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# Evidence for pharmacological similarity between $\alpha_2$-adrenoceptors in the vas deferens and central nervous system of the rat

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**1** Seven $\alpha_2$-adrenoceptor antagonists with diverse chemical structures have been examined for their effects at $\alpha_2$-adrenoceptors in the vas deferens and central nervous system of the rat.

**2** Antagonist potency assessed against the presynaptic $\alpha_2$-adrenoceptor agonist action of clonidine in the isolated vas deferens (RX 781094 > Wy 26703 > yohimbine > rauwolscine > piperoxan > mianserin > RS 21361) was highly correlated with the ability of these drugs to displace saturable [$^3$H]-RX 781094 binding from cerebral cortex membranes.

**3** Similarly, antagonist potency in the vas deferens was highly correlated with antagonist activity in reversing the centrally-mediated mydriasis induced by the selective $\alpha_2$-adrenoceptor agonist, guanoxabenz, in pentobarbitone-anaesthetized rats.

**4** The results indicate that the presynaptic $\alpha_2$-adrenoceptors in the vas deferens are pharmacologically similar to characterized these $\alpha_2$-adrenoceptors in the central nervous system of the rat.

## Introduction

A substantial body of evidence now exists demonstrating the presence of $\alpha_2$-adrenoceptors in a wide variety of mammalian tissues (for reviews see Langer, 1981; Starke, 1981). In the majority of studies these $\alpha_2$-adrenoceptors have been classified according to their relative sensitivity to various $\alpha_2$-adrenoceptor agonists and antagonists. This approach has allowed the characterization of $\alpha_2$-adrenoceptors in a number of peripheral tissues and one in particular, the rat isolated vas deferens, is now widely used for screening drugs with potential $\alpha_2$-adrenoceptor activity (Doxey & Roach, 1980). In order to assess the general predictive value of this screen, especially with regard to central activity, it is important to investigate whether the $\alpha_2$-adrenoceptors in the vas deferens resemble presumed $\alpha_2$-adrenoceptors in the CNS.

Central $\alpha_2$-adrenoceptors have been widely investigated by radioligand binding techniques using various selective $\alpha_2$-adrenoceptor agents (U'Prichard, Greenberg & Snyder, 1977; Perry & U'Prichard, 1981; Jarrott, Louis & Summers, 1982). Recently RX 781094, a highly selective $\alpha_2$-adrenoceptor antagonist (Doxey, Roach & Smith, 1983) has proved to be a particularly useful ligand for studying $\alpha_2$-adrenoceptor sites (Howlett, Taylor and Walter, 1982). In addition, RX 781094 has been used to characterize functional $\alpha_2$-adrenoceptors in the CNS (Dettmar, Lynn & Tulloch, 1981), including those mediating the mydriatic effect of $\alpha_2$-adrenoceptor agonists (Berridge, Gadie, Roach & Tulloch, 1983).

The availability of RX 781094 and other recently discovered selective $\alpha_2$-adrenoceptor antagonists such as RS 21361 (Michel & Whiting, 1981) and Wy 26703 (Lattimer, Rhodes, Ward, Waterfall & White, 1982) makes it possible to perform a detailed characterization of various $\alpha_2$-adrenoceptors. This approach, as an alternative to using $\alpha_2$-adrenoceptor agonists for the characterization of this receptor subtype, should have the advantage of obviating the interpretative problems caused by differences in intrinsic activity between agonists. The present study has compared the prejunctional $\alpha_2$-adrenoceptors in the rat vas deferens with central $\alpha_2$-adrenoceptors in the same species; namely (1) the functional receptors responsible for mediating the mydriatic effect of $\alpha_2$-adrenoceptor agonists, and (2) the receptor sites in cerebral cortex labelled by [$^3$H]-RX 781094. Receptor characterization was achieved by determining the relative activities of a range of selective $\alpha_2$-

adrenoceptor antagonists at these various receptor sites. A preliminary account of this work has been published (Doxey, Gadie, Lane & Tulloch, 1982).

