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α_2-Adrenoceptor agonists induce mydriasis in the rat by an action within the central nervous system

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1 The effects of intravenous administration of the selective α_2-adrenoceptor agonists clonidine, UK 14,304 and guanoxabenz on rat pupil diameter were investigated.

2 In rats anaesthetized with pentobarbitone, each agonist produced a marked dose-related increase in pupil diameter; the rank order of potency was: clonidine > UK 14,304 > guanoxabenz.

3 Pretreatment with the selective α_2-adrenoceptor antagonist, RX 781094 (0.5 mg/kg, i.v.), produced a parallel 30–40 fold shift to the right of the dose-pupil dilator response curves for the three agonists. Yohimbine (1.5 mg/kg, i.v.) produced about a 10 fold rightward shift of the dose-response curve for guanoxabenz. In contrast, the α_1-selective antagonist, prazosin (0.5 mg/kg, i.v.), failed to affect the dose-response relation for guanoxabenz.

4 Several antagonists of varying selectivities towards α_1- and α_2-adrenoceptors were tested for their ability to reverse the maximal mydriasis induced by guanoxabenz (0.3 mg/kg, i.v.). The rank order of potency of the antagonists producing a 50% reversal of this effect was: RX 781094 > yohimbine > piperoxan = rauwolscine > mianserin > RS 21361. Neither corynanthine nor prazosin reversed the guanoxabenz-induced mydriasis.

5 Topical application of RX 781094 (0.1 to 3% w/v solutions) onto one eye produced a slow reversal of guanoxabenz-induced mydriasis; the time course and degree of reversal were virtually the same in both eyes.

6 Intracerebroventricular administration of RX 781094 (1.25–15 μg total dose) caused a rapid dose-related reversal of the maximal mydriasis induced by guanoxabenz (0.3 mg/kg, i.v.).

7 Guanoxabenz (0.3 and 1.0 mg/kg, i.v.) did not produce any dilation of the physostigmine-constricted undamaged pupil of the pithed rat. Intravenous adrenaline was found to produce a small mydriatic effect, while atropine completely antagonized the effects of physostigmine in this preparation.

8 These results indicate that α_2-adrenoceptor agonists induce mydriasis in the rat through a central α_2-adrenoceptor mechanism. However, the site of action within the central nervous system remains to be determined.

Introduction

Clonidine is known to produce mydriasis in several animal species, including the cat and rat. Initial studies indicated that this effect was due to a direct action of clonidine on the iris musculature (Walland & Kobinger, 1971; Kobinger, 1973). However, more recent studies in the anaesthetized cat have provided evidence that a central site or action for clonidine is more likely. In particular, clonidine has been found to produce a marked dose-related decrease of the efferent parasympathetic constrictor tone to the iris which is highly correlated with an increase in pupil diameter (Koss & San, 1976; Koss, 1979a,b).

The mydriatic effect of clonidine in the cat has been shown to be competitively antagonized by yohimbine (Koss, 1979a); this, in the light of the above results, would indicate that the mydriasis is mediated by a central α_2-adrenoceptor mechanism. Clonidine also produces a similar yohimbine-sensitive mydriasis in the rat (Gherezghiher & Koss, 1979), but as yet there is no direct evidence that this is a centrally-mediated effect. Consequently, the present study set out to investigate the mydriatic action of selective α_2-adrenoceptor agonists in the rat, and to examine the sensitivity of this effect to selective α-adrenoceptor antagonists. In most experiments RX 781094 [2-(2-(1,4-benzodioxanyl))-2-

imidazoline HCl], a new, potent, selective α_2-adrenoceptor antagonist (Chapleo, Doxey, Myers & Roach, 1981; Dettmar, Lynn & Tulloch, 1981) was used. RX 781094 has a high specificity for α_2-adrenoceptors and a 7-times greater $\alpha_2:\alpha_1$ selectivity than yohimbine in peripheral tissues (Chapleo *et al.*, 1981), making it a powerful pharmacological agent with which to investigate α_2-adrenoceptor mechanisms. This antagonist has been administered by different routes in an attempt to localize the mydriatic action of α_2-adrenoceptor agonists to a peripheral or central site, and to investigate whether an α_2-adrenoceptor mechanism is involved. Part of this work has been presented to the British Pharmacological Society (Berridge, Gadie, Roach & Tulloch, 1982).

Methods

Male Sprague-Dawley rats weighing 250–350 g were used in these experiments.

Anaesthetized rats

(a) *Intravenous administration of α-adrenoceptor agonists and antagonists* Rats were anaesthetized with sodium pentobarbitone (Sagatal; 60 mg/kg, i.p.) and a polyethylene catheter was inserted into the femoral vein for drug administration. Subsequent drug experiments were all performed within an 80 min period after induction of anaesthesia.

