

Rats were prepared as described above, and then pithed with a 16 gauge stainless steel rod which had been lacquered with polyurethane varnish over its entire length, except for a 3 mm section at its tip. The rod was used to stimulate selectively the preganglionic nerves to the heart. Tubocurarine (1.0 mg kg^{-1}) was administered before stimulation to provide neuromuscular blockade.

The pithing rod was wired to a Grass SD9 stimulator, with an indifferent electrode placed dorsally, and during a period of constant stimulation was positioned at the spinal level C_7–T_1 so as to evoke a maximal tachycardia (Gillespie *et al.*, 1970). The preganglionic nerves to the heart were then stimulated at 45 s intervals for periods of 10 s with monophasic rectangular pulses of 20 V, 1 ms pulse width and 1 Hz frequency. When the evoked tachycardia response became constant, saline vehicle (1.0 ml kg^{-1}) or test agents were administered intravenously. Fifteen min later the selective α_2-adrenoceptor agonist B-HT 933 was administered cumulatively following each evoked tachycardia to obtain an inhibitory dose-response curve. Shifts in the inhibitory dose-response curve to the right following the administration of 0.9% w/v NaCl solution (saline) or drug were calculated and expressed as the ratio of the dose of B-HT 933 required to produce 50% inhibition of the response in the presence and absence of the antagonist.

Calculation of selectivity index The dose-ratios

(DR) calculated at the pre- and postjunctional α_2-sites and the postjunctional α_1-sites were expressed as DR pre α_2/DR α_1, DR post α_2/DR α_1 or DR pre α_2 /DR post α_2 to give selectivity indices. For the α_2/α_1 ratios, values greater than unity indicate selectivity for the α_2-receptor site whereas those less than unity indicate selectivity for the α_1-receptor site. All dose-ratios were calculated from curves drawn from at least four experiments.

Statistical evaluation of dose-response curves was performed by probit transformation and an analysis of covariance on regression (Snedecor & Cochran, 1980; Sokal & Rohlf, 1981).

Central administration of clonidine

Groups of 4 female Sprague-Dawley rats (250–300 g) were anaesthetized with pentobarbitone sodium (50 mg kg^{-1} i.p.) The trachea was intubated. Arterial blood pressure was monitored via a cannula introduced into the left femoral artery and recorded using a Statham P231D pressure transducer connected to a Grass 7D polygraph. The femoral vein was cannulated for the peripheral administration of antagonists. The skull was exposed by a midline incision and the connective tissue cleared. A hole was drilled in the skull (1 mm lateral and 1 mm posterior to the bregma) and was used for direct injection of the α_2-adrenoceptor agonist, clonidine, into the lateral ventricles of the brain. Blood pressure was allowed to stabilize (15 min) and then the rats were injected with clonidine (500 ng) intracerebroventricularly (i.c.v.). Thirty min later saline vehicle or the α_2-antagonists Wy 25309, Wy 26392 or Wy 26703 (1 μg kg^{-1}–10 mg kg^{-1} i.v.) were administered cumulatively at 5 min intervals. Reversal of the hypotension and bradycardia induced by clonidine was then measured. Two controls were used; one group was given saline (10 μl i.c.v.) and the second saline (10 μl i.c.v.) and chlorisondamine (1 mg kg^{-1} i.v.) followed 30 min later by peripheral administration of the antagonists or saline vehicle as described above.

The results were analysed statistically using a 2-way analysis of variance from which a t value was derived to compare data within groups, and a nested analysis of variance to compare data between groups.