## Methods

### $\alpha_2$-Adrenoceptor antagonist potency in the CNS

(a) *Antagonism of guanoxabenz-induced mydriasis* Male Sprague-Dawley rats, 250–350 g, were anaesthetized with sodium pentobarbitone (Sagatal; 60 mg $kg^{-1}$, i.p.) and subsequent drug experiments were all performed within the following 40 min period. Antagonist potency was determined as described by Berridge *et al.* (1983). Maximal pupil dilation (> 4 mm) was produced by injection of the selective $\alpha_2$-adrenoceptor agonist guanoxabenz (0.3 mg $kg^{-1}$, i.v.). Fifteen minutes later increasing doses (half-log increments) of an antagonist were injected i.v. at 5 min intervals; antagonist potency was assessed by determining the cumulative dose to produce a 50% reversal of the mydriatic response. These $AD_{50}$ values, expressed as $\mu$mol $kg^{-1}$, were determined graphically from the log dose-response curves for each antagonist.

(b) *Displacement of [$^3$H]-RX 781094 binding in rat cortex* The displacement of [$^3$H]-RX 781094 was determined in rat brain cerebral cortex preparations essentially as described by Howlett *et al.*, (1982). Male Sprague-Dawley rats, 250–300 g, were killed by decapitation, the brains quickly removed, and the cerebral cortex dissected free from striatal tissue but including the hippocampus. The tissue was homogenized in ice-cold buffered sucrose (20 ml $g^{-1}$ tissue, 300 mmol $l^{-1}$ sucrose in Tris/HCl buffer, 50 mmol $l^{-1}$, pH 7.4) using a Potter-type glass-teflon homogenizer. A crude synaptosomal $P_2$ fraction was prepared according to Whittaker (1969). The washed $P_2$-pellet was resuspended in a physiological salt solution to give a final protein concentration of about 1 mg $ml^{-1}$. The physiological salt solution had the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $KH_2PO_4$ 1.2, $MgSO_4$ 1.2, $NaHCO_2$ 25 and was equilibrated with 95% $0_2$; 5% $CO_2$ (Starke & Montel, 1973).

A typical incubation mixture (in triplicate) contained: membrane preparation (970 $\mu$l), saturating ligand or varying concentrations of an $\alpha_2$-adrenoceptor antagonist (10 $\mu$l), and [$^3$H]-RX 781094 (20 $\mu$l, to give a final concentration of 1nM). Non-saturable binding, representing about 30% of the total binding, was defined by the addition of an excess of phentolamine (1uM). Samples were incubated for 15 min at 25°C and then rapidly filtered under vacuum through Whatman GFB filters and washed with 2 × 4 ml of ice-cold Tris/HCl buffer (0.05 M, pH 7.4). Filters were transferred to scintillation vials and NE 260 micellar scintillation fluid (3.5 ml; Nuclear Enterprises) was added prior to scintillation counting. Competition binding curves using up to eight concentrations of test antagonist were constructed and $IC_{50}$ values, defined as the concentrations which displaced 50% of saturable [$^3$H]-RX 781094 binding, were determined. Hill plots were constructed for each antagonist. These were linear regressions of $\log (B - B_L)/B_L$ against log (antagonist concentration) where B was specific binding of [$^3$H]-RX 781094 in the absence of competing antagonist and $B_L$ was specific binding at a particular concentration of antagonist. Inhibitor constants ($K_i$ values) were derived from the $IC_{50}$ values according to the method of Cheng & Prusoff (1973). Saturation binding studies were also performed separately under these conditions using a range of [$^3$H]-RX 781094 concentrations (0.1–30 nM). Specific binding of [$^3$H]-RX 781094 was characterized using Scatchard analysis.

### *Rat vas deferens*

The detailed method has been described elsewhere (Doxey, Smith & Walker, 1977). In brief, the prostatic half of the vas deferens was suspended under an initial tension of 0.5 g in an organ bath of 8–10 ml capacity. Tissue was bathed in a Krebs solution (composition, mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $KH_2PO_4$ 1.2, $MgSO_4$ 0.6, $NaHCO_3$ 25 and glucose 11.1) containing corticosterone (40 $\mu$M), desmethylimipramine (10 nM) and propranolol (100 nM) and gassed with 95% $O_2$ and 5% $CO_2$. Bath fluid, at 30°C, was renewed continuously at a rate of 3–5 ml $min^{-1}$.

