Br. J. Pharmac. (1984), **83**, 707–712

2-Alkyl analogues of idazoxan (RX 781094) with enhanced antagonist potency and selectivity at central α_2-adrenoceptors in the rat

B. Gadie, A.C. Lane, P.S. McCarthy, I.F. Tulloch & D.S. Walter

Department of Pharmacology, Reckitt and Colman p.l.c., Pharmaceutical Division, Dansom Lane, Hull, HU8 7DS

1 Four 2-alkyl (methyl, ethyl, *n*-propyl and isopropenyl) analogues of idazoxan (RX 781094) have been synthesized and assessed in terms of their central α_2/α_1-adrenoceptor selectivity and α_2-adrenoceptor antagonist potency using both *in vitro* and *in vivo* tests in the rat.

2 In cortical binding assays using [^{3}H]-idazoxan and [^{3}H]-prazosin, idazoxan had a 5 times greater α_2/α_1-selectivity than yohimbine. The 2-alkyl substituted analogues all showed improved selectivity, being between 17 and 29 times more selective than yohimbine for [^{3}H]-idazoxan binding sites.

3 In terms of central antagonist potency *in vivo*, the most favourable substitutions were 2-ethyl (RX 811033) and 2-*n*-propyl (RX 811054). Compared with yohimbine, these analogues were, respectively, 36 and 18 times more potent intravenously and 5 and 7.5 times more potent orally in their antagonism of guanoxabenz-induced mydriasis in the pentobarbitone-anaesthetized rat.

4 All the analogues had a duration of action similar to that of idazoxan, which was significantly shorter than that of yohimbine.

5 The results indicate that introduction of alkyl groups in the 2-position of idazoxan greatly increases the α_2/α_1-adrenoceptor selectivity as measured in binding studies. Improved α_2-adrenoceptor affinity and antagonist potency were particularly associated with the 2-ethyl and 2-*n*-propyl analogues.

Introduction

Idazoxan (RX 781094; 2-(2-(1,4 benzodioxanyl))-2-imidazoline HCl) has been shown to be a highly potent and selective antagonist at peripheral α_2-adrenoceptors (Chapleo *et al.*, 1981). In addition, idazoxan possesses central α_2-adrenoceptor antagonist activity as demonstrated by its ability to block the neuropharmacological effects induced by α_2-adrenoceptor agonists in rodents; these agonist effects include cortical EEG synchronization, hypothermia and behavioural sedation (Dettmar *et al.*, 1983) as well as mydriasis (Berridge *et al.*, 1983). Competition binding studies in rat cerebral cortex have confirmed that idazoxan also has a high α_2/α_1-selectivity in the CNS (Howlett *et al.*, 1982).

In the present study we have examined several 2-alkyl analogues of idazoxan for central α_2-adrenoceptor activity in the rat (for structures see Figure 1). The α_2/α_1-selectivities of the analogues were assessed in cortical binding studies using [^{3}H]-idazoxan and [^{3}H]-prazosin as selective ligands for α_2- and α_1-adrenoceptors, respectively. To provide a

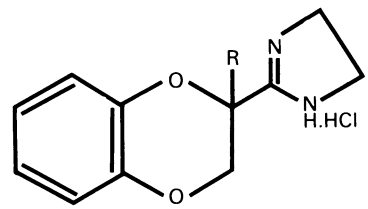

Figure 1 Chemical structures of idazoxan (RX 781094) and its 2-alkyl analogues:

R substituent	*Compound*
-H	Idazoxan (RX 781094)
-CH_3	RX 801079
-CH_2CH_3	RX 811033
-$CH_2CH_2CH_3$	RX 811054
-C(CH_2)(CH_3)	RX 811005

© The Macmillan Press Ltd 1984

measure of central α_2-adrenoceptor antagonist potency *in vivo*, the analogues were tested against guanoxabenz-induced mydriasis in the anaesthetized rat (Berridge *et al.*, 1983). A comparison is made between the 2-alkyl analogues of idazoxan and two α_2-adrenoceptor antagonists from different chemical series, namely yohimbine and Wy 26703 (Lattimer *et al.*, 1982).

A preliminary account of these findings has been presented to the British Pharmacological Society (Gadie *et al.*, 1983).

