J. Pharm. Pharmacol. 1982, 34: 644–647
Received February 17, 1982

0022–3573/82/100644–04 $02.50/0

Involvement of presynaptic dopamine receptors in the antihypertensive response to 2-*NN*-dimethylamino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene(M-7)

J. C. CLAPHAM* AND T. C. HAMILTON

Beecham Pharmaceuticals, Medicinal Research Centre, Coldharbour Road, The Pinnacles, Harlow, Essex, CM19 5AD, U.K.

M-7, 1 and 3 mg kg^{-1} s.c., elicits an antihypertensive response and bradycardia in conscious spontaneously hypertensive rats (SHR) and causes inhibition of stimulation-evoked pressor responses and tachycardia in pithed SHR. Metoclopramide (30 mg kg^{-1} i.p.), but not piperoxan (5 mg kg^{-1} i.p.), abolishes the antihypertensive effect and inhibition of stimulation-evoked pressor responses produced by M-7 (1 mg kg^{-1} s.c.) in SHR. Conversely, piperoxan, but not metoclopramide, reduces the bradycardia and inhibition of stimulation-evoked tachycardia produced by M-7. Metoclopramide (30 mg kg^{-1} i.p.) did not affect the cardiovascular responses elicited by intracerebroventricular administration of either clonidine (1 μg) or M-7 (3 μg). These results suggest that the antihypertensive effect of M-7 may be mediated by stimulation of presynaptic dopamine receptors on sympathetic nerves to the vasculature and is independent of the bradycardia, which is probably due to stimulation of presynaptic α_2-adrenoceptors on cardiac sympathetic nerve endings.

From studies in rabbit isolated ear artery (Brown et al 1979) and the pithed rat (Clapham & Hamilton 1981) it has been found that M-7 (2-*NN*-dimethylamino-5,6,-dihydroxy-1,2,3,4-tetrahydronaphthalene), stimulates inhibitory presynaptic dopamine receptors on sympathetic nerve endings in the vasculature. M-7 also stimulates inhibitory presynaptic dopamine receptors on sympathetic nerves to the heart of the cat and dog (Long et al 1975; Ilhan et al (1976) though in the rat inhibitory presynaptic α_2-adrenoceptors have been implicated (Hicks & Cannon 1979; Clapham & Hamilton 1981).

The inhibitory action of M-7 on sympathetic neurotransmission to the heart and blood vessels suggested to us that the compound may reduce both blood pressure and heart rate in the conscious animal. We have now shown that M-7 has such actions in conscious spontaneously hypertensive rats (SHR) and determined the role, and nature, of presynaptic receptors involved in mediating these responses.

MATERIALS AND METHODS

Male spontaneously hypertensive rats (SHR) (14 to 24 weeks old), derived from the Japanese strain, and male Sprague Dawley rats were used.

Indirect measurement of blood pressure and heart rate in conscious rats

Rats, prewarmed in an incubator (32–35 °C) for 20 to 30 min, were restrained to measure systolic blood pressure and heart rate indirectly by the tail cuff method using a W and W 8005 B.P. recorder. Each determination was the mean of at least six recordings. Groups of six animals were used, measurements being made predose (zero) 1, 2 and 4 h after M-7, 1 and 3 mg kg^{-1} subcutaneously (s.c.) or vehicle, 2 ml kg^{-1} s.c.

In studies using antagonists, metoclopramide (30 mg kg^{-1}) or piperoxan (5 mg kg^{-1}) were given intraperitoneally (i.p.) immediately before M-7.

Stimulation of sympathetic outflow in pithed rats

Groups of 6 to 12 SHR were dosed with vehicle (2 ml kg^{-1}) s.c. and i.p., a submaximal antihypertensive dose of M-7 (1 mg kg^{-1} s.c.) alone or with metoclopramide (30 mg kg^{-1} i.p.) or piperoxan (5 mg kg^{-1} i.p.). Two hours later the animals were anaesthetized with methohexitone sodium (50 mg kg^{-1} i.p.), pithed through one orbit, and given (+)-tubocurarine (1·5 mg kg^{-1} i.v.) immediately. Changes in diastolic blood pressure and heart rate produced by electrical stimulation of the entire sympathetic outflow from the spinal cord (0·25–8 H_z in random order, 0·5 ms pulse width, supramaximal voltage for 20 s) were recorded from a carotid artery using a Bell and Howell pressure transducer (type 4-422-0001). Heart rate was measured using a Devices instantaneous ratemeter which was triggered by the arterial pulse wave. A jugular vein was cannulated to allow intravenous (i.v.) dosing.

* Correspondence.