The intramural nerves of the vas deferens were stimulated by rectangular 40 V pulses of 3 ms duration at a frequency of 0.1 Hz. Clonidine inhibits this stimulation-evoked twitch response. Cumulative clonidine concentration-response curves were constructed in the absence and presence of a range of concentrations of each antagonist. Antagonists were allowed to equilibrate for 15 min before clonidine administration. Each antagonist (except RX 781094) was tested at three concentrations in the range indicated: RX 781094 (4.2 nM – 4.2 $\mu$M, four concentrations), Wy 2663 (27 nM – 2.7 $\mu$M), yohimbine (26 nM – 260 $\mu$M), rauwolscine (26 nM – 2.6 $\mu$M), piperoxan (4 nM – 400 nM), mianserin (33 nM – 3.3 $\mu$M), RS 21361 (360 nM – 3.6 $\mu$M).

Antagonist concentrations (*K*e values in nM) to produce a 2 fold parallel shift of the clonidine concentration-response curve were calculated according to the method of van Rossum (1963). Schild plots of log (clonidine concentration ratio – 1) against log. (molar concentration of antagonist) were

also constructed (Arunlakshana & Schild, 1959) and the slopes of the plots determined.

*Drugs*

The following drugs were used in the study: clonidine hydrochloride (Boehringer Ingelheim), corticosterone (Sigma), desmethylimipramine hydrochloride (Ciba Geigy), guanoxabenz hydrochloride (Roussel), mianserin hydrochloride (Organon), piperoxan hydrochloride (synthesized in the Medicinal Chemistry Department, Reckitt and Colman), (±)-propranolol hydrochloride (ICI), rauwolscine hydrochloride (Roth), RS 21361 hydrochloride [2-(1-ethyl-2-imidazolyl methyl)-1,4-benzodioxan] (Syntex), RX781094 [2-(2(1,4-benzodiozanyl)) Wy 26703 [N-methyl-N-(1,3,4,6,7,11ba-hexahydro-2H-benzo (α)-quinolizin-2β-yl) isobutanesulphonamide] 2-imidazoline hydrochloride] (Reckitt and Col-man), Wy 26703 [N-methyl-N-(1,3,4,6,7,11ba-hexahydro-2H-benzo (α)-quinolizin-2β-yl) isobutanesulphonamide] (Wyeth), yohimbine hydrochloride (Sigma). [$^3$H]-RX 781094 was prepared by catalytic bromine-tritium exchange (Radiochemical Centre, Amersham) and purified (> 99%) by preparative thin layer chromatography on silica gel 60 $F_{254}$ plates (Merck) using chloroform : methanol : 880 ammonia (91 : 8 : 1 by volume) as the solvent system. The specific activity was 30 Ci mmol$^{-1}$.

In the binding studies, drug solutions were made up in distilled water or dilute hydrochloric acid. Drugs applied to the vas deferens were dissolved in distilled water or 0.9% w/v sodium chloride solution (saline). For i.v. administration, drugs were dissolved in sterile saline or distilled water (dilutions in sterile saline) and given in a dose-volume of 1 ml kg$^{-1}$.

## Results

*[$^3$H]-RX 781094 binding in rat cerebral cortex*

The binding of [$^3$H]-RX 781094 to rat cerebral cortex membranes was a saturable process over the concentration range studied (0.1 to 30 nM) (Figure 1a). Non-saturable binding, defined by the addition of an excess of phentolamine (10 μM), represented about 30% of the total binding. Scatchard analysis of the data yielded linear plots consistent with binding to a single homogeneous population of binding sites with an equilibrium dissociation constant ($K_D$) of 4.4 ± 0.4 nM (mean ± s.e.mean of 5 determinations). The maximal number of binding sites was 144.4 ± 14 fmol mg$^{-1}$ protein. A typical Scatchard plot is shown in Figure 1 (b).

