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α-Methyldopa produces mydriasis in the rat by stimulation of CNS α_2-adrenoceptors

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1 The effects of i.v. administration of α-methyldopa (MD) on rat pupil diameter were investigated. All experiments were carried out in rats in which vagosympathetic nerve trunks were sectioned bilaterally at the cervical level.

2 In anaesthetized rats MD produced a marked dose-related increase in pupil diameter. The onset of pupillary response to MD was gradual and reached maximal levels 2–3 h after administration.

3 Pretreatment with α_2-adrenoceptor antagonists yohimbine (1.5 mg kg^{-1}, i.v.) or idazoxan (0.5 mg kg^{-1}, i.v.) blocked the pupillary response to MD. In contrast, the α_1-antagonists prazosin (1.0 mg kg^{-1}, i.v.) and phenoxybenzamine (1.5 mg kg^{-1}, i.v.) did not significantly alter the pupillary effects of MD.

4 Selective enzymatic blockade with 3-hydroxy-benzyl-hydrazine (NSD-1015; 25 mg kg^{-1}, i.p.), a dopa-decarboxylase inhibitor, as well as *bis* (4-methyl-homopiperazinyl-thiocarbonyl) disulphide (FLA-63, 5.0 mg kg^{-1}, i.p.), a dopamine-β-hydroxylase inhibitor, prevented the mydriatic effect of MD.

5 The above findings support the hypothesis that MD produces a clonidine-like CNS mydriasis in the rat. This effect appears to be mediated primarily by the MD metabolite, α-methylnoradrenaline.

6 These results indicate that MD produces mydriasis in the rat by a CNS action. The mydriatic action of MD appears to be produced by its metabolite α-methylnoradrenaline which in turn stimulates CNS postsynaptic α_2-adrenoceptors.

Introduction

We have demonstrated previously that α-methyldopa (MD) produces pupillary dilatation in the cat (Koss, 1980; Gherezghiher *et al.*, 1982). The mydriatic action of MD appears to be mediated by a clonidine-like effect of its metabolite, α-methylnoradrenaline, acting directly on CNS postsynaptic α_2-adrenoceptors (Koss, 1980; Gherezghiher *et al.*, 1982; 1983). Other investigators have shown that MD produces hypotension by inhibiting CNS sympathetic outflow (Ingenito *et al.*, 1970; Henning & Rubenson, 1971; Day *et al.*, 1973). This central hypotensive action is analogous to the central parasympathoinhibitory effects of MD enabling the use of the pupil regulatory system as a model to assess autonomic effects of centrally acting antihypertensive drugs (Koss, 1986).

The present study was undertaken to characterize the mode of action of MD in producing mydriasis in the rat. The information obtained might enable us to determine whether the pupillary response to MD is qualitatively similar to the cat, and to investigate whether α_2-adrenoceptors are involved. The information obtained from this study combined with the earlier reports that clonidine and other α_2-adrenoceptor agonists produce a similar yohimbine-sensitive mydriasis in the rat (Gherezghiher & Koss, 1979; Koss, 1986) may provide further insight in evaluating the central autonomic effects of other centrally acting antihypertensive drugs. Use of the yohimbine-sensitive pupillary dilatation in the rat might be a simple and relatively inexpensive model for assessing CNS α_2-adrenoceptor activity.

Methods

General

Male Sprague-Dawley rats (250–450 g) were anaesthetized with urethane (2.0 g kg^{-1}, i.p.), dial urethane

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(0.6 mg kg^{-1}, i.p.), or a combination of chloral hydrate and pentobarbitone (180 mg kg^{-1} and 40 mg kg^{-1}, i.p., respectively). The dose of anaesthetic was reduced by 50% in monoamine depleted preparations. In all experiments the vagosympathetic nerve trunks were sectioned bilaterally at the cervical level. Following cannulation of the trachea, the rats were placed on a respirator and warmed with a heating pad. One femoral vein was cannulated for i.v. drug administration and one femoral artery was cannulated for blood pressure and heart rate recordings. In some studies, methyldopa was administered by the tail vein. Blood pressure and heart rate were monitored by means of a Statham P23Dd blood pressure transducer and tachograph and recorded on a Grass 7B polygraph. Pupil diameter was measured at the point of greatest horizontal diameter with a ruler (0.1 mm graduations) and a binocular microscope. Measurements were made under green light to minimize the light reflex pupillary constriction and to add contrast to the iris.