Pupil diameter was measured by means of a Beck Luminex magnifier, with a 10 × magnification, held close to but not touching the corneal surface. Drug-induced changes in pupil diameter were assessed by measurement against a 10 mm graduated line (0.1 mm divisions) on the magnifier lens. The magnifier had an internal light source which was maintained at a steady intensity throughout the experiments. During the time period employed in these anaesthetized rat experiments, pupil diameter was always small, normally less than 0.5 mm, and independent of changes in background illumination. All the experiments were performed in the same well-illuminated laboratory.

Pupillary responses to the α_2-adrenoceptor agonists clonidine, UK 14,304 and guanoxabenz were measured after intravenous administration of increasing doses (half-log scale increments) at 5 min intervals. UK 14,304 and guanoxabenz were chosen for this study because of their high degree of selectivity for the α_2-adrenoceptor (Cambridge, 1981; Doxey, Frank & Hersom, 1981). Agonist dose-response curves were constructed after intravenous pretreatment (10 min) with either drug vehicle (sterile 0.9% w/v NaCl solution) or one of the following α-adrenoceptor antagonists, RX 781094 (0.5 mg/kg), yohimbine (1.5 mg/kg), or prazosin (0.5 mg/kg). The results are plotted as cumulative log dose-pupillary response curves.

A further series of interaction studies was performed with the α_2-adrenoceptor agonist, guanoxabenz and a number of α-adrenoceptor antagonists (the α_2-selective antagonists RX 781094, yohimbine, RS 21361, piperoxan, rauwolscine; the non-selective α_1/α_2-antagonist mianserin; and the α_1-selective antagonists corynanthine and prazosin). Maximal pupil dilation was first produced by a single injection of guanoxabenz (0.3 mg/kg, i.v.). Fifteen minutes later logarithmically increasing doses of α-adrenoceptor antagonists were injected at 5 min intervals; antagonist potency was assessed by determining the cumulative dose which produced a 50% reversal of the guanoxabenz-induced mydriasis. These AD_{50} values were determined graphically from the log dose-response curves for each antagonist.

Experiments were also performed to examine the effect of RX 781094 on the mydriatic response induced by atropine. Atropine (0.1 mg/kg, i.v.) was administered 15 min before RX 781094 (0.03–3.0 mg/kg, i.v.) which was injected in logarithmically increasing doses at 5 min intervals.

(b) *Topical application to the eye* Experiments in anaesthetized rats were performed in which RX 781094 was applied topically to the eye after maximal pupil dilation had first been produced by an intravenous injection of guanoxabenz (0.3 mg/kg, i.v.). RX 781094 was applied in solutions of increasing strength (0.1–3% w/v) onto the corneal surface of the right eye; the dose volume added was 25 μl, measured by a calibrated dropper. Between changes of RX 781094 solutions the cornea was gently rinsed with a few drops of sterile isotonic saline. Pupil diameter was measured periodically in both eyes. Control experiments were performed in which sterile saline was applied instead of RX 781094.

(c) *Intracerebroventricular (i.c.v.) administration* Rats were anaesthetized with sodium pentobarbitone, (60 mg/kg, i.p.), and secured in a David Kopf stereotaxic frame. A stainless-steel guide cannulae (20 gauge) was implanted into the left cerebral ventricle; the coordinates for implantation (De Groot, 1959) were: P, −0.9 mm; L, 2.5 mm; and V (from dura surface), 3 to 3.5 mm. Correct placement of the cannula was ensured by connecting it to a saline-filled polyethylene tube, which served as a manometer, and adjusting the vertical position of the cannula until saline flowed freely into the ventricle. The cannula was then secured to the skull with dental acrylic cement. The cannulae were kept patent with stainless-steel wire stylets (25-gauge). Rats were allowed a 7-day recovery period.

After recovery, rats were anaesthetized with sodium pentobarbitone (60 mg/kg, i.p.), and guanoxabenz (0.3 mg/kg) was injected via a tail vein to produce maximal pupil dilation. Fifteen minutes later RX 781094 (1.25, 5 or 15 μg in 10 μl sterile saline) or drug vehicle was administered i.c.v. using a 25-gauge needle, over a period of 1 min; groups of 5–6 rats were used for each dose. Pupil diameter was recorded in both eyes at 1 min intervals after antagonist injection. The rats were used repeatedly with at least a 7-day interval between experiments.