*Comparison between peripheral and central $\alpha_2$-adrenoceptor antagonist activity*

A structurally diverse group of $\alpha_2$-adrenoceptor antagonists covering a fairly wide potency range was assessed against clonidine-induced inhibition of the rat isolated vas deferens, [$^3$H]-RX 781094 cortical binding and guanoxabenz-induced mydriasis in the anaesthetized rat. The antagonists were: RX 781094, Wy 26703, yohimbine, rauwolscine, piperoxan, mianserin and RS 21361.

In the vas deferens, each antagonist produced a parallel concentration-dependent shift to the right of the clonidine concentration- response curve. Plots of log (clonidine concentration ratio − 1) against log (antagonist concentration) gave linear regressions with slopes close to unity (see Table 1), consistent with competitive antagonism. Schild plots for

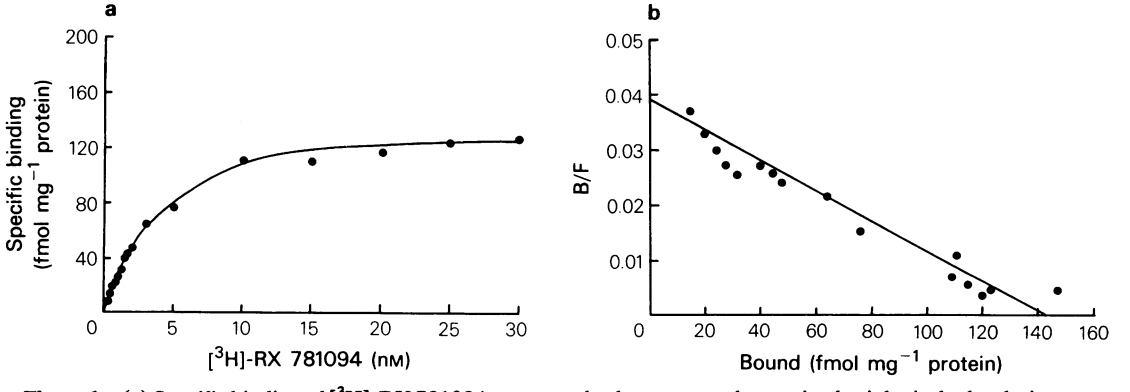


**Figure 1** (a) Specific binding of [$^3$H]-RX 781094 to rat cerebral cortex membranes in physiological salt solution at 25°C. Non-specific binding was determined with 10 μM phentolamine. Each point is the mean of three determinations. (b) Scatchard plot of the data shown in (a). Each point is the mean of three determinations and the line was fitted by linear regression analysis. The maximum number of binding sites ($B_{max}$) indicated by the intercept on the abscissa scale was 142.7 fmol mg$^{-1}$ protein and the dissociation constant (minus 1/slope) 3.7 nM. A Hill plot of these data has a slope of 0.94 indicating a lack of cooperativity in binding.

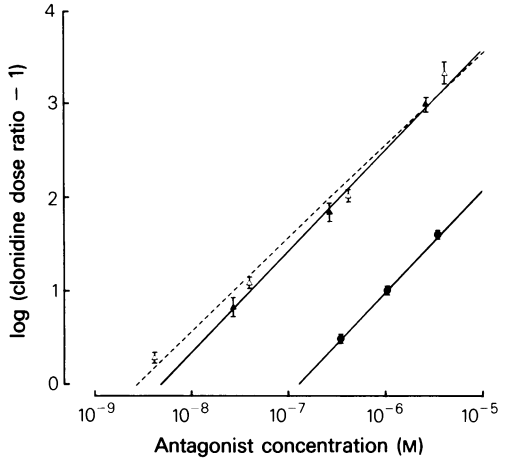


**Figure 2** Schild plots for the interaction between selective $\alpha_2$-adrenoceptor antagonists and clonidine on the electrically stimulated (0.1 Hz) rat isolated vas deferens. Antagonists: RX781094 (△, dashed line), Wy 26703 (▲) and RS 21361 (●). The lines were fitted by linear regression analysis of the data. Each point and vertical bar represents the mean and s.e.mean of a minimum of three determinations. The slope of these Schild plots are presented in Table 1.

