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Rilmenidine produces mydriasis in cats by stimulation of CNS α_2-adrenoceptors

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Summary

1 Experiments were undertaken to determine if the imidazoline/α_2-adrenoceptor agonist, rilmenidine, would produce mydriasis in cats and, if so, to delineate its site of action and determine if this effect is mediated by imidazoline receptors or α_2-adrenoceptors.

2 Rilmenidine produced dose-related pupillary dilator responses in pentobarbital anaesthetized cats that were independent of sympathetic innervation to the iris but were dependent upon intact parasympathetic neuronal tone. The ED_{50} for rilmenidine-induced pupillary dilation was approximately 200 μg kg^{-1}, i.v., and was sustained for at least 1 h.

3 The highly selective α_2-adrenoceptor antagonist, RS-79948, administered either before or after rilmenidine, antagonized rilmenidine-induced mydriasis. Neuronally induced reflex inhibition of parasympathetic nerve activity was also inhibited by administration of RS-79948.

4 These results suggest that rilmenidine acts like clonidine to produce pupillary dilation by inhibition of parasympathetic tone to the iris sphincter and that this central nervous system parasympatho-inhibition is mediated by α_2-adrenoceptors, rather than imidazoline receptors.

Keywords: mydriasis, parasympatho-inhibition, α_2-adrenoceptors, imidazoline receptors, RS-79948, pupil, rilmenidine, cats

Introduction

Rilmenidine represents a newer class of centrally acting anti-hypertensive drugs that are claimed to have a more advantageous side effect profile when compared with drugs like clonidine (Feldman *et al*., 1998). There is, however, some dispute as to whether rilmenidine produces its effects by acting primarily by stimulation of central nervous system (CNS) imidazoline receptors or by stimulation of CNS α_2-adrenoceptors (Ernsberger & Haxhiu, 1997; Guyenet, 1997; Eglen *et al*., 1998; Szabo, Bock, Nordheim & Niederhoffer, 1999).

We have previously shown that clonidine and clonidine-like drugs produce dose-related pupillary dilation in cats and rats by a CNS action to inhibit ongoing parasympathetic neuronal tone to the iris sphincter (Koss & San, 1976; Koss, 1986). It appears that these drugs mimic release of an inhibitory neurotransmitter from pathways ascending from the periphery to the region of the Edinger Westphal preganglionic cell bodies as seen in response to stimulation of afferent fibres in the sciatic nerve (Koss, Gherezghiher & Nomura, 1984).

This study was undertaken to determine if rilmenidine also produces mydriasis in anaesthetized cats and, if so, to determine the underlying mechanism involved. The site of action and specific involvement of sympathetic or parasympathetic nerves was studied using selective nerve sections. The relative involvement of α_2-adrenoceptors was determined using a highly selective α_2-adrenoceptor antagonist, RS-79948 (Hume *et al*., 1996; Uhlen *et al*., 1998).

Materials and methods

All animals were treated in a manner consistent with the regulations of the US Public Health Service, with experimental protocols approved by the University of Oklahoma Institutional Animal Use and Care Committee. Adult cats of either sex were anaesthetized with pentobarbital (36 mg kg^{-1}, i.p.). A femoral artery and vein were cannulated for measurement of systemic arterial blood pressure (model P23 transducer; Statham Hato Rey, PR, USA) and for i.v. drug administration, respectively. Heart rate was derived from the femoral arterial pulse wave by means of a cardiotachograph (Grass 7P4; Grass instruments, Quincy, MA, USA). Blood pressure and heart rate responses were recorded on a polygraph (Grass model 7).

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Following cannulation of the trachea, the animals were mounted in a stereotaxic apparatus (David Kopf, Tujunga, CA, USA). Body temperature was maintained at 37 °C using a heating pad connected to a thermistor measuring rectal temperature and controlled with a feed-back circuit (Yellow Springs Instrument Co., Yellow Springs, OH, USA). Basal pupil size and changes in pupillary diameter were measured at the point of greatest horizontal diameter using a clear millimetre ruler. All observations were made under the same general ambient lighting conditions.