Monoamine depletion

Some of the rats were depleted of CNS monoamines by intraperitoneal injection of reserpine (5 mg kg^{-1}) and two doses of the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (300 mg kg^{-1}). The dose of reserpine and one dose of α-methyl-*p*-tyrosine were administered 20 h before the experiments. The second dose of α-methyl-*p*-tyrosine was administered 2 h before the experiment.

Statistics

The evaluation of experimental results was by use of two-way analysis of variance (ANOVA). The test of significance for the experimental findings was at the $P < 0.05$ level.

Drugs

The following drugs were dissolved in physiological sodium chloride solution: α-methyl-*p*-tyrosine methylester hydrochloride (Sigma); L-α-methyldopa (Merck-Sharpe Dohme), 3-hydroxy-benzylhydrazine (NSD-1015) and *bis*(4-methyl-homopiperazinyl thiocarbonyl) disulphide (FLA-63) (Sigma); phenoxybenzamine hydrochloride (Smith, Kline and French); idazoxan hydrochloride (Reckitt and Colman). Yohimbine hydrochloride (Aldrich) was dissolved in distilled water. Prazosin hydrochloride (Pfizer) was dissolved in a 5% glucose–5% glycerol (w/v) vehicle. A few drops of dilute HCl were added to solutions of MD and FLA-63 to improve solubility. The vehicle control was saline brought to the same pH. Reserpine was given as Serpasil (Ciba-Geigy).

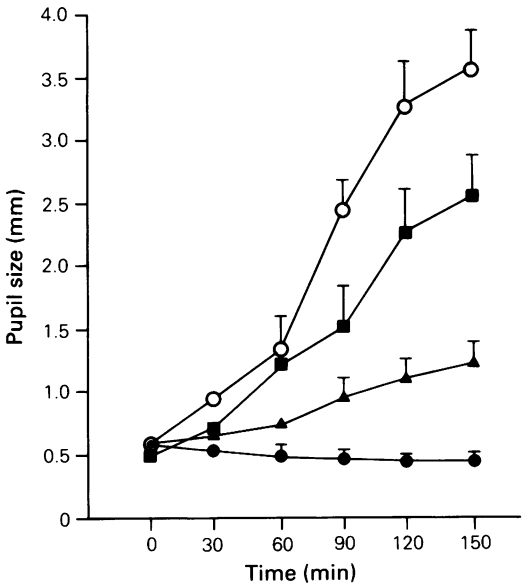

Figure 1 Effect of graded i.v. doses of α-methyldopa (10 mg kg^{-1}; ▲), (30 mg kg^{-1}; ■), (100 mg kg^{-1}; ○) on pupil diameter of the anaesthetized rat. Saline vehicle (●) was without effect. Values are means for 5 rats in each group with vertical bars showing s.e. mean. The three experimental curves are significantly different from the response to saline; $P < 0.05$.

Results

Effects of intravenous administration of α-methyldopa

Control responses Intravenous administration of MD (10, 30 and 100 mg kg^{-1}) produced a dose-dependent pupillary dilatation in anaesthetized rats (Figure 1). With all three doses the onset was gradual and reached a maximum plateau in about 150 min. The saline vehicle did not show significant pupillary responses over the duration of the experiment. At the time of injection the initial pupillary diameters were between 0.5–0.7 mm for all four experimental groups.