RX 781094, Wy 26703 and RS 21361 are shown in Figure 2.

Each antagonist caused a concentration-dependent displacement of [$^3$H]-RX 781094 binding from cortical membranes. Competition binding curves (Hill plots) were linear with Hill coefficients

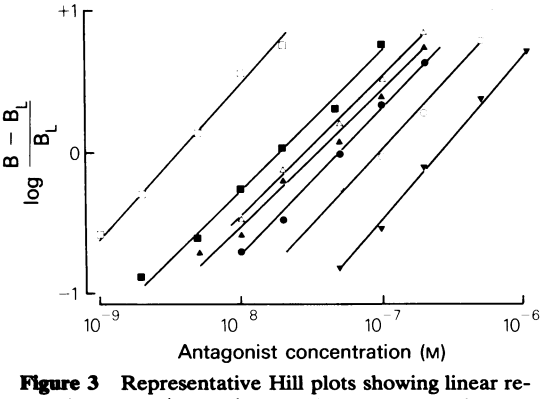


**Figure 3** Representative Hill plots showing linear regression of log $(B - B_L)/B_L$ against log antagonist concentration. B is specific [$^3$H]-RX 781094 binding in the absence of competing antagonists and $B_L$ specific binding at a particular concentration of antagonist. Antagonists: RX 781094 (□), Wy 26703 (■), piperoxan (△), yohimbine (▲), rauwolscine (●), mianserin (○), RS 21361 (▼). Each point is the mean of three determinations. The respective Hill coefficients of these plots are presented in Table 1.

($n_H$) close to 1 (Figure 3, Table 1), indicating that all the antagonists were acting in a competitive manner.

The $\alpha_2$-adrenoceptor antagonist potencies in the rat vas deferens and central nervous system (assessed against [$^3$H]-RX 781094 cortical binding and guanoxabenz-induced mydriasis) are presented in Table 1. The relative potencies of the compounds were similar in the three tests. Linear regression analysis of the data revealed that antagonist activity in the vas deferens was positively correlated with the ability of these agents to reverse guanoxabenz-induced mydriasis (Figure 4a) and to displace [$^3$H]-RX 781094 binding (Figure 4b). There was also a highly significant correlation between the antagonist effects in the two tests of central activity ($r = 0.97$; $P < 0.001$).

## Discussion

This study has compared the pharmacological activities of several structurally heterogeneous $\alpha_2$-adrenoceptor antagonists at presumed $\alpha_2$-adrenoceptors in the vas deferens and CNS of the rat. As far as is known from published data, all the agents used are thought to act as pure antagonists at $\alpha_2$-adrenoceptors, though it should be noted that not all of them are selective for this particular sub-type. RX 781094, RS21361 and Wy 26703 possessed the highest $\alpha_2$-adrenoceptor selectivities relative to their effects at $\alpha_1$-adrenoceptors (see Introduction for references).

The pharmacological activity of the antagonists in the vas deferens was highly correlated with both their ability to displace [$^3$H]-RX 781094 binding from cortical sites and their ability to reverse the centrally-mediated mydriasis induced by the selective $\alpha_2$-adrenoceptor agonist guanoxabenz (Berridge *et al.*, 1983). These results therefore provide convincing evidence that these peripheral and central $\alpha_2$-adrenoceptors are similar in terms of their pharmacological responsiveness. Furthermore the significant correlation between the antagonist activities in the two central tests indicates that [$^3$H]-RX 781094 binding sites are pharmacologically similar to the functional $\alpha_2$-adrenoceptors responsible for mediating mydriasis. Whether this close relationship holds for other functional $\alpha_2$-adrenoceptors in the CNS remains to be determined.