Pithed rats

Rats were anaesthetized with halothane (4% v/v in room air) and pithed according to the method of Shipley & Tilden (1947). The brain and spinal cord were destroyed by passing a metal rod through the orbit of one eye into the brain and then advancing the rod down the spinal cord. The animals were immediately respired with room air (1 ml/100g body weight; 100 strokes/min; Palmer Small Animal Respirator) and body temperature maintained at 37°C with a thermostatically controlled heating table. A femoral vein was cannulated to enable drug injections.

The rats were allowed a stabilization period of 45–60 min before starting the experiments. At this time the undamaged eye was fully dilated. Pupillary constriction was produced by topical administration of physostigmine (1% w/v, 2 drops). The effect of guanoxabenz (0.3 and 1.0 mg/kg, i.v.) was studied. The pupil dilator responses to adrenaline (30 μg/kg, i.v.) and atropine (0.3 mg/kg, i.v.) were also assessed.

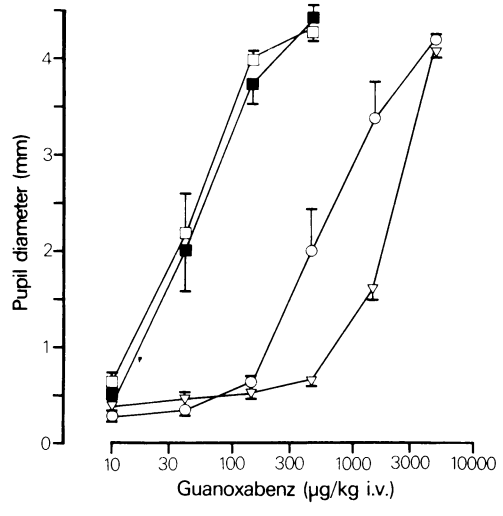

Figure 1 Effects of RX 781094, yohimbine and prazosin on the cumulative dose-pupillary response curves for guanoxabenz in the anaesthetized rat. RX 781094 (0.5 mg/kg; ▽), yohimbine (1.5 mg/kg; ○), prazosin (0.5 mg/kg; □) or saline (1 ml/kg; ■) was injected i.v. 10 min before constructing the dose-response curves for guanoxabenz. Each point and vertical bar represents the mean and s.e. mean obtained from 4–6 rats.

Drugs

The following drugs were used in the study: adrenaline hydrogen tartrate (BDH), atropine sulphate (Burroughs Wellcome), clonidine hydrochloride (Boehringer Ingelheim) corynanthine (Roth), guanoxabenz hydrochloride (Roussel), mianserin hydrochloride (Organon), physostigmine sulphate (BDH), piperoxan hydrochloride (synthesized in the Medicinal Chemistry Department, Reckitt and Colman), prazosin hydrochloride (Pfizer), rauwolscine hydrochloride (Roth), RS21361 [2-(1-ethyl-2-imidazolyl methyl)-1,4-benzodioxan] (Syntex), RX 781094 [2-(2-(1,4-benzodioxanyl))-2-imidazoline hydrochloride](synthesized in the Medicinal Chemistry Department, Reckitt and Colman), UK 14,304 tartrate [5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline], Pfizer). All drugs were dissolved in sterile saline (0.9% w/v NaCl solution) or distilled water immediately before use; all dilutions were made in sterile saline. Drugs were administered i.v. in a dose-volume of 1 ml/kg. Intracerebroventricular injections were given in a dose-volume of 10 μl. With the exception of corynanthine and unless otherwise stated, all drug doses refer to the respective salts.

For comparison of potencies of agonists and antagonists, ED_{50} and AD_{50} doses were expressed in molar terms (nmol/kg and μmol/kg, respectively).

Results

Effect of intravenous administration of α_2-adrenoceptor agonists on pupil diameter

Intravenous administration of the α_2-adrenoceptor agonists clonidine, UK 14,304 and guanoxabenz produced a marked dose-dependent pupillary dilation in the pentobarbitone-anaesthetized rat (Figures 1 and 2). With each of these agonists the mydriatic effect was rapid in onset, becoming apparent within 1 min after injection, and of long duration (> 60 min). Initial mean pupil diameter was usually less than 0.5 mm before agonist administration; each of the agonists produced a maximum dilation of the pupil. The rank order of potency for these agonists was: clonidine > UK 14,304 > guanoxabenz. The cumulative doses to produce a pupil dilation of 2 mm (ED_{50}; approximately half maximum effect) were: 28.7, 57.5 and 139.7 nmol/kg.