Pretreatment with α_2-adrenoceptor antagonists To determine whether the MD-induced mydriasis was mediated by activation of α_2-adrenoceptors, yohimbine (1.5 mg kg^{-1}, i.v.) or idazoxan (0.5 mg kg^{-1}, i.v.) was given to 4–5 rats 15 min before the administration of MD (30 mg kg^{-1}, i.v.). As seen in Figure 2, both yohimbine and idazoxan pretreatment effectively blocked the MD-induced mydriasis. In these preparations, pretreatment with both α_2-antagonists produced an initial miotic effect evident before the

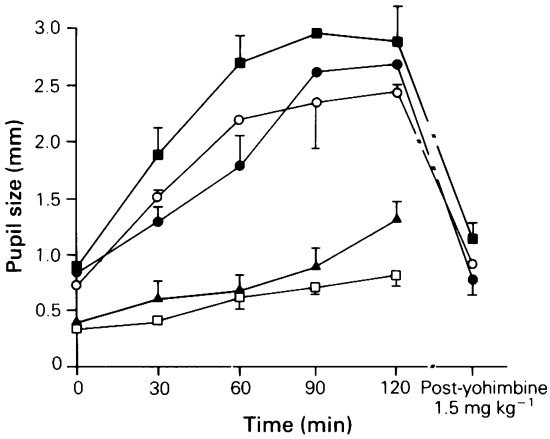

Figure 2 Pupillary effects of α-methyldopa ($30 mg kg^{-1}$, i.v.) in five groups of rats: control (●), yohimbine pretreated ($1.5 mg kg^{-1}$, i.v.; ▲); idazoxan pretreated ($0.5 mg kg^{-1}$, i.v.; □); prazosin pretreated ($1.0 mg kg^{-1}$, i.v.; ○), and phenoxybenzamine pretreated ($2 mg kg^{-1}$, i.v.; ■). Note that only yohimbine and idazoxan pretreatment antagonized α-methyldopa-induced mydriasis. Yohimbine administered at 120 min (after MD) to the control, prazosin and phenoxybenzamine pretreated rats completely reversed the mydriatic effects of α-methyldopa. Values are means for 5 rats in each group with vertical bars showing s.e. mean. Responses to pretreatment with both α_2-adrenoceptor antagonists are statistically different from control responses; $P < 0.02$. The differences between control responses and responses to pretreatment with either of the α_1-adrenoceptor antagonists are not statistically different; $P > 0.05$.

administration of MD (0.40 ± 0.06 mm compared with 0.82 ± 0.11 mm for the MD controls).

Pretreatment with α_1-adrenoceptor antagonists

Experiments were performed to determine whether MD-induced mydriasis was mediated by activation of peripheral α_1-adrenoceptors of the iris. In contrast to the α_2-antagonists, the α_1-adrenoceptor antagonists prazosin ($1.0 mg kg^{-1}$) and phenoxybenzamine ($2 mg kg^{-1}$, i.v.) given 30 min before MD ($30 mg kg^{-1}$) failed to block the pupillary effects of MD. As illustrated in Figure 2, phenoxybenzamine pretreatment caused an apparent potentiation of the MD-induced mydriasis. The greatest increase in pupil diameter above control was seen at 60 min when the phenoxybenzamine pretreated rats showed a mydriatic response to MD that was 50% greater than the control response (2.64 ± 0.22 vs 1.76 ± 0.26 mm).

After treatment with yohimbine ($1.5 mg kg^{-1}$) the MD-induced mydriasis in both control, prazosin and phenoxybenzamine pretreated rats was completely reversed; this provided further evidence that

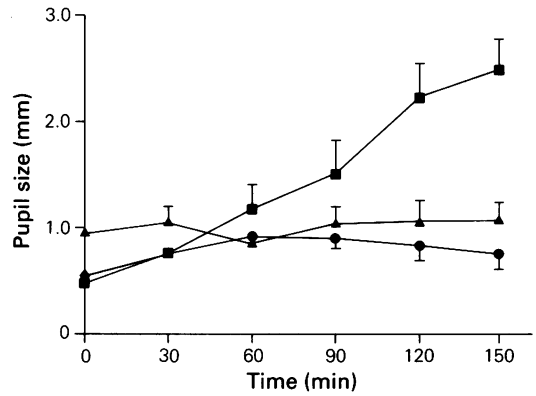

Figure 3 Effect of α-methyldopa ($30 mg kg^{-1}$, i.v.; ■) on pupil diameter before and after administration of NSD-1015 ($25 mg kg^{-1}$, i.p.; ▲) and FLA-63 ($5.0 mg kg^{-1}$, i.p.; ●). Note that pretreatment with either NSD-1015 or FLA-63 antagonized the pupillary effects of α-methyldopa. Values are means for 5 rats in each group. The two experimental curves are significantly different from the control responses; $P < 0.01$.