Previous studies, mainly employing $\alpha_2$-adrenoceptor agonists, have also demonstrated a pharmacological similarity between several identified central and peripheral $\alpha_2$-adrenoceptors. For instance, in a study with a series of three agonists, Hammer, Kobinger & Pichler (1980) found central $\alpha_2$-adrenoceptor sites labelled by [$^3$H]-clonidine to be similar to postsynaptic $\alpha_2$-adrenoceptors on vascu-

**Table 1** $\alpha_2$-Adrenoceptor antagonist activity assessed against clonidine-induced inhibition of the rat isolated vas deferens, [$^3$H]-RX 781094 cortical binding and guanoxabenz-induced mydriasis in the anaesthetized rat

| | *$\alpha_2$-Adrenoceptor antagonist potency* | | | | |
|---|---|---|---|---|---|
| Antagonist | *Vas deferens* $K_e$ (nM) | *Schild plot slope* | *[$^3$H]-RX 781094 binding* $K_i$ (nM) | *Hill coefficient* $n_H$ | *Guanoxabenz-induced mydriasis* $AD_{50}$ ($\mu$mol kg$^{-1}$, i.v.) |
| RX 781094 | 3.0± 0.2 (21) | 1.00±0.04 | 3.1± 0.4 (3) | 1.04±0.10 | 0.2±0.05 (5) |
| Wy 26703 | 4.1± 0.6 (10) | 1.07±0.08 | 17.6± 2.5 (3) | 0.87±0.07 | 0.2±0.01 (5) |
| Yohimbine | 8.0± 0.7 (8) | 0.92±0.10 | 40.0± 5.5 (3) | 0.86±0.07 | 2.2±0.04 (5) |
| Rauwolscine | 16.0± 3.0 (14) | 1.03±0.08 | 43.0± 8.2 (3) | 0.92±0.08 | 3.2±0.2 (5) |
| Piperoxan | 20.0± 1.6 (14) | 0.96±0.04 | 34.0± 6.5 (3) | 1.03±0.07 | 3.1±0.2 (5) |
| Mianserin | 53.0±12.0 (6) | 0.90±0.16 | 82.0± 2.4 (3) | 0.95±0.03 | 10.0±0.9 (5) |
| RS 21361 | 107.0± 5.0 (10) | 1.09±0.03 | 190.0±21.0 (3) | 1.02±0.03 | 16.2±2.5 (5) |

Values are mean ± s.e.mean of the number of experiments indicated in parentheses.
$K_e$ values for yohimbine, rauwolscine, piperoxan and RS 21361 were calculated from $pA_2$ values from Doxey *et al.* (1983).
$AD_{50}$ values against guanoxabenz-induced mydriasis are from Berridge *et al.* (1983), except Wy 26703.

lar tissue. Similarly, Kobinger & Pichler (1980) concluded on the basis of the rank order of potency of eight clonidine analogues, that central $\alpha_2$-adrenoceptors mediating sympatho-inhibition resemble those on vascular tissue.

Attempts to correlate pharmacological activity at central and peripheral $\alpha_2$-adrenoceptors may be influenced by factors other than differences in the drug responsiveness of the particular receptors under study. For example one of the major determinants of a drug's potency in the CNS is its ability to penetrate the blood brain barrier. All the antagonists used in this study seemed to have ready access to the CNS as shown by the excellent correlation between central $\alpha_2$-adrenoceptor affinity *in vitro* and central antagonist activity *in vivo*. The ability of these drugs to penetrate into the CNS should allow other putative central $\alpha_2$-adrenoceptors to be characterized by use of this pharmacological approach.