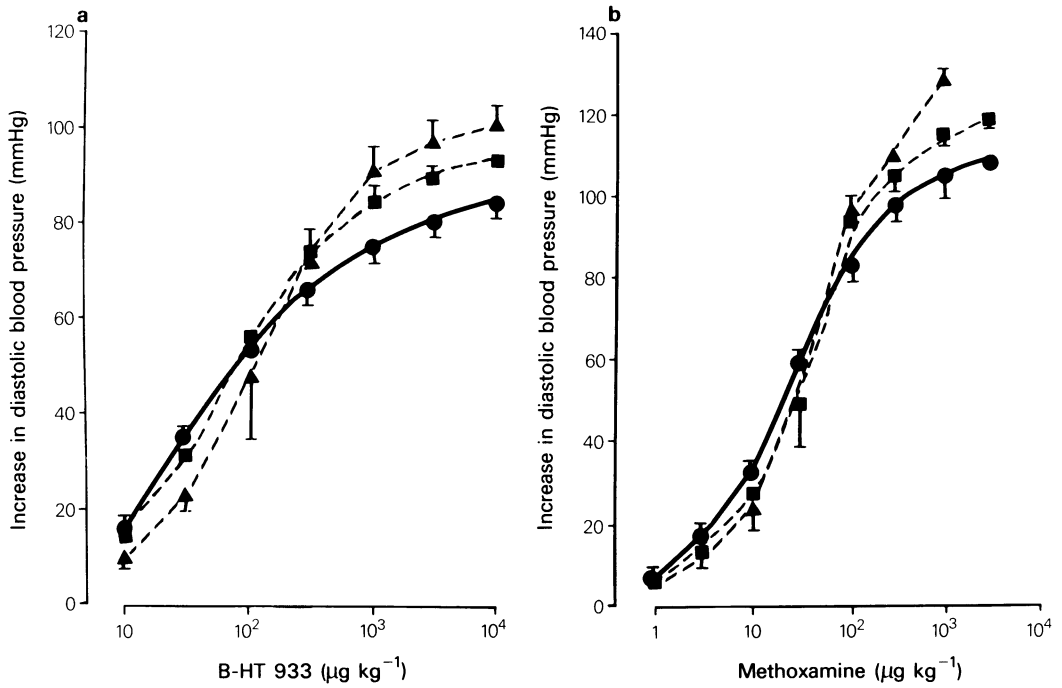


Figure 2 The pressor response evoked by intravenous administration of B-HT 933 (a) or methoxamine (b) in the pithed rat. All preparations were given saline (i.v.) 15 min before constructing the dose-response curve. No other treatment (■); adrenalectomized rats (▲); rats pretreated with reserpine 24 hrs before pithing and given propranolol (1 mg kg^{-1}) 15 min before constructing the curves (●).

Drugs

The following compounds were employed in this study; Wy 26703 (N-((2β, 11bα)-1,3,4,6,7,11b-hexahydro-2*H*-benzo(a) quinolizin-2-yl)-N-methyl-isobutanesulphonamide, hydrochloride); Wy 26392 (N - ((2β, 11bα) - 1,3,4,6,7,11b - hexahydro - 2*H*-benzo-(a)-quinolizin-2-yl)- N -methylpropane-sulphonamide, hydrochloride); Wy 25309 (N-((2β, 11bα)-1,3,4,6,7,11b-hexahydro-2*H*-benzo(a) quinolizin-2-yl)-N-methylmethanesulphonamide hydrochloride), synthesised by the Department of Chemistry, Wyeth Laboratories. The structures of these compounds are given in Figure 1. B-HT 933 (2 - amino - 6 - ethyl - 4,5,7,8 - tetrahydro - 6H - oxazolo - [5,4 - d] - azepin - dihydrochloride) (Boehringer Ingelheim), clonidine HCl (Department of Chemistry, Wyeth Laboratories), methoxamine HCl (Wellcome Laboratories), propranolol HCl (Sigma), indoramin HCl (Wyeth Labs.), yohimbine HCl (Sigma), chlorisondamine chloride, (Ciba-Geigy), reserpine (Koch-Light).

Results

Postjunctional α_1- and α_2-adrenoceptors

The rats, after being pithed, had a diastolic blood pressure of 40.1 ± 0.4 mmHg ($n = 128$). In the presence of saline vehicle (1.0 ml kg^{-1}) the maximum pressor response evoked by methoxamine (3 mg kg^{-1} i.v.) was 119 ± 2 mmHg and that evoked by B-HT 933 (10 mg kg^{-1}) 93 ± 1 mmHg.

Adrenalectomy, or pretreatment with reserpine and propranolol before constructing the dose-response curves to methoxamine or B-HT 933 had no significant effect on the responses to these agonists (Figure 2.).

Yohimbine (0.3–3.0 mg kg^{-1} i.v.) produced dose-related shifts to the right in the dose-response curve to B-HT 933; in contrast the dose-response curve to methoxamine was shifted only at the highest dose level. The post α_2/α_1 selectivity ratio for yohimbine reached a maximum of 12.9 at 1 mg kg^{-1} (Table 1.)

Indoramin (1.0 mg kg^{-1}) evoked a parallel shift to the right of the dose-response curve to methoxamine, but had no significant effect on the dose-response curve to B-HT 933 (Table 1.).