In some preparations the preganglionic cervical sympathetic nerve trunk was sectioned at the midcervical level. In others, efferent parasympathetic innervation to the iris was eliminated by surgical removal of the ciliary ganglion via an intra-orbital approach (Koss & San, 1976). In this second set of experiments, the subsequently dilated pupil was constricted by topical application of several drops of a 2% solution of pilocarpine hydrochloride. The ability of this parasympathectomized, pilocarpine-treated iris to respond to activation of the dilator muscle was tested by direct electrical stimulation of the preganglionic sympathetic nerve.

For preganglionic activation of the cervical sympathetic nerve, a bipolar electrode was placed beneath the sympathetic nerve trunk at the midcervical level and insulated using mineral oil. Mydriatic responses were evoked using a Grass S88 stimulator and Grass SIU5 isolation unit (Grass Instruments). Typical stimulation parameters for preganglionic nerve activation were 6–10 V, 10 s trains and 2 ms duration. The frequency was varied from 2 to 32 Hz.

In order to produce reflex mydriasis, afferent nerve fibres in a peripheral nerve were activated to produce inhibition of parasympathetic neuronal tonic activity (Koss *et al*., 1984). In these experiments, graded pupillary dilations were elicited by electrical stimulation of the proximal portion of the ligated sciatic nerve trunk using a bipolar electrode covered with mineral oil. The stimuli, generated using a Grass stimulator and isolation unit, consisted of pulses of 2 ms duration and 20 V presented in 10 s trains with the frequency varied between 1 and 64 Hz.

Pilocarpine hydrochloride was obtained from Alcon Ophthalmic, Humacao, PR, USA, as *Isopto Carpine* 2%. Rilmenidine hemifumarate and RS-79948 hydrochloride were purchased from Tocris Cookson, Inc., Ballwin, MO, USA. Rilmenidine and RS-79948 solutions were prepared in physiological saline with the drug dosages indicating the respective salts. Both agonists and antagonists were given intravenously with 10–15 min allowed between administrations in order to reach steady state values.

Data concerning the pupil are reported as mean ± SEM and represent either basal pupil size or the change in pupillary diameter. Basal blood pressure and heart rate values before and after drug administration were analysed using Student's *t*-test for paired comparisons. Dose–response and frequency–response relationships were compared using analysis of variance with values of $P < 0.05$ considered statistically significant.

Results

Increasing cumulative doses of rilmenidine were administered to two groups of animals. In one group, the cervical sympathetic nerve trunk was sectioned on one side leaving one eye with dual sympathetic and parasympathetic innervation and the opposite eye with only parasympathetic innervation. In the other group, both cervical sympathetic nerves were cut and the ciliary ganglion was also removed on one side leaving only one eye with autonomic (parasympathetic) innervation. The subsequently dilated pupil on the parasympathectomized side was reconstricted using topical pilocarpine hydrochloride.

In the first group of animals (with section of one sympathetic nerve), i.v. administration of rilmenidine (33–1000 μg kg^{-1}) produced a dilation of both pupils in a dose-related fashion that was equivalent to both eyes (Fig. 1). This rilmenidine-induced mydriatic effect remained constant for at least 1 h after administration of the highest dose. In contrast, rilmenidine produced unilateral mydriasis in the cats having only parasympathetic tonic innervation on one side. Fig. 2 shows that only the iris with the intact parasympathetic nerve dilated in response to rilmenidine. Direct preganglionic sympathetic nerve stimulation, however, was able to produce pupillary effects in the parasympathectomized, pilocarpine-treated eyes.

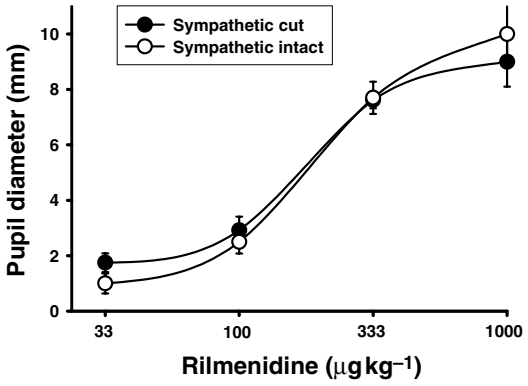

Figure 1 Effects of intravenous administration of increasing cumulative doses of rilmenidine (33–1000 μg kg^{-1}) on pupil size in six pentobarbital anaesthetized cats. Parasympathetic innervation intact to both eyes. Sympathetic innervation intact on right side (open circles) and cervical sympathetic nerve sectioned on left side (solid circles). Values represent mean ± SEM. Note equivalence of rilmenidine-induced mydriasis independent of sympathetic innervation to the iris.