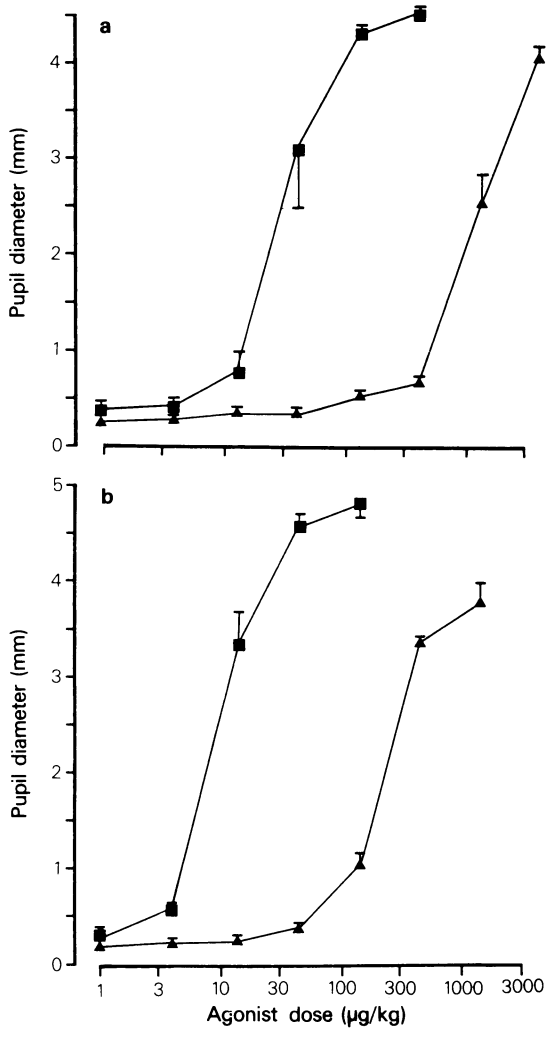

Figure 2 Effects of RX 781094 on the cumulative dose-pupillary response curves for UK 14,304 (a) and clonidine (b) in the anaesthetized rat. RX 781094 (0.5 mg/kg; ▲) or saline (ml/kg; ■) was injected i.v. 10 min before constructing the agonist dose-response curves. Each point and vertical bar represents the mean and s.e. mean obtained from 4–6 rats.

Effects of α-adrenoceptor antagonists on the mydriatic response to α_2-adrenoceptor agonists

(a) *Pretreatment with α-adrenoceptor antagonists* Pretreatment with the highly selective α_2-adrenoceptor antagonist RX 781094 (0.5 mg/kg, i.v.) produced a 30 to 40 fold parallel shift to the right of the log dose-pupillary response curves for the agonists guanoxabenz, UK 14,304 and clonidine (Figures 1 and 2). Yohimbine was found to be a less effective antagonist; 1.5 mg/kg pretreatment producing about a 10 fold rightward shift of the guanoxabenz dose-response curve (Figure 1). Neither of these antagonists given alone had any measurable effect on pupil diameter in the anaesthetized rat.

A further series of experiments was performed in which rats were pretreated with prazosin. This antagonist, at 0.5 mg/kg, i.v. failed to produce any significant displacement of the guanoxabenz dose-response curve (Figure 1).

(b) *Reversal of guanoxabenz-induced mydriasis* In these experiments, maximal mydriasis was first produced by administration of a single dose of guanoxabenz (0.3 mg/kg, i.v.; see Figure 1). Several i.v. injections of saline had no effect on the mydriasis produced by guanoxabenz. Increasing doses of various α-adrenoceptor antagonists were then administered in an attempt to reverse this mydriatic effect. The α_2-adrenoceptor antagonists RX 781094, yohimbine and RS 21361 each produced a dose-related reversal of guanoxabenz-induced pupil dilation (Figure 3); the slopes of the dose-response curves were essentially parallel. The ability of other α-adrenoceptor antagonists to reverse guanoxabenz-induced mydriasis was also examined. The AD_{50} values for all the antagonists tested are shown in Table 1. The rank order of potency of antagonists producing full reversal of guanoxabenz was: RX781094 > yohimbine > piperoxan = rauwolscine > mianserin > RS 21361. In this test situation, corynanthine (0.03–10 mg/kg, i.v.) and prazosin (0.03–5 mg/kg, i.v.) failed to produce any reversal of the mydriasis. Owing to solubili-