Wy 25309, 26392 and 26703 (0.3–3.0 mg kg^{-1}) all evoked similar dose-related shifts to the right of the dose-response curve to B-HT 933 (Table 1). A representative series of dose-response curves (Wy 26703) are shown in Figure 3a. Over this dose range all the antagonists had only minimal effects on the dose-response curves to methoxamine (Table 1), Figure 3b).

The selectivities of the Wy compounds for the postjunctional α_2-adrenoceptor increased as the dose of antagonists was increased (Table 1).

Prejunctional α_2-adrenoceptors

Following pithing and before drug administration

Table 1 Shifts of the dose-response curves to α-adrenoceptor agonists in pithed rats (expressed as the dose-ratio) evoked by adrenoceptor antagonists, and the selectivity of these antagonists expressed as a ratio of these shifts

		Dose-ratio			*Selectivity ratio*		
	Dose	*Postjunctional*		*Prejunctional*	$\frac{\alpha_2\ post}{\alpha_1\ post}$	$\frac{\alpha_2\ pre}{\alpha_1\ post}$	$\frac{\alpha_2\ pre/}{\alpha_1\ post}$
Antagonist	(mg kg^{-1})	α_2	α_1	α_2			
Yohimbine	0.3	11.0*	2.1	11.8*	5.2	5.6	1.1
	1.0	39.0*	3.0	22.7*	12.9	7.5	0.6
	3.0	76.8*	8.7*	91.6*	8.9	10.6	1.2
Indoramin	1.0	1.7	6.7*	1.1	0.3	0.2	0.6
Wy 26703	0.3	9.8*	2.2	7.7*	4.4	3.4	0.8
	1.0	17.1*	2.8	28.9*	6.1	10.4	1.7
	3.0	36.6*	3.3	77.1*	11.1	23.4	2.1
Wy 26392	0.3	7.8*	1.2	17.4*	6.5	14.3	2.2
	1.0	23.2*	2.5	29.5*	9.3	11.8	1.3
	3.0	65.9*	5.0	130.1*	13.2	26.0	2.0
Wy 25309	0.3	8.3*	2.2	16.4*	3.7	7.3	2.0
	1.0	39.0*	2.4	53.6*	16.5	22.6	1.4
	3.0	78.1*	3.4	192.8*	22.8	56.4	2.5
Saline	1.0†	1.09	0.95	1.1	—	—	—

† ml kg^{-1}, administered 15 min before constructing the dose-response curve.
* $P < 0.05$, t ratio derived from analysis of covariance.

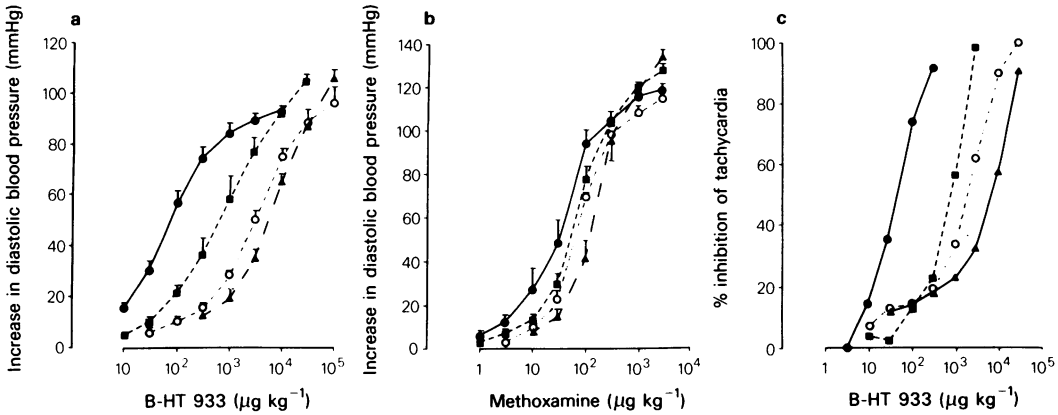


Figure 3 The effect of Wy 26703 on (a) the pressor responses to B-HT 933, (b) the pressor response to methoxamine and (c) the inhibition by B-HT 933 of neurally induced tachycardia in the pithed rat. Dose-response curves to the agonists were constructed 15 min after administration of saline (1.0 ml kg^{-1} i.v.) (●), 0.3 mg kg^{-1} Wy 26703 (■), 1.0 mg kg^{-1} Wy 26703 (○) or 3.0 mg kg^{-1} Wy 26703 (▲).