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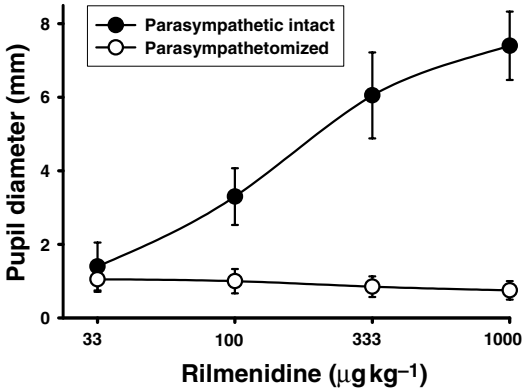

Figure 2 Effects of intravenous administration of increasing cumulative doses of rilmenidine (33–1000 μg kg^{-1}) on pupil size in five pentobarbital anaesthetized cats. Sympathetic innervation sectioned for both eyes. Parasympathetic innervation intact on right side (solid circles) and ciliary ganglion (parasympathetic) removed on left side with subsequently dilated pupil constricted with topical 2% pilocarpine hydrochloride (open circles). Values represent means ± SEM. Note dependence of rilmenidine-induced mydriasis on intact parasympathetic innervation to the iris.

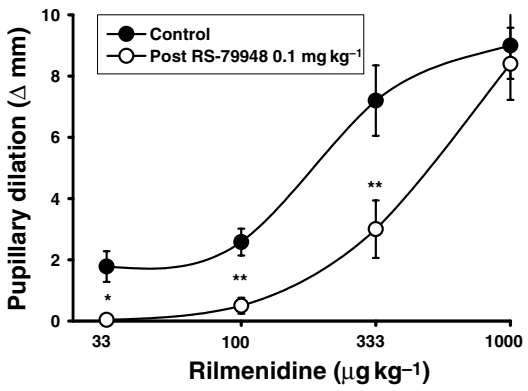

Figure 3 Inhibition of mydriatic effects of rilmenidine by selective α_2-adrenoceptor antagonist, RS-79948. Pupillary response to administration of increasing cumulative doses of rilmenidine (33–1000 μg kg^{-1}, i.v.) in six non-treated control cats (solid circles) and in seven animals pretreated with RS-79948 (100 μg kg^{-1}, i.v.; open circles). Preganglionic sympathetic nerves cut in all preparations. Values represent mean ± SEM. $^*P < 0.05$; $^{**}P < 0.01$.

In three of these preparations, subsequent stimulation of the preganglionic nerve trunk resulted in a statistically significant pupillary dilation of 3.3 ± 1.9, 6.9 ± 1.5 and 7.7 ± 1.4 mm at 2, 8 and 32 Hz, respectively.

Blood pressure responses to rilmenidine were comprised of a short-lived hypertension followed by a more sustained hypotensive response. Basal mean systemic arterial blood pressure was 155 ± 9 mmHg prior to rilmenidine administration and was 123 ± 11 mmHg after the 1000 μg kg^{-1} dose of rilmenidine ($P < 0.05$; $n = 8$). Heart rate was 238 ± 15 beats min^{-1} prior to rilmenidine administration and was 187 ± 12 beats min^{-1} after the 1000 μg kg^{-1} dose of rilmenidine ($P < 0.01$; $n = 8$).