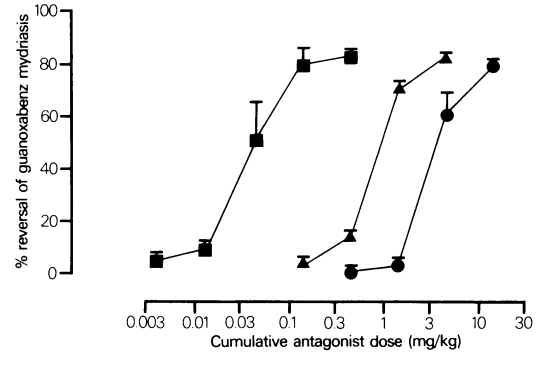

Figure 3 The effects of selective α_2-adrenoceptor antagonists on the mydriatic effect produced by guanoxabenz in anaesthetized rats: (■) RX 781094; (▲) yohimbine; (●) RS 21361. Antagonist doses were injected i.v. at 5 min intervals starting 15 min after injection of guanoxabenz (0.3 mg/kg, i.v.). This agonist dose produced a maximum mydriasis and the results are plotted as percentage reversal of this effect versus cumulative antagonist dosage. The points plotted are means obtained from 4–6 rats. Vertical bars indicate s.e. mean.

Table 1 AD_{50} values for α-adrenoceptor antagonists against guanoxabenz-induced mydriasis in the rat

α-Adrenoceptor antagonist	*AD_{50} (μmol/kg)*
RX 781094	0.2 ± 0.05
Yohimbine	2.2 ± 0.04
Piperoxan	3.1 ± 0.2
Rauwolscine	3.2 ± 0.2
Mianserin	10.0 ± 0.9
RS 21361	16.2 ± 2.5
Corynanthine	No reversal
Prazosin	No reversal

Antagonist potency (AD_{50}) was assessed by determining graphically the cumulative i.v. dose necessary to cause a 50% reversal of the maximal mydriatic effect produced by guanoxabenz (0.3 mg/kg, i.v.)

Corynanthine (0.03–10 mg/kg) and prazosin (0.03–5 mg/kg) were ineffective in this test. Values are means ± s.e. mean obtained from 4–6 experiments.

ty problems, higher doses of these antagonists were not tested.

Effects of intravenous guanoxabenz, adrenaline and atropine on the physostigmine-constricted pupil in pithed rats

Over the 30 to 40 min period after the pithing procedure, the pupil diameter of the undamaged eye gradually increased to its maximum extent, indicating an effective destruction of the parasympathetic innervation to the iris. Application of physostigmine (1% w/v) to the eye produced a slow (over 10 min) pupil constriction (Figure 4). Administration of maximally-effective mydriatic doses of guanoxabenz (0.3 and 1.0 mg/kg, i.v.) (see Figure 1) failed to