rats had a basal heart rate of 303 ± 3 beats min^{-1} ($n = 64$). The increase in heart rate following electrical stimulation was 74 ± 1 beats min^{-1} ($n = 64$). In the presence of saline vehicle (1.0 ml kg^{-1}) the dose of B-HT 933 required to produce a 50% inhibition of electrically stimulated tachycardia was 47 ± 4 μg kg^{-1}. In adrenalectomized rats the B-HT 933 dose-response curve was moved slightly to the right with a dose-ratio of 1.4. In vagotomized rats the B-HT 933 dose-response curve was marginally shifted to the left (dose-ratio 0.9).

Indoramin (1.0 mg kg^{-1}) had little effect on the dose-response curve to B-HT 933 (Table 1). Yohimbine (0.3–3.0 mg kg^{-1}), Wy 25309, Wy 26392 and Wy 26703 (0.3–3 mg kg^{-1}) evoked similar dose-related shifts to the right of the B-HT 933 dose-response curve (Table 1 and Figure 3c).

The selectivity of each of the antagonists for the prejunctional α_2-adrenoceptor compared to the postjunctional α_1-adrenoceptor increased with the dose administered (Table 1). None of the antagonists showed preference for either the pre- or postjunctional α_2-adrenoceptors (Table 1).

The effect of saline pretreatment

At the end of the series of experiments described above an additional group of saline controls was obtained for each experimental group in order to estimate any changes which might be related to the time required to complete the experiments. The ED_{50} values obtained from these experiments were compared with those employed as the control for the antagonist-treated groups. There was no significant difference between these two saline groups (Table 1).

Reversal of clonidine-induced hypotension

Administration of clonidine (500 ng) into the lateral cerebral ventricle (i.c.v.) lowered diastolic blood pressure (DBP) by 50 ± 1 mmHg ($n = 20$). Subsequent intravenous administration of saline (1 ml kg^{-1}) at five min intervals resulted in a slow reversal of the clonidine-induced response (Figure 4).

Administration of saline (10 μl i.c.v.) had no significant effect on DBP. Subsequent i.v. administration of saline, Wy 25309, Wy 26392 or Wy 26703 evoked slight falls in DBP (Figure 4). A comparison of the drug- and saline-treated groups by nested analysis of variance techniques revealed that only the higher doses of the antagonists (3 and 10 mg kg^{-1} i.v.) induced falls which were significantly ($P < 0.05$) larger than those in the saline treated group.

Following i.c.v. administration of clonidine, i.v. administration of each of the benzoquinolizines (1 μg–10 mg kg^{-1}) reversed the hypotension induced by clonidine in a dose-dependent manner (Figure 4). None of the benzoquinolizines completely reversed the clonidine-induced hypotension. A maximum reversal was obtained with doses of 30–100 μg kg^{-1}; increasing the dose further resulted in a reduction in the DBP (Figure 4).

Chlorisondamine (1 mg kg^{-1} i.v.) lowered DBP by 59 ± 4 mmHg ($n = 16$). Subsequent administration of Wy 25309, Wy 26703 or Wy 26392 had no significant effect on this depressor response by comparison with the saline-treated group (Figure 4).