Methods

Competition binding studies

Tissue preparation Cerebral membranes were prepared from male Sprague-Dawley rats as described previously (Lane *et al.*, 1983). The membranes were suspended in a physiological salt solution containing (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, KH_2PO_4 1.2, $MgSO_4$ 1.2 and $NaHCO_3$ 25, equilibrated with 95% O_2/5% CO_2 at 25°C before use; final pH, 7.8. The final protein concentration was approximately 1 mg ml^{-1}.

[^{3}H]-prazosin binding Preliminary experiments showed that in the physiological incubation medium, [^{3}H]-prazosin (33 Ci $mmol^{-1}$) over the concentration range 0.06 nM to 5 nM bound to a single population of high affinity sites: $B_{max} = 62 \pm 2$ fmol mg^{-1} protein, $K_D = 0.27 \pm 0.05$ nM (mean ± s.e.mean of 3 experiments). Specific binding of [^{3}H]-prazosin was defined using an excess of WB 4101.

The incubation mixture used in competition binding experiments contained: membrane preparation (970 µl), saturating ligand or varying concentrations of test compound (10 µl), and [^{3}H]-prazosin (20 µl) at a final concentration of 0.3 nM. Samples were incubated for 30 min at 25°C and then rapidly filtered under vacuum through Whatman GFB filters which were then washed with 2 × 4 ml of ice-cold Tris HCl buffer (0.05 M, pH 7.4). Membrane-bound radioactivity trapped by the filters was counted by a scintillation counter following the addition of NE 260 micellar scintillation fluid (3.5 ml; Nuclear Enterprises). Specific binding of [^{3}H]-prazosin was defined using an excess of the selective α_1-adrenoceptor antagonist WB 4101 (2 µM). Hill plots were constructed from up to eight concentrations of test compound. These were linear regressions of log concentration versus log $(B - B_L)/B_L$ where B was the amount of specific [^{3}H]-prazosin binding in the absence of test compound and B_L the specific binding at each concentration of test compound. The concentrations producing 50% displacement of specific [^{3}H]-prazosin binding were calculated and the K_i values derived according to the method of Cheng & Prusoff (1973).

[^{3}H]-idazoxan binding The method has been described previously (Doxey *et al.*, 1983). This was similar to that used for [^{3}H]-prazosin but differed in the following respects: the final concentration of [^{3}H]-idazoxan was 1 nM; the incubation time was 15 min. Specific binding of [^{3}H]-idazoxan was defined by the addition of an excess of phentolamine (1 µM).

A previous study showed that in the physiological incubation medium, [^{3}H]-idazoxan over the range 0.1 to 35 nM bound to a single population of high affinity sites: $B_{max} = 144 \pm 14$ fmol mg^{-1} protein, $K_D = 4.4 \pm 0.4$ nM (mean ± s.e.mean of 5 determinations).

Central α_2-adrenoceptor antagonist potency, in vivo

Determination of α_2-adrenoceptor antagonist potency after intravenous administration Rats (male, Sprague-Dawley, 250–350 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). Fifteen minutes later the tail vein was cannulated for drug administration. Drug-induced changes in pupil diameter were measured with a Beck Luminex magnifier as described previously (Berridge *et al.*, 1983).

Animals were injected with a maximally effective mydriatic dose of the α_2-adrenoceptor agonist, guanoxabenz (0.3 mg kg^{-1}, i.v.) ($n = 5-6$ per group). Fifteen minutes later, increasing doses (half-log increments) of idazoxan or a 2-alkyl analogue were injected intravenously at 5 min intervals. The α_2-adrenoceptor antagonist potency was determined by calculation of the cumulative dose that reversed the mydriatic effect of guanoxabenz by 50% (AD_{50} value).

Determination of α_2-adrenoceptor antagonist potency after oral administration Groups ($n = 5-6$) of conscious, fasted (18 h) rats were dosed orally either with one of a range of doses of α_2-adrenoceptor antagonist, or with vehicle. Thirty minutes later the animals were anaesthetized and the tail vein cannulated as described above. Forty-five minutes after oral dosing the rats were challenged with a test dose of guanoxabenz (0.3 mg kg^{-1}, i.v.). This dose caused a maximal mydriasis (> 4.0 mm) in the vehicle-treated rats. The mydriatic response in each of the antagonist treated groups was then expressed as a percentage of the maximum response observed in the vehicle-pretreated group. The AD_{50} and AD_{99} values were determined from a graph of percentage antagonism against log dose of antagonist.