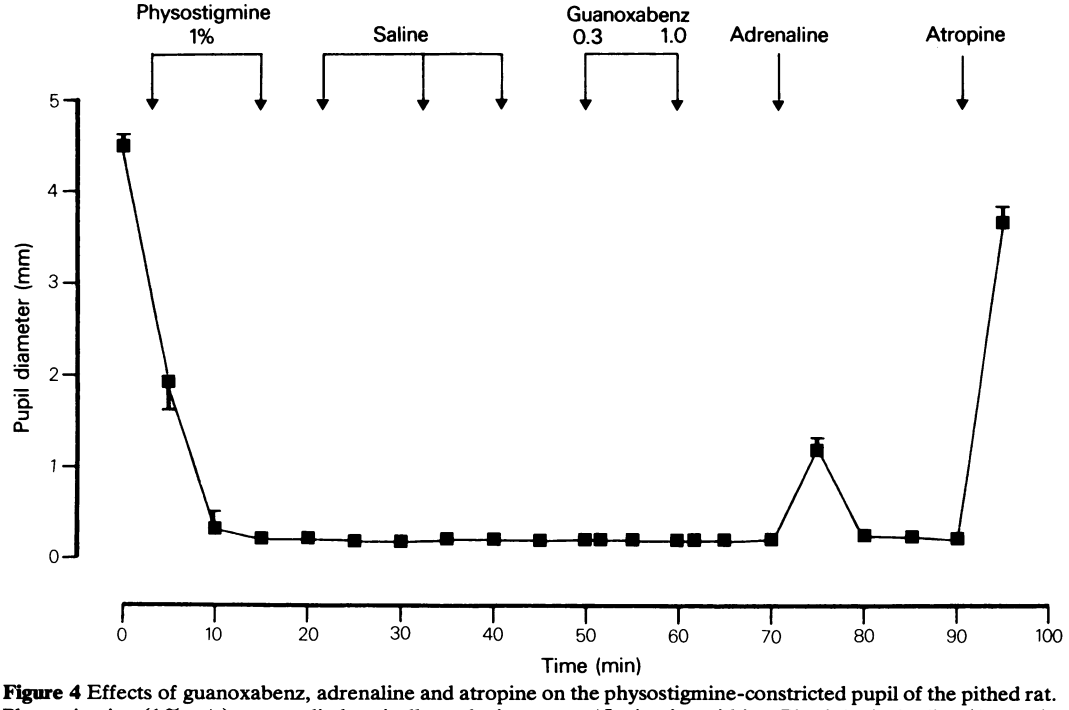

Figure 4 Effects of guanoxabenz, adrenaline and atropine on the physostigmine-constricted pupil of the pithed rat. Physostigmine (1% w/v) was applied topically to the intact eye 45 min after pithing. Physiological saline (1 ml/kg), guanoxabenz (0.3 and 1.0 mg/kg), adrenaline (30 μg/kg) and atropine (0.3 mg/kg) were administered i.v. at the times indicated by the arrows. Each point and vertical bar represents the mean and s.e. mean obtained from a group of 4 rats.

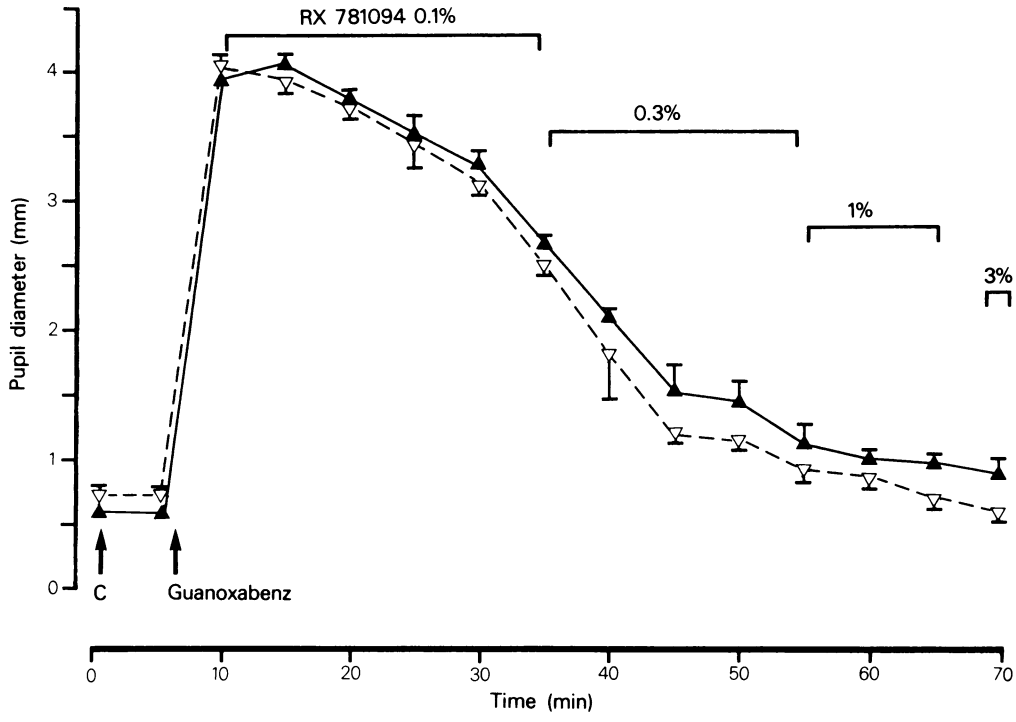

Figure 5 Effect of topical application of RX 781094 on to the eye on maximal mydriasis injuced by guanoxabenz (0.3 mg/kg, i.v.) in anaesthetized rats. Increasing concentrations (0.1 to 3% w/v) of RX 781094 (25 μl dose volume) were applied on to the cornea of the right eye for the periods indicated: (▲——▲) ipsilateral pupil; (▽- - -▽) contralateral pupil. (C) indicates i.v. injection of saline. Six rats were used in each group; vertical bars indicate s.e. mean.

produce any detectable dilation of the constricted pupil (Figure 4). In contrast, adrenaline (30 μg/kg, i.v.) produced a small transient mydriatic effect (Figure 4). Injection of atropine (0.3 mg/kg, i.v.) caused a total reversal of the miotic effect of physostigmine (Figure 4).

The effect of RX 781094 on the pupil dilation produced by the pithing procedure was also examined. In 3 preparations, pithing dilated the pupil to a maximum mean value of 4.4 ± 0.03 mm; this value was unchanged after injection of RX 781094 (1.0 mg/kg, i.v.).