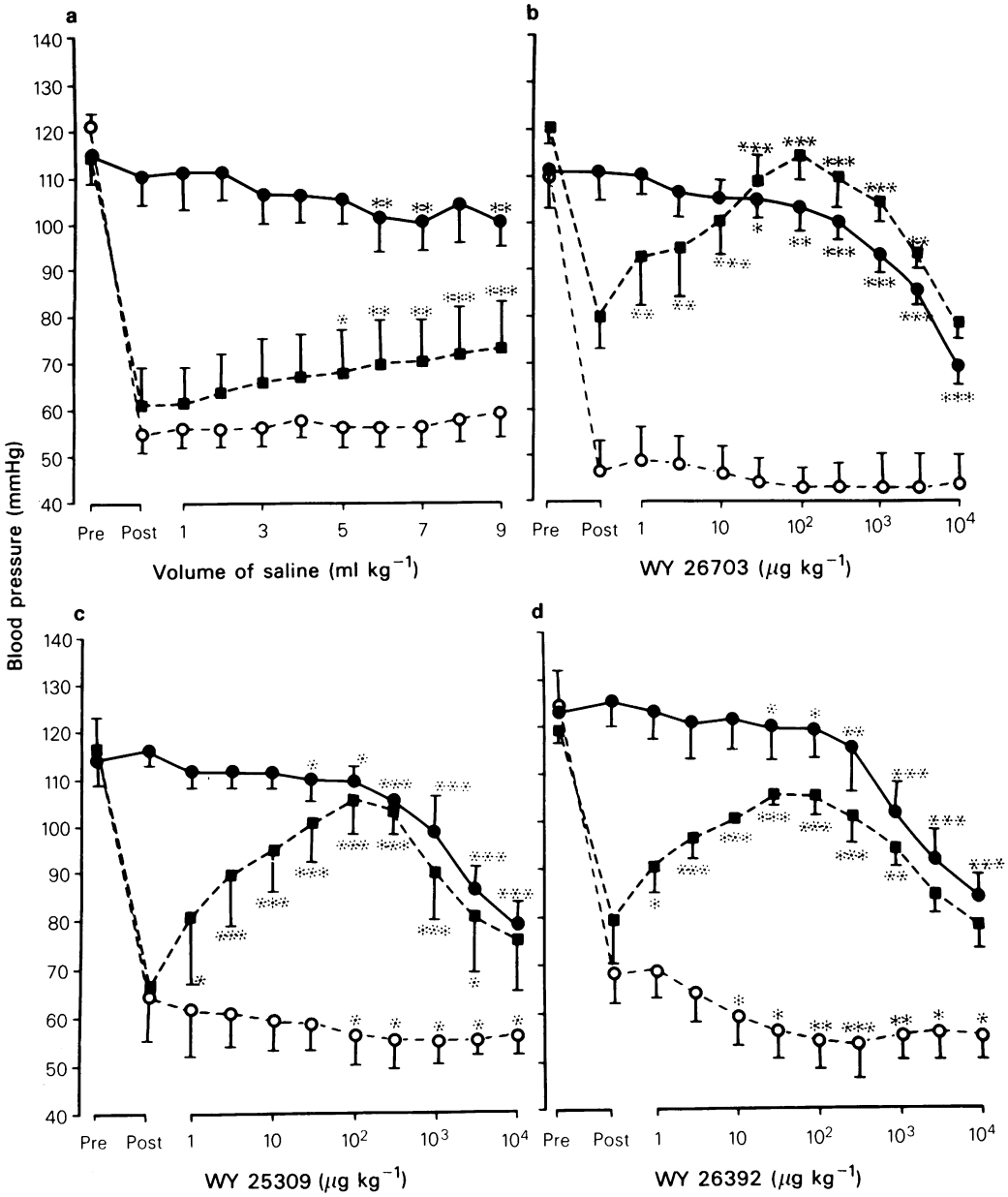


Figure 4 The effect of intravenous administration of (a) saline, (b) Wy 26703 (c) Wy 25309 and (d) Wy 26392 on blood pressure. Pre—indicates blood pressure before administration of saline or drug. Post—indicates blood pressure 30 min after saline (10 μl i.c.v.) (●), clonidine (500 ng in 10 μl i.c.v.) (■) or chlorisondamine. (1 mg kg^{-1} i.v.) (○). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicates level of significance of the difference between each time point and the 'post' blood pressure reading (2 way analysis of variance).

Discussion

The α-adrenoceptor blockade produced by a series of novel substituted benzoquinolizines, Wy 25309, Wy 26392 and Wy 26703 and the preferential α_2-adrenoceptor antagonist yohimbine has been compared in pithed rats, a preparation which has been used extensively in the study of α-adrenoceptors.

Two selective α-adrenoceptor agonists have been employed to define the responses mediated via α_1- and α_2-adrenoceptors in this preparation. The α_2-adrenoceptor agonist B-HT 933 (Timmermans & Van Zwieten, 1980) evoked a pressor response (postjunctional α_2-adrenoceptor) and inhibited the tachycardia produced by spinal cord stimulation (prejunctional α_2-adrenoceptor). The selective α_1-adrenoceptor agonist methoxamine (Kobinger & Pichler, 1981) evoked a pressor response mediated via postjunctional α_1-adrenoceptors. The specificity of these agonists was checked under the experimental conditions employed in this study by administering the selective α_2-adrenoceptor antagonist yohimbine (Starke *et al.*, 1975; Timmermans *et al.*, 1980) and the selective α_1-adrenoceptor antagonist indoramin (Rhodes & Waterfall, 1978). Yohimbine inhibited both the pre- and postjunctional responses to B-HT 933 and had a relatively small effect on the response to methoxamine.