Table 1 Affinities and selectivities of idazoxan analogues, yohimbine and Wy 26703 for α-adrenoceptors in rat cerebral cortex

Compound	*2-substituent*	*[³H]-idazoxan* (α_2) K_i (nM)	*[³H]-prazosin* (α_1) K_i (nM)	$K_i(\alpha_1)/K_i(\alpha_2)$
Idazoxan	-H	3.1 ± 0.4	91 ± 3	29
RX 801079	-methyl	4.4 ± 0.4	449 ± 90	102
RX 811033	-ethyl	1.0 ± 0.1	174 ± 29	172
RX 811054	-n-propyl	1.0 ± 0.1	107 ± 31	108
RX 811005	-isopropenyl	16.3 ± 1.8	1794 ± 185	110
Yohimbine		40.0 ± 5.5	230 ± 16	6
Wy 26703		18 ± 2.5	833 ± 137	46

K_i values are mean ± s.e.mean of three experiments, each performed in triplicate. Hill plots were constructed for each antagonist and slopes were close to unity (range 0.84–1.11), indicating competitive interaction at both α_1- and α_2-sites.

Time course of α_2-adrenoceptor antagonist activity

The AD_{99} dose of an antagonist, or distilled water vehicle, was administered orally to groups of 5 to 6 conscious rats at various times before challenge with guanoxabenz. Guanoxabenz was injected intravenously in incremental doses starting 10 min after induction of anaesthesia with sodium pentobarbitone (60 mg kg^{-1}, i.p.). Pupil diameter was measured 5 min after each dose of guanoxabenz. The mean change in pupil diameter (post-guanoxabenz minus pre-guanoxabenz value) was plotted graphically against log-dose of guanoxabenz for each antagonist-treated group. By calculating the percentage antagonism of the mydriatic effect of 0.3 mg kg^{-1} guanoxabenz (the ED_{99} dose in vehicle-treated rats) for each pretreatment group, a time-effect relationship was established. This in turn permitted the measurement of the time taken for the antagonist effect of the AD_{99} oral dose to fall by half (to the AD_{50} level).

Drugs

Idazoxan [2-(2-(1,4-benzodioxanyl))-2-imidazoline HCl] and four 2-alkyl analogues of idazoxan – RX 801079 (2-methyl), RX 811033 (2-ethyl), RX 811054 (2-n-propyl) and RX 811005 (2-isopropenyl) were synthesized in the Medicinal Chemistry Department at Reckitt and Colman. Other agents included: guanoxabenz HCl (Roussel), WB 4101 HCl (2,6-dimethyloxyphenoxyethyl) aminomethyl-1,4benzodioxane Hcl (Ward Blenkinsop), Wy 26703 [N-methyl-N-(1, 3, 4, 6, 7, 11 bα-hexahydro-2H-benzo(a)-quinolizin-2β-yl) isobutane sulphonamide (Wyeth), yohimbine HCl (Sigma). [³H]-prazosin (33 Ci mmol^{-1}) was kindly supplied by Pfizer Ltd.

In the binding studies, drug solutions were made up either in distilled water or dilute hydrochloric acid. For intravenous administration, drugs were dissolved in sterile saline or distilled water (dilutions in sterile saline) and given in a dose volume of 1 ml kg^{-1}. For oral administration drugs were dissolved in distilled water and given in a dose volume of 2 ml kg^{-1}. Antagonist doses are expressed in terms of base (mol kg^{-1}). Guanoxabenz doses are in terms of the hydrochloride salt.

Results

α-Adrenoceptor binding studies in rat cerebral cortex

The mean apparent affinities (K_i values) of the compounds in radioligand binding studies are shown in Table 1. All of the compounds had higher affinities for α_2-adrenoceptor sites labelled with [³H]-idazoxan than for α_1-adrenoceptor sites labelled with [³H]-prazosin. Degree of selectivity for the α_2-adrenoceptor site was expressed as the ratio of the K_i values at the α_1- and α_2-sites. The 2-alkyl substituted analogues of idazoxan were between 3 and 6 times more selective than idazoxan for α_2-adrenoceptors. Optimum selectivity was seen with the 2-ethyl analogue (RX 811033). The rank order of α_2-adrenoceptor selectivity was: RX 811033 > RX 811005 > RX 811054 > RX 801079 > Wy 26703 > idazoxan > yohimbine. In addition, idazoxan and its analogues showed higher affinities for the α_2-adrenoceptor than did yohimbine or Wy 26703.

The rank order of potency at α_1-adrenoceptors was: idazoxan > RX 811054 > RX 811033 > yohimbine > RX 801079 > Wy 26703 > RX 811005.