Effect of topical application of RX 781094 to the eye on guanoxabenz-induced mydriasis

Maximal pupil dilation was induced in both eyes of anaesthetized rats by a single dose of guanoxabenz (0.3 mg/kg, i.v.). RX 781094 solutions of increasing concentration were then applied topically onto the cornea of the right eye. As shown in Figure 5, RX 781094 produced a gradual reversal of guanoxabenz-induced mydriasis, with both pupils responding with the same time course and magnitude of effect. Relatively high concentrations (1–3% w/v) of the antagonist were necessary to effect a full reversal of the agonist-induced dilation. In control experiments ($n = 4$), repeated application of saline instead of RX 781094 to one eye failed to produce any reversal of the maximal mydriatic effect in either eye over the 70-min period studied.

Effect of i.c.v. administration of RX 781094 on guanoxabenz-induced mydriasis

These experiments were performed in separate groups of 6 rats. As before, guanoxabenz (0.3 mg/kg, i.v.) was administered initially to cause a maximal pupil dilation (> 4 mm). Intracerebroventricular injection of RX 781094 (1.25, 5 and 15 μg/rat) 15 min later produced a clear dose-related reversal of the mydriasis (Figure 6). This antagonism was fairly rapid in onset, becoming manifest in less than 1 min after injection and observable in both eyes. Injection of 10 μl saline i.c.v. did not produce any reversal of the mydriatic effect.

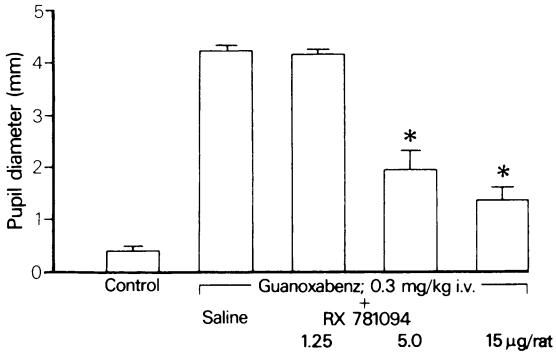

Figure 6 Effect of i.c.v. administration of RX 781094 on the maximum mydriasis induced by guanoxabenz in anaesthetized rats. RX 781094 or saline (10 μl dose volume) was administered 15 min after guanoxabenz (0.3 mg/kg, i.v.). Control rats received i.v. saline instead of guanoxabenz. The values shown were measured 5 min after antagonist injection. Each value is the mean of 5–6 rats; vertical bars indicate s.e. mean. Significant differences from rats injected with guanoxabenz plus i.c.v. saline were evaluated by Student's *t* test for unpaired data: $^*P < 0.01$.

Effect of RX 781094 on atropine-induced mydriasis

A sustained maximal pupil dilation (4.2 ± 0.05 mm; $n = 4$ rats) was induced by injection of atropine (0.1 mg/kg, i.v.). Administration of RX 781094 (0.03–3 mg/kg, i.v.) failed to produce any detectable antagonism of this mydriatic effect.

Discussion

The present study demonstrates that intravenous administration of clonidine as well as UK 14304 and guanoxabenz, two highly selective α_2-adrenoceptor agonists (Cambridge, 1981; Doxey *et al.*, 1981) causes a marked dose-related mydriasis in the pentobarbitone anaesthetized rat. The finding that the selective α_2-adrenoceptor antagonists RX 781094 (Chapleo *et al.*, 1981), RS 21361 (Michel & Whiting, 1981) and yohimbine competitively antagonized this mydriatic effect strongly suggests that it is mediated through an α_2-adrenoceptor mechanism. This is further supported by the fact that the selective α_1-adrenoceptor antagonists prazosin and corynanthine were ineffective against this mydriatic response. These results confirm and extend the findings of Gherezghiher & Koss (1979) who showed that yohimbine, but not phenoxybenzamine (a selective α_1-adrenoceptor antagonist), inhibited clonidine-induced mydriasis in the rat.

Koss & San (1976) proposed that clonidine-induced mydriasis in the cat was produced passively following a central inhibition of the parasympathetic innervation to the constrictor pupillae of the iris sphincter. However, in contrast to this well-documented effect of clonidine in the cat there is only limited information in the rat concerning the site of mydriatic action of this agent or other more selective α_2-adrenoceptor agonists. Consequently, the present studies of the interaction between guanoxabenz and RX 781094 were designed mainly to try and localise more specifically the site of the α_2-adrenoceptor mechanism underlying this mydriatic effect.