the pupillary response to MD is mediated by central α_2-adrenoceptors. The pupil diameter following treatment with yohimbine ($1.5 mg kg^{-1}$) was 0.70 ± 0.08 mm for the MD-control group, 1.05 ± 0.10 mm for the phenoxybenzamine pretreated group and 0.91 ± 0.16 mm for the prazosin pretreatment group. These results indicate that α_1-adrenoceptors are not involved in the pupillary effects of MD.

Effect of enzyme inhibitors on the mydriatic response to α-methyldopa

Experiments were performed to determine whether the pupillary response to MD was related to MD itself or to one or both of its primary metabolites, α-methyl-dopamine (MDA) or α-methynoradrenaline (MNA). Pretreatment with either NSD-1015 ($25 mg kg^{-1}$ i.p.) or FLA-63 ($5 mg kg^{-1}$, i.p.) administered 30 min before MD ($30 mg kg^{-1}$, i.v.) completely prevented the MD-induced mydriasis (Figure 3). Both enzyme inhibitors were devoid of intrinsic pupillary effects. The results of the present study demonstrate that blockade of either the decarboxylation of MD to MDA (NSD-1015) or the β-hydroxylation of MDA to MNA (FLA-63) prevents the pupillary response to MD. These findings are in agreement with earlier studies demonstrating that MNA is the active metabolite that mediates the mydriatic effect of MD in the cat (see Discussion).

Discussion

The results of the present study demonstrate that α-methyldopa, administered intravenously, produces a

dose-dependent pupillary dilatation in anaesthetized rats. Similar findings have been reported in earlier studies using cats (Koss, 1980; Gherezghiher *et al.*, 1982; 1983). These results are consistent with the hypothesis that MD produces mydriasis in both rats and cats by a common mechanism involving activation of postsynaptic α_2-adrenoceptors.

In studies on both rats and cats we have demonstrated that the pupillary dilatation in response to clonidine is mediated by a direct activation of CNS postsynaptic α_2-adrenoceptors producing a reduction in parasympathetic tone to the iris (Koss & San, 1976; Gherezghiher & Koss, 1979; Hey *et al.*, 1985). A CNS site of mydriatic action for several α_2-adrenoceptor agonists has also been demonstrated in the rat (Berridge *et al.*, 1983). In the above mentioned studies, the mydriatic action of direct α_2-adrenoceptor agonists was antagonized by selective α_2-adrenoceptor antagonists such as yohimbine and idazoxan. In contrast, the pupillary effects of clonidine have been shown to be refractory to phenoxybenzamine and prazosin (Gherezghiher & Koss, 1979; Berridge *et al.*, 1983; Koss, 1986). Taken together these findings indicate that in cats and rats α_2-adrenoceptor agonists elicit similar responses. It is suggested that these species share a common underlying central autonomic pupillo-regulatory system.

More recently we have demonstrated, also in the cat, that the pupillary response induced by α-methyldopa correlates exclusively with α-methyl-noradrenaline oculomotor nucleus perfusate concentration (Gherezghiher *et al.*, 1983). In these studies pretreatment with the dopa-decarboxylase inhibitor NSD-1015 ($25\,mg\,kg^{-1}$, i.p.) prevented the accumulation of α-methylnoradrenaline in the perfusate and blocked the pupillary responses to MD. These results are consistent with the hypothesis that α-methylnoradrenaline is the active metabolite producing MD-induced mydriasis in the cat.