Central α_2-adrenoceptor antagonist potency in vivo

The 2-alkyl analogues of idazoxan were all effective antagonists of the centrally-mediated mydriasis induced by guanoxabenz (Table 2). After intravenous administration the rank order of antagonist potencies

Table 2 Antagonist potencies and duration of action of idazoxan analogues, yohimbine and Wy 26703 at central α_2-adrenoceptors in the rat

		Antagonism of guanoxabenz-induced mydriasis		
Compound	*2-substituent*	AD_{50} (i.v.) $\mu mol\ kg^{-1}$	AD_{50} (p.o.) $\mu mol\ kg^{-1}$	*Duration of antagonism (h)*
Idazoxan	-H	0.18 ± 0.05	16.6 ± 3.4	2.9
RX 801079	-methyl	0.25 ± 0.06	17.7 ± 3.8	3.0
RX 811033	-ethyl	0.06 ± 0.01	2.9 ± 0.5	2.4
RX 811054	-*n*-propyl	0.12 ± 0.004	2.0 ± 0.3	—
RX 811005	-isopropenyl	0.54 ± 0.04	24.2 ± 3.4	2.9
Yohimbine		2.2 ± 0.04	15.3 ± 1.5	> 8
Wy 26703		0.2 ± 0.02	ND	—

AD_{50} values (mean ± s.e.mean, $n = 5-6$) represent the antagonist doses required to inhibit by 50% the maximal mydriatic response induced by guanoxabenz (0.3 mg kg^{-1}, i.v.) (see Methods for details). To determine oral potency, antagonists were administered to conscious rats; they were anaesthetized 30 min later and then challenged with guanoxabenz after 15 min. The duration refers to the time taken for the antagonist effect of an AD_{99} oral dose to fall to the AD_{50} level. ND = not detectable : oral doses up to 54 $\mu mol\ kg^{-1}$ (20 mg kg^{-1}) were devoid of antagonist effect.

to cause a 50% reversal of the mydriatic response was: RX 811033 > RX 811054 > idazoxan > Wy 26703 > RX 801079 > RX 811005 > yohimbine.

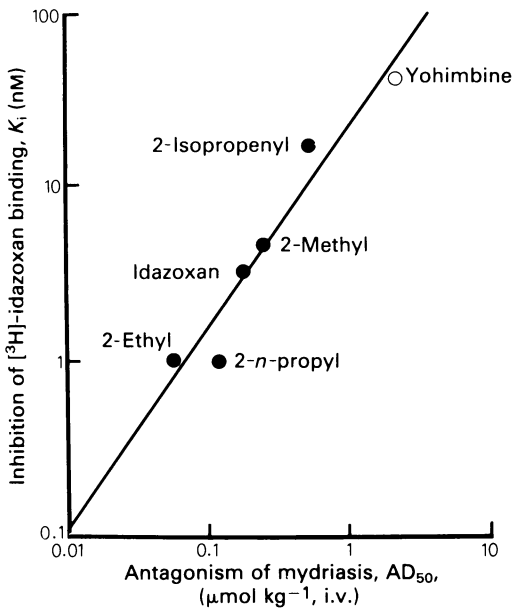

Figure 2 Correlation between α_2-adrenoceptor affinity determined against [^{3}H]-idazoxan binding in rat cortical membranes and central α_2-adrenoceptor antagonist potency assessed against guanoxabenz-induced mydriasis in the pentobarbitone-anaesthetized rat. The correlation uses data taken from Tables 1 and 2. Labelling next to the points refers to the 2-alkyl substituent in the idazoxan molecule. Correlation coefficient, $r = 0.97$ ($P < 0.001$).

The central α_2-adrenoceptor antagonist potencies after intravenous administration were closely correlated with the affinities displayed for cortical α_2-adrenoceptors labelled with [^{3}H]-idazoxan (Figure 2).

With the oral route of administration, the 2-ethyl (RX 811033) and 2-*n*-propyl (RX 811054) analogues were 7–13 times more potent than either yohimbine or the other compounds related to idazoxan (Table 2). Wy 26703 was devoid of oral activity in doses up to 54 $\mu mol\ kg^{-1}$ (20 mg kg^{-1}).