Both pre- and post-treatment with the selective α_2-adrenoceptor antagonist, RS-79948 antagonized the mydriatic response to rilmenidine. Pretreatment with RS-79948 (0.1 mg kg^{-1}, i.v.) significantly shifted the rilmenidine pupillary dose–response curve to the right (Fig. 3). There was no statistically significant antagonism of rilmenidine-induced hypotension or bradycardia by RS-79948. In these experiments, basal mean systemic arterial blood pressure was 169 ± 11 mmHg prior to rilmenidine administration and was 126 ± 13 mmHg after the 1000 μg kg^{-1} dose of rilmenidine ($P < 0.01$; $n = 7$). Heart rate was 250 ± 9 beats min^{-1} prior to rilmenidine administration and was 198 ± 11 beats min^{-1} after the 1000 μg kg^{-1} dose of rilmenidine ($P < 0.01$; $n = 8$).

For a more quantitative assessment of RS-79948 blockade, cats were administered a maximal mydriatic dose of rilmenidine (1000 μg kg^{-1}, i.v.)

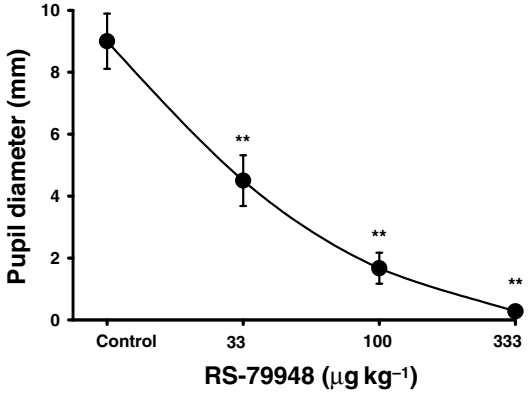

Figure 4 Pupillary effects of administration of increasing cumulative doses of RS-79948 (33–333 μg kg^{-1}) in cats where pupils were previously dilated in response to rilmenidine (1000 μg kg^{-1}, i.v.). RS-79948 was administered intravenously at 10–15 min intervals. Values represent mean ± SEM for seven cats. $^*P < 0.05$; $^{**}P < 0.01$.

and sequentially challenged with cumulative doses of the antagonist (at 10–15 min intervals). RS-79948 (33–333 μg kg^{-1}, i.v.) produced a dose-dependent reversal of rilmenidine-induced parasympatho-inhibition that was complete at the highest dose of RS-79948 (Fig. 4).

The final series of experiments were undertaken in order to determine if RS-79948 would act like other α_2-adrenoceptor antagonists and block reflex pupillary dilation (parasympatho-inhibition) seen in response to peripheral afferent nerve stimulation.

Electrical stimulation of the central end of the ligated sciatic nerve produced a frequency-dependent reflex pupillary dilation in cats having only the parasympathetic innervation to the iris

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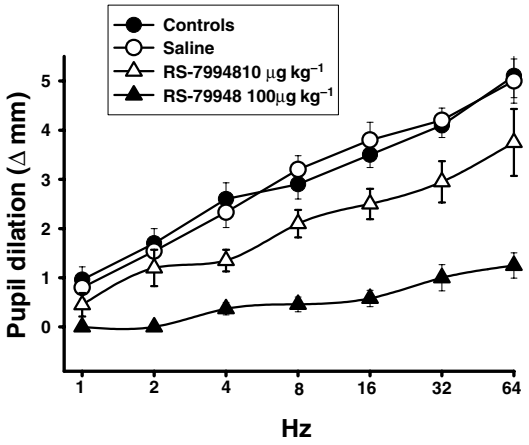

Figure 5 Effects of RS-79948 (10 and 100 μg kg^{-1}, i.v.) on graded mydriatic responses elicited by stimulation of the afferent sciatic nerve in anaesthetized cats. Pupillary dilation is a result of withdrawal of parasympathetic neural tone to the iris as the sympathetic nerve trunks have been sectioned at the mid-cervical level. Values represent mean ± SEM for six cats. Controls represented by circles (before and after saline). Triangles represent responses evoked after two sequential doses of the α_2-adrenoceptor antagonist, RS-79948.

intact. Peak pupillary dilation was seen within 3–5 s and was generally maintained throughout the 10-s period of stimulation. Administration of the selective α_2-adrenoceptor antagonist, RS-79948 (10 and 100 μg kg^{-1}, i.v.), produced a dose-dependent antagonism of the parasympatho-inhibitory effect of afferent sciatic nerve stimulation (Fig. 5).