Direct application of RX 781094 on to the cornea of one eye caused a slow and similar reversal of guanoxabenz-induced mydriasis in both eyes. This result is consistent with a central site of action for RX 781094 following slow absorption of the antagonist into the systemic circulation, rather than a local effect of the drug in the eye. If RX 781094 acted by displacing guanoxabenz from α_2-adrenoceptors located in the iris sphincter or on the cholinergic terminals of the ciliary nerve, it is reasonable to predict that the antagonism should have been quicker in onset and more potent, and that the ipsilateral pupil should have been preferentially constricted. The fact that none of these predicted effects was observed indicates that RX 781094 did not exert a local effect after topical application on to the cornea. These findings are consistent with experimental data from the cat showing that application of clonidine to one eye produces a slow onset, bilateral inhibition of ciliary nerve activity and concomitant increase in pupil diameter, an effect ascribed to a central site of action following drug absorption from the eye (Koss, 1979b). This finding and our results conflict to some extent with Hsu, Lee & Betts (1981) who reported that the selective α_2-adrenoceptor agonist, xylazine, produced its mydriatic effect in rats by stimulation of α_2-adrenoceptors located both in the CNS and in the iris. They reported in their discussion that topical administration of the agonist to one eye induced a mydriasis in both eyes; however, the ipsilateral eye showed a more pronounced mydriasis than did the opposite eye. Intravenous yohimbine reduced, but did not abolish, the mydriasis in the ipsilateral eye although it fully reversed the effect in the opposite eye. This would suggest that xylazine produces part of its mydriatic effect by a direct action in the eye which does not involve α_2-adrenoceptors.

Experiments in the pithed rat preparation provided additional evidence against the possibility that α_2-adrenoceptor agonists produce mydriasis by a local sympathomimetic action on the iris, a mechanism originally proposed for clonidine by Walland & Kobinger (1971). It was presumed that the maximal pupil dilation observed in the pithed rat was a consequence of the destruction of the parasympathetic

innervation to the iris, as this effect could be readily overcome by topical application of physostigmine. Guanoxabenz failed to produce any pupil dilation following constriction by physostigmine. This is in keeping with clonidine's reported lack of effect on the parasympathectomized, physostigmine-constricted cat iris (Koss & San, 1976; Koss, 1979a).

It should be emphasised that RX 781094 does not reverse guanoxabenz-induced mydriasis by a direct pupillo-constrictor effect: maximal pupil dilation produced by pithing (effective parasympathetic denervation) or atropine was completely unaffected by RX 781094.

Administration of small amounts of RX 781094 into the lateral cerebral ventricle caused a rapid dose-related antagonism of the mydriasis induced by guanoxabenz. This is direct evidence that the mydriatic effect is mediated via a central α_2-adrenoceptor mechanism. It is unlikely that leakage of RX 781094 from the central nervous system into the periphery could have accounted for this result: 5 μg of RX 781094 i.c.v. (equivalent to 0.058 to 0.066 μmol/kg depending on body weight) caused a 54% reversal of the mydriasis, whereas on almost 3 fold greater intravenous dose (0.2 μmol/kg; Table 1) was necessary to produce a 50% reversal against the same agonist dose.

The location of the central α_2-adrenoceptor mechanism mediating this pronounced mydriasis remains obscure. It has been found previously in the cat that the mydriatic effect of clonidine is unaltered by almost complete depletion of central monoamine stores (Koss & Christensen, 1979), indicating that the agonist effect is mediated via post-synaptically located receptors. The observed results could be explained simply by postulating the existence of α_2-adrenoceptors on neurones in the Edinger-Westphal nucleus, the purported source of the preganglionic fibres of the parasympathetic innervation to the iris (Warwick, 1954). An agonist effect at these receptors would be predicted to reduce the firing rate of the parasympathetic neurones which in turn would decrease pupillo-constrictor tone and lead to pupil dilation.

The rank order of potency of the α_2-adrenoceptor antagonists, as assessed against guanoxabenz-induced mydriasis, is consistent with the relative antagonist potencies of these agents reported by others (Brown, Doxey & Handley, 1980; Chapleo *et al.*, 1981; Drew, 1981; Hedler, Stamm, Weitzell & Starke, 1981; for review, see Starke, 1981). This supports the view that the central receptors involved in the mydriatic action of α_2-adrenoceptor agonists closely resemble α_2-adrenoceptors present in a variety of other tissues. On the basis of these results, therefore, it is apparent that the central activity of α_2-adrenoceptor agonists and antagonists may be quantitatively assessed by determining their effects on pupil diameter in the rat, using the approach outlined in this study.

In summary, we have observed that selective α_2-adrenoceptor agonists produce a marked increase in pupil diameter in the rat. In the light of the results of the interaction studies with highly selective α_2-adrenoceptor antagonists, we suggest that this mydriatic effect is mediated by a central α_2-adrenoceptor mechanism.

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