Duration of antagonist action

There was a close similarity in the time course of the effects of the idazoxan-related antagonists after oral dosing, as typified by the 2-methyl analogue RX 801079 (Figure 3). The time taken for the antagonist effect of AD_{99} doses of idazoxan and analogues to fall by half was between 2 and 3 h whereas an equieffective dose of yohimbine was virtually fully effective for at least 8 hours (Table 2).

Discussion

Alkylation of the 2-position of idazoxan increased central α_2/α_1-selectivity as measured by radioligand binding studies in rat cerebral cortex. The improved selectivity of the analogues ranged from 3.5 to 6 times that of idazoxan (α_2/α_1-selectivity = 29) which itself was five times more selective than yohimbine. The selectivity of the antagonist Wy 26703 for α_2-adrenoceptors was intermediate between that of idazoxan and its 2-alkyl analogues.

The increased selectivity of the 2-alkyl derivatives

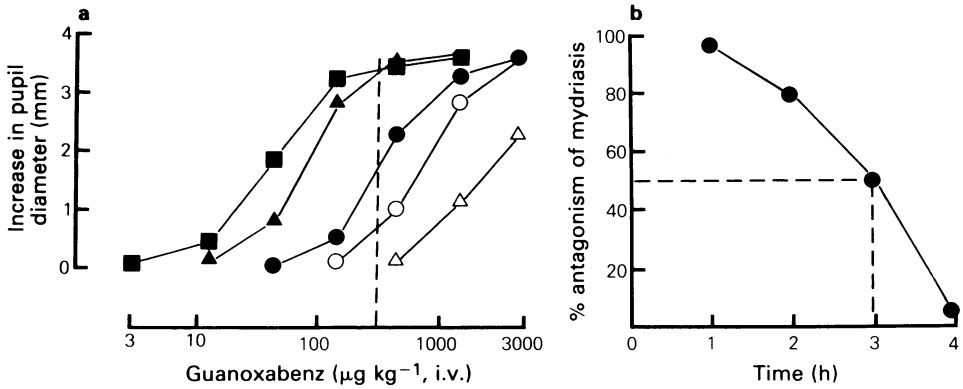

Figure 3 Duration of central α_2-adrenoceptor antagonist effect of the 2-methyl analogue (RX 801079) after administration of an oral AD_{99} dose (39 μmol kg^{-1}). (a) Effect of antagonist (AD_{99}) pretreatment time (1 h, △; 2 h, ○; 3 h ●; 4 h, ▲) on guanoxabenz-induced mydriasis. Pretreated rats were anaesthestized 10 min before injection of guanoxabenz. The degree of antagonism in each group was determined by measuring the mydriatic response after 0.3 mg kg^{-1} guanoxabenz (dashed line) and expressing it as a percentage of the antagonist vehicle response (■). Each point is the mean of 5–6 rats. (b) Time course of antagonism. The degree of antagonism at each time point was calculated as described in (a) above. The time for the antagonist effect to fall to the AD_{50} level was read off from the graph and taken as a measure of the duration of action (see Table 2).

of idazoxan for central α_2-adrenoceptors occurred as a result of two factors: either increased affinity for the α_2-adrenoceptor or decreased affinity for the α_1-adrenoceptor. Compared with idazoxan, the 2-ethyl (RX 811033) and 2-*n*-propyl (RX 811054) analogues showed a marked enhancement of affinity for α_2-adrenoceptors. In addition, part of the high selectivity of RX 811033 resulted from its reduced affinity for α_1-adrenoceptors. RX 811005 (2-isopropenyl) was also considerably more selective than idazoxan for α_2-adrenoceptors, chiefly as a consequence of its weak affinity for α_1-adrenoceptors. The increased selectivity of the 2-methyl analogue (RX 801079) was achieved mainly through a decreased affinity for α_1-adrenoceptors.

The affinities of the 2-alkyl analogues for α_2-adrenoceptors *in vitro* correlated with α_2-adrenoceptor antagonist potencies assessed *in vivo*. The most potent compounds after intravenous or oral administration were RX 811033 and RX 811054. It is noteworthy that both these analogues showed considerably enhanced oral bioavailability compared with idazoxan. For instance, RX 811033 was at least five times more potent than idazoxan by the oral route but only three times more potent by the intravenous route. The close correlation between α_2-adrenoceptor affinity determined in binding studies and intravenous antagonist potency assessed against guanoxabenz-induced mydriasis indicates that these agents have similar access to central α_2-adrenoceptors.