Discussion

Rilmenidine and moxonidine are 'second generation' centrally acting anti-hypertensive drugs that are claimed to produce their CNS sympatholytic actions by stimulation of CNS I_1 imidazoline receptors rather than by stimulation of CNS α_2-adrenoceptors as seen with drugs like clonidine and guanabenz (Bousquet, Feldman & Schwartz, 1984; Ernsberger & Haxhiu, 1997). Although these newer agents also stimulate CNS α_2-adrenoceptors, it is suggested that their relative selectivity for I_1 imidazoline receptors give them the advantage of lowering systemic arterial blood pressure with fewer untoward side effects than seen with CNS α_2-adrenoceptor agonists (Feldman *et al.*, 1998). Although extensive research has been undertaken in this arena, controversy concerning I_1 imidazoline *vs.* α_2-adrenoceptor mechanisms persists (Ernsberger & Haxhiu, 1997; Guyenet, 1997; Eglen *et al.*, 1998; Szabo *et al.*, 1999).

Clonidine and clonidine-like drugs produce pupillary dilation in cats, rats and mice by inhibition of tonic parasympathetic neuronal activity by an apparent stimulation of CNS α_2-adrenoceptors (Koss & San, 1976; Koss, 1981; Berridge, Gadie, Roach & Tulloch, 1983; Hey, Gherezghiher & Koss, 1985; Heal, Prow & Buckett, 1989). Clonidine-induced mydriasis appears to be a result of direct stimulation of postsynaptic α_2-adrenoceptors located on preganglionic parasympathetic pupilloconstrictor neurones as the parasympatho-inhibitory effect is blocked by α_2-adrenoceptor antagonists like yohimbine and rauwolscine (Berridge *et al.*, 1983; Koss, 1986), but is still seen in monoamine depleted preparations (Koss, 1979; Koss & Christensen, 1979). In contrast, CNS administration of amphetamine produces comparable mydriatic effects that are dependent on the presence of CNS monoamines (Koss, 1980a; Hey, Ito & Koss, 1989). CNS administration of α-methyl-dopa also produces a similar, although delayed mydriatic response, but only when the enzymes needed for conversion to α-methyl-noradrenaline in the CNS are functional (Koss, 1980b; Gherezghiher, Christensen & Koss, 1982). The normal physiological substrate for this parasympatho-inhibitory mydriasis appears to be tonic ascending inhibition from the periphery as these effects can be mimicked by electrical stimulation of afferent sciatic nerve fibres or by electrical stimulation of ascending pathways in the lower brain stem (Koss *et al.*, 1984).

In the present study, we demonstrated that rilmenidine also produces a long lasting, dose-dependent mydriasis that is more likely caused by CNS parasympatho-inhibition. This conclusion is based on experimental results showing that (like clonidine) section of the parasympathetic, but not the sympathetic innervation to the pupil, totally prevents the mydriatic actions of rilmenidine. Lack of rilmenidine effect on the parasympathectomized iris was not due to pharmacological antagonism with the pilocarpine used to constrict the pupil as sympathetic nerve stimulation produced mydriatic responses that were not significantly different from responses seen in non-treated eyes. It is of interest that rilmenidine dose–response relationships for CNS parasympatho-inhibition are virtually identical to those found to decrease systemic arterial blood pressure and to inhibit renal nerve activity in rabbits (Szabo *et al.*, 1999).

The present results of RS-79948 antagonism of rilmenidine-induced mydriasis offer strong support for a clear-cut α_2-adrenoceptor mechanism of action, at least for this autonomic system, as there is abundant evidence for α_2-adrenoceptor selectivity of RS-79948 (Hume *et al.*, 1996; Milligan *et al.*, 1997; Uhlen *et al.*, 1998). In contrast, a compound showing high selectivity for binding to I_1 imidazoline sites was devoid of all physiological effect when administered (even in very high doses) to rabbits and monkeys (Munk *et al.*, 1996). In this regard, it is of note that a similar CNS-induced mydriatic response is seen following systemic

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administration of the relatively selective α_2-adrenoceptor agonists α_2-methyl-noradrenaline and guanabenz (Koss, 1980b; Gherezghiher *et al*., 1982; Koss, 1983).