The mydriatic effect of MD was gradual, requiring more than 2 h before the maximal mydriatic effect was observed. The time course to achieve this effect probably reflects the time needed for the CNS conversion of MD to α-methylnoradrenaline (Gherezghiher *et al.*, 1983). It is well known that MD is metabolized to α-methyldopamine and α-methylnoradrenaline (Carlsson & Lindquist, 1962) and earlier studies reported that the hypotensive effects of MD could be prevented by blocking the enzymatic conversion to these substances (Davis *et al.*, 1963; Henning, 1969; Day *et al.*, 1973). Studies from this laboratory using cats demonstrated that the mydriatic effect of MD could be prevented by blocking the decarboxylation or β-hydroxylation steps with NSD-1015 or FLA-63, respectively (Gherezghiher *et al.*, 1982). The results from the present study are in full agreement with this hypothesis and strongly suggest that the mydriatic response to MD in the rat also requires enzymatic conversion in the CNS to its active metabolite α-methylnoradrenaline.

There are several other reports supporting a CNS adrenergic inhibitory modulation of parasympathetic constrictor tone to the iris. Reserpine-induced miosis in both the rat and cat is most probably caused by removal of tonic inhibitory influences to the pupillo-constrictor neurones in the oculomotor nuclear complex (Dahlström *et al.*, 1964). Earlier studies of Ury & Gelhorn (1939) demonstrated that section of parasympathetic (but not sympathetic) innervation will interrupt reserpine miosis in the cat. Similar findings have been reported by Hauesler (1974) who showed that monoamine depletion causes an increase in tonic sympathetic nerve activity possibly the consequence of removal of an endogenous inhibitory monoaminergic input. In the present study, both α_2-adrenoceptor antagonist pretreatment and monoamine depletion produced a small but significant miosis in the untreated rat suggesting a blockade of tonic α_2-adrenoceptor-mediated inhibitory inputs to the pupilloconstrictor areas of the rat brain.

Physiological manipulations in rats and cats can also produce inhibition of parasympathetic constrictor tone to the iris and consequent pupillary dilatation. Activation of an ascending pathway (e.g. afferent sciatic nerve stimulation) or a descending pathway (e.g. posterior hypothalamic stimulation) produce pupillary dilatation in sympathectomized cats and rats almost exclusively mediated by inhibition of parasympathetic activity to the iris sphincter muscle (Koss *et al.*, 1984; Hey *et al.*, 1985). Reflex mydriasis (e.g. by stimulation of afferent sciatic nerve) was abolished both by administration of yohimbine as well as by depletion of monoamine stores. These findings suggests that pupillary dilatation produced by activation of the ascending inhibitory pathways utilizes a catecholamine as an inhibitory transmitter acting on postsynaptic α_2-adrenoceptors. In contrast, pupillary dilatation evoked by hypothalamic stimulation was not antagonized by either yohimbine or monoamine depletion which is suggestive of a non-monoaminergic descending inhibiting system (Koss, 1986). The present observations on the α_2-adrenoceptor-mediated mydriasis observed with MD in the rat are identical to those observed in the cat (Koss, 1986) and consistent with the hypothesis postulating the existence of postsynaptic α_2-adrenoceptors located on the neurones of the oculomotor nuclear complex.

There are other CNS autonomic components that interact similarly to the effects of α_2-adrenoceptor

activation. For example, both clonidine and amphetamine (given i.c.v.) decrease sympathetic nerve activity and potentiate vagal reflexes in dogs and cats (Hoyer & van Zwieten, 1972; Kobinger & Pichler, 1978; Schmitt *et al.*, 1979). In these studies, the actions of both drugs were prevented by α_2-adrenoceptor antagonists. Other investigators have reported that MD acts centrally rather than peripherally to inhibit sympathetic outflow (Ingenito *et al.*, 1970; Henning & Rubenson, 1971; Day *et al.*, 1973) and have presented evidence that α-methylnoradrenaline mediates the hypotensive effect of MD via CNS sympatholytic action (Davis *et al.*, 1963; Henning & Rubenson, 1971; Day *et al.*, 1973; Torchiana *et al.*, 1973). The hypotensive effects of MD were abolished selectively with yohimbine (Heise & Kroneberg, 1973). Taken together, these earlier reports in addition to the present findings, support the view that CNS α_2-adrenoceptor activation mediates a functional adrenergic inhibition involved in regulation of central autonomic function.

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