Comparison of oral AD_{99} doses of idazoxan and its analogues showed that the degree of antagonism exerted by each of these compounds had fallen by half in the space of two to three hours. This would suggest that 2-alkyl substitution in this chemical series has little effect on the duration of pharmacological action in the central nervous system. In contrast, the antagonist action of yohimbine was only slightly reduced after eight hours pretreatment. It is possible that the high lipophilicity of yohimbine (octanol/water partition coefficient of 143 at pH 7.4 against a value of 1.5 for idazoxan – unpublished data), coupled with a slow rate of elimination of the drug from the brain and other tissues, may be contributing to its long duration of action. An additional factor may be the slower receptor kinetics of yohimbine (manifested by sluggish onset and offset times at α_2-adrenoceptors) which are apparent in isolated peripheral tissues such as the mouse vas deferens (C.F.C. Smith, personal communication).

Although the 2-alkyl analogues have a high selectivity for α_2-adrenoceptors, further studies are required to investigate their actions at other receptor types in the periphery and central nervous system. Idazoxan has previously been shown to have only very weak affinity for peripheral receptors other than α-adrenoceptors (Doxey *et al.*, 1983) and it will be of interest to determine whether this high receptor specificity is also exhibited by the present series of analogues.

The enhanced central α_2/α_1-selectivity and antagonist potency of these 2-alkyl substituted analogues of idazoxan reflect the favourable phar-

macological profiles of these compounds observed in peripheral tissues (Doxey *et al.*, 1984). These pharmacological agents should prove of value in the elucidation of α_2-adrenoceptor-mediated noradrenergic mechanisms in both the periphery and the central nervous system.

References

BERRIDGE, T.L., GADIE, B., ROACH, A.G. & TULLOCH, I.F. (1983). α_2-Adrenoceptor agonists induce mydriasis in the rat by an action within the central nervous system. *Br. J. Pharmac.*, **78,** 507–515.

CHAPLEO, C.B., DOXEY, J.C., MYERS, P.L. & ROACH, A.G. (1981). RX 781094, a new potent, selective antagonist at α_2-adrenoceptors. *Br. J. Pharmac.*, **74,** 942P.

CHENG, Y. & PRUSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmac.*, **22,** 3099–3108.

DETTMAR, P.W., LYNN, A.G. & TULLOCH, I.F. (1983). Neuropharmacological studies in rodents on the action of RX 781094, a new selective α_2-adrenoceptor antagonist. *Neuropharmacology*, **22,** 729–737.

DOXEY, J.C., GADIE, B., LANE, A.C., & TULLOCH, I.F. (1983). Evidence for pharmacological similarity between α_2-adrenoceptors in the vas deferens and central nervous system of the rat. *Br. J. Pharmac.*, **80,** 155–161.

DOXEY, J.C., ROACH, A.G. & SMITH, C.F.C. (1983). Studies on RX 781094: a selective, potent and specific antagonist of α_2-adrenoceptors. *Br. J. Pharmac.*, **78,** 489–505.

DOXEY, J.C., ROACH, A.G., STRACHAN, D.A. & VIRDEE, N.K. (1984). Selectivity and potency of 2-alkyl analogues of the α_2-adrenoceptor antagonist idazoxan (RX 781094) in peripheral systems. *Br. J. Pharmac.*, **83,** 713–722.

GADIE, B., LANE, A.C., McCARTHY, P.S., TULLOCH, I.F. & WALTER, D.S. (1983). 2-Alkyl analogues of RX 781094: potent, selective antagonists at central α_2-adrenoceptors. *Br. J. Pharmac.*, **79,** Proc. Suppl., 312P.

HOWLETT, D.R., TAYLOR, P. & WALTER, D.S. (1982). α-Adrenoceptor selectivity studies with RX 781094 using radioligand binding to central membranes. *Br. J. Pharmac.*, **76,** Proc. Suppl., 294P.

LANE, A.C., HOWLETT, D.R. & WALTER, D.S. (1983). The effects of metal ions on the binding of a new α_2-adrenoceptor antagonist radioligand [^{3}H]-RX 781094 in rat cerebral cortex. *Biochem. Pharmac.*, **32,** 3122–3125.

LATTIMER, N., RHODES, K.F., WARD, T.J., WATERFALL, J.F. & WHITE, J.F. (1982). Selective α_2-adrenoceptor antagonist activity of novel substituted benzoquinolizines. *Br. J. Pharmac.*, **75,** Proc. Suppl., 154P.

(*Received March 23, 1984. Revised June 7, 1984.*)