Systemic administration of RS-79948 also greatly reduced reflex mydriasis seen with afferent sciatic nerve stimulation. This further supports an α_2-adrenoceptor mechanism of action as similar effects are seen with other CNS acting α_2-adrenoceptor antagonists such as yohimbine (Koss *et al*., 1984).

Controversy remains regarding the primary mechanism of action of 'second generation' anti-hypertensive drugs such as rilmenidine and moxonidine on sympathetic regulation of the cardiovascular system (Ernsberger & Haxhiu, 1997; Guyenet, 1997; Eglen *et al*., 1998; Szabo *et al*., 1999). The present observations seem to suggest that α_2-adrenoceptors are not responsible for rilmenidine-induced hypotension and bradycardia as these effects were not significantly different before and after treatment with the selective α_2-adrenoceptor antagonist, RS-79948. These observations must be interpreted with caution, as (unlike rilmenidine-induced mydriasis) there was insufficient time between doses of rilmenidine to reach steady-state with regard to the more complex cardiovascular responses.

In contrast, other evidence supports an exclusive α_2-adrenoceptor mechanism of action. Only results from experiments using microinjection techniques seem to suggest a primary imidazoline mechanism (Bousquet *et al*., 1984), whereas i.v. administration produces hypotensive effects that are blocked by α_2-adrenoceptor agents such as SKF-86466 that are nearly devoid of affinity for imidazoline binding sites (Hieble & Kolpak, 1993). Perhaps the controversy is a product of the mode of administration, as microinjection techniques have inherent problems such as knowledge of local tissue accessibility and concentrations. It also is possible, as has been proposed, that there is an interaction between adrenergic and imidazoline mechanisms at the CNS level that could account for these differences where two different brain stem sites of action may be involved (Ernsberger & Haxhiu, 1997; Feldman *et al*., 1998). However, there is no such evidence for multiple sites of control in the present model system using tonic parasympathetic outflow to the iris sphincter.

The most convincing support for lack of a primary imidazoline mechanism in the physiological actions of rilmenidine and moxonidine comes from experiments utilizing direct recordings of electrical activity of rat brain stem neurones and from studies of *in vivo* gene manipulation.

In the first case, noradrenaline, clonidine, rilmenidine and moxonidine dose-dependently decrease firing of neurones of the locus coeruleus, an effect that is almost totally abolished with the α_2-adrenoceptor antagonist, SKF-86466 (Szabo, Frohlich & Illes, 1996). Similar results are seen in recordings of neurones of the rostral ventrolateral medulla in brain stem slices of neonatal rats where moxonidine-induced neuronal firing is also largely prevented by administration of SKF-86466 (Hayar & Guyenet, 2000). The overall conclusion from both of these studies is that the neuronal actions of these agonists can be fully explained as an interaction with inhibitory α_2-adrenoceptors.

More recently, mice with deletions or point mutations of specific α_2-adrenoceptor subtypes have been extensively studied (MacMillan, Hein, Smith, Piascik, & Limbird, 1996; Hunter *et al*., 1997; Lakhlani *et al*., 1997; Zhu *et al*., 1999). These investigations clearly demonstrate that most clinically important CNS actions of imidazoline-like drugs (i.e. hypotension, sedation and analgesia) are mediated exclusively by α_{2A}-adrenoceptors (for review see Kable, Murrin & Bylund, 2000).

In conclusion, the combined I_1 imidazoline receptor/α_2-adrenoceptor agonist, rilmenidine, produced dose-related pupillary dilation in anaesthetized cats. Rilmenidine, like clonidine, produced mydriasis by inhibition of tonic parasympathetic neuronal activity to the iris sphincter muscle. The selective α_2-adrenoceptor antagonist, RS-79948, blocked reflex-induced mydriasis in response to afferent sciatic nerve stimulation as well as rilmenidine-induced mydriasis. Taken together, these results suggest that rilmenidine acts in the CNS to produce parasympatho-inhibition by acting as an α_2-adrenoceptor agonist with no evidence for an action *via* imidazoline receptors.

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