Indoramin inhibited responses to methoxamine but not those to B-HT 933. The responses to the agonists were not significantly affected by saline pretreatment, adrenalectomy, depletion of neuronal noradrenaline by reserpine, or β-adrenoceptor blockade.

All three benzoquinolizines exhibited a marked degree of selectivity for the α_2-adrenoceptor, both at the pre- and postjunctional sites. The rightward shift of the dose-response curve to B-HT 933 (α_2 blockade) evoked by the benzoquinolizines and yohimbine increased in a dose-related manner. The methoxamine dose-response curves were also dose-dependently shifted to the right (α_1 blockade) but the increase in the shift of the curve for a three fold increase in antagonist dose was less than that observed at the α_2-adrenoceptor site. As a consequence, the selectivity of each compound increased in a dose-related manner. The compound exhibiting the greatest overall selectivity for α_2-adrenoceptors was Wy 25309 and generally the relative order of selectivity of the compounds was Wy 25309 > Wy 26392 > Wy 26703 > yohimbine.

The changes in the order of selectivity at the α_2-adrenoceptor are generally small, and probably of little pharmacological significance. These results, however, demonstrate that the selectivity of a compound may vary with the dose employed and an accurate appraisal of selectivity can only be made by comparing the effects at a number of doses.

The finding that α_2-adrenoceptors are located both pre- and postjunctionally in peripheral noradrenergic neuroeffector junctions raises the question of whether these two populations are identical. Two studies have reached the conclusion that there are differences between these receptors (Hicks, 1981; De Jonge *et al.*, 1981a). The series of benzoquinolizines investigated here exhibited a small degree of selectivity for the prejunctional α_2-adrenoceptor (approximately two fold for each compound). This degree of selectivity is small compared to that exhibited for the α_2-versus the α_1-adrenoceptor and we believe it is unlikely to represent a true difference between the receptors. In addition the less selective α_2-adrenoceptor antagonist yohimbine exhibited no preference for either α_2-adrenoceptor. B-HT 933 was significantly more potent at the pre- than the postjunctional site. The differences in potency of agonists and antagonists at pre- and postjunctional sites has recently been demonstrated for α_1-adrenoceptors (Docherty, 1983).

Following intravenous administration, all three benzoquinolizines reversed the hypotension evoked by the administration of clonidine (i.c.v.). It has been demonstrated in a number of species, including the rat, that this hypotensive response is due to an agonist effect of clonidine mediated via α_2-adrenoceptors within the CNS. (Berthelsen & Pettinger, 1977; Timmermans *et al.*, 1981). The benzoquinolizines did not reverse the hypotension resulting from ganglion blockade suggesting a specificity for the clonidine-induced hypotension. These results are consistent therefore with the hypothesis that benzoquinolizines are able to cross the blood brain barrier and block central α_2-adrenoceptors. All three benzoquinolizines were potent central α_2-adrenoceptor antagonists producing a significant reversal of the effects of clonidine at doses of $1\,\mu g\,kg^{-1}$ i.v. Despite this high potency none of the compounds completely reversed the effect of clonidine. This incomplete reversal is probably due to two factors. Firstly the blood pressure of the preparation fell during the experiment, even in preparations treated with saline alone and secondly, the antagonists alone decreased the blood pressure of control animals at doses of 3 and $10\,mg\,kg^{-1}$. From the pithed rat experiments it is clear that these compounds produced a degree of α_1-adrenoceptor blockade at doses of $3.0\,mg\,kg^{-1}$. As α_1-adrenoceptor blockade is known to lower blood pressure in the rat it is likely that this property of the benzoquinolizines was responsible for the fall in blood pressure at the higher doses. Although clonidine did evoke a bradycardia in this preparation (data not shown) we have not considered this effect in

our analysis because there is considerable evidence that this effect is mediated via several populations of receptors which limits its use as an index of central α_2-adrenoceptor blockade (De Jonge *et al.*, 1981b).

In conclusion, the results presented in this paper demonstrate that three novel benzoquinolizines, Wy 25309, 26703 and 26392 are potent and selective α_2-adrenoceptor antagonists in the pithed rat. These compounds readily cross the blood brain barrier and block α_2-adrenoceptors in the CNS.

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