There is still considerable uncertainty as to whether central $\alpha_2$-adrenoceptors are located predominantly on noradrenergic terminals and cell bodies or on postsynaptic neuronal structures. It has been proposed that activation of presynaptic $\alpha_2$-adrenoceptors inhibits $K^+$- and electrically-induced [$^3$H]-noradrenaline release from brain slices (Dis-

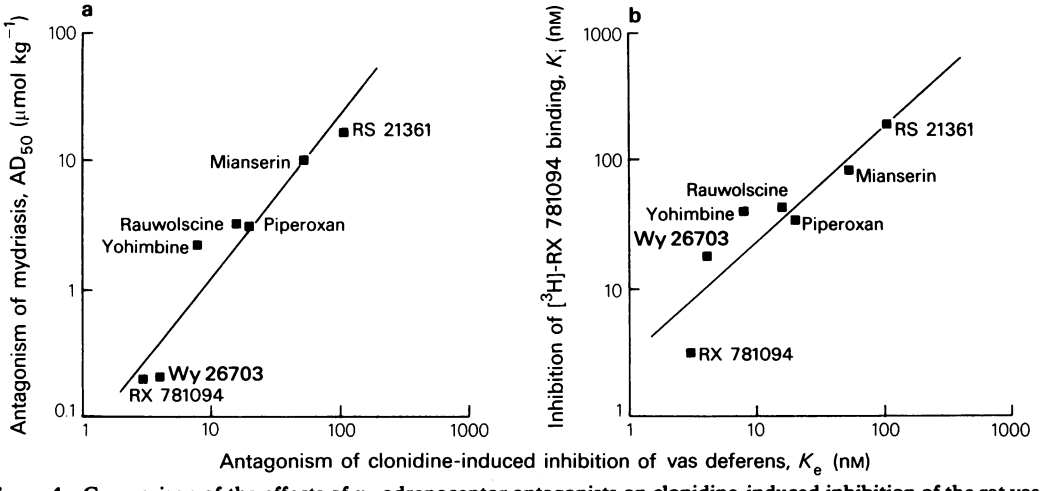


**Figure 4** Comparison of the effects of $\alpha_2$-adrenoceptor antagonists on clonidine-induced inhibition of the rat vas deferens ($K_e$ values) and either (a) guanoxabenz-induced mydriasis in the pentobarbitone-anaesthesized rat ($AD_{50}$ values) or (b) [$^3$H]-RX 781094 binding in rat cortical membranes ($K_i$ values). Linear regression analysis of the untransformed data (shown in Table 1) gives positive correlation coefficients of $r = 0.98$ ($K_e$ versus $K_i$ values; $P < 0.001$) and $r = 0.99$ ($K_e$ versus $AD_{50}$ values: $P < 0.001$).

mukes, De Boer & Mulder 1977; Taube, Starke & Borowski, 1977; Wemer, Frankhuyzen & Mulder 1982). The existence of functional presynaptic $\alpha_2$-adrenoceptors could also account for the increased turnover of cortical noradrenaline seen after RX781094 administration (Flockhart, Haynes & Walter, 1982; Scatton, Dedek & Zivkovic, 1983). This would be analogous to the presynaptic, negative feedback mechanism that is thought to modulate noradrenaline release from sympathetic nerve endings in some peripheral tissues (Langer, Adler-Graschinsky & Giorgi 1977; Story, McCulloch, Rand & Standford-Starr, 1981).

It appears unlikely, however, that [$^3$H]-RX781094 labels mainly presynaptic $\alpha_2$-adrenoceptor sites in the cerebral cortex. If a proportion of $\alpha_2$-adrenoceptors were located presynaptically then removal of cortical noradrenergic terminals would be expected to reduce [$^3$H]-RX781094 binding. However, extensive destruction of the noradrenergic innervation to the rat cerebral cortex has no effect on either the number ($B_{max}$) or affinity ($K_D$) of [$^3$H]-RX781094 binding sites in this brain region (Langer, Pimoule & Scatton, 1983; present authors, unpublished observations). This result is partly consistent with the finding that [$^3$H]-clonidine binding in cerebral cortex is not reduced, but increased, after lesioning the dorsal noradrenergic bundle (U'Prichard, Reisine, Mason, Fibiger & Yamamura 1980). There is thus no evidence to date from radioligand binding studies that $\alpha_2$-adrenoceptors are located on noradrenergic terminals in the cerebral cortex. Further studies are clearly necessary in order to determine the anatomical location of these receptor sites and to investigate the mechanism by which they regulate central noradrenergic activity under normal physiological conditions.

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