Intracerebroventricular (i.c.v.) administration of drugs in anaesthetized rats

Groups of 7–8 Sprague Dawley rats were dosed with metoclopramide (30 mg kg^{-1} i.p.), piperoxan (5 mg kg^{-1} i.p.) or vehicle (2 ml kg^{-1} i.p.) and 2 h later the animals were anaesthetized with pentobarbitone sodium (65 mg kg^{-1} i.p.). Cannulae were inserted into the lateral cerebral ventricles according to Hayden et al (1966). Blood pressure changes were recorded from a carotid artery, as described for the pithed rat. Injections of clonidine (1 μg in 10 μl) or M-7 (3 μg in 10 μl) were made by using a Hamilton microlitre syringe. At the end of each experiment about 30 μl of methylene blue was injected via the i.c.v. cannulae: the brain was then dissected to confirm that the cannulae had been accurately placed in the lateral ventricles.

Statistical analysis

Student's *t*-test for group data was used to evaluate statistical significance; *P* values <0·05 were considered significant.

Drugs

The drugs used were: clonidine hydrochloride (Boehringer), 2-*NN*-dimethyl-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene(M-7) hydrobromide (synthesized by Dr R. E. Markwell, Beecham, Harlow), metoclopramide hydrochloride (Beecham), piperoxan hydrochloride (May and Baker), and (+)-tubocurarine hydrochloride (Burroughs Wellcome). Doses are expressed as base. All drugs were dissolved in 0·9% w/v NaCl (saline).

RESULTS

Effect of M-7 on systolic blood pressure and heart rate in conscious SHR

M-7 (1 mg kg^{-1} s.c.) produced progressive falls in blood pressure during the 4 h test (Fig. 1). Heart rate was also reduced by M-7, and the maximum response at 1 h clearly preceded the maximum antihypertensive effect. Furthermore, whereas systolic blood pressure fell progressively over 4 h, the bradycardia gradually diminished between 1 and 4 h (Fig. 1). The cardiovascular effects of M-7, 3 mg kg^{-1} s.c., were slightly greater than those shown in Fig. 1 for M-7, 1 mg kg^{-1} s.c.

Metoclopramide (30 mg kg^{-1} i.p.) abolished the antihypertensive effect, but did not affect the bradycardia, elicited by M-7 (1 mg kg^{-1} s.c.); metoclopramide itself had no effect on blood pressure or heart rate at the dose used (Fig. 1). Conversely, piperoxan (5 mg kg^{-1} i.p.) did not affect the antihypertensive effect of M-7 (1 mg kg^{-1} s.c.) but significantly reduced the bradycardia whilst piperoxan itself had little effect on blood pressure or heart rate at the dose used (Fig. 1).

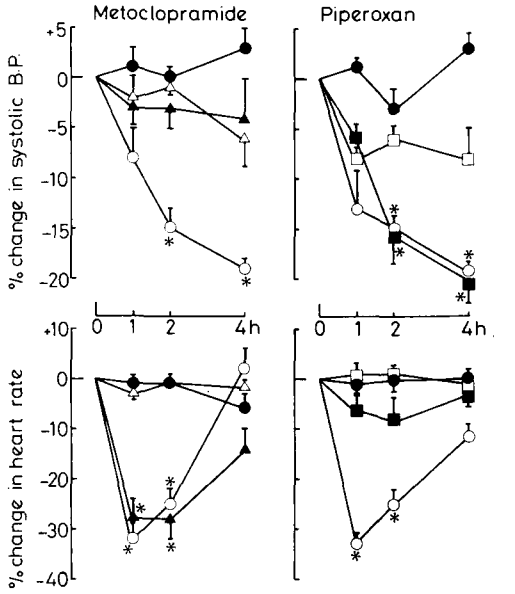

FIG. 1. Percentage changes produced in systolic blood pressure and heart rate by M-7 alone and in combination with metoclopramide or piperoxan in conscious SH-rats. (●——●) vehicle (2 ml kg^{-1} s.c.); (○——○) M-7 (1 mg kg^{-1} s.c.); (△——△) metoclopramide (30 mg kg^{-1} i.p.); (▲——▲) M-7 (1 mg kg^{-1} s.c.) and metoclopramide (30 mg kg^{-1} i.p.); (□——□) piperoxan (5 mg kg^{-1} i.p.); (■——■) M-7 (1 mg kg^{-1} s.c.) and piperoxan (5 mg kg^{-1} i.p.). Values are ± s.e. means. Groups of six rats were used. The basal BP (and HR) for each group was for vehicle, M-7, metoclopramide, M-7 and metoclopramide groups respectively, 230 ± 7 mm Hg (512 ± 4 bts min^{-1}) 227 ± 7 mm Hg (509 ± 12 bts min^{-1}); 243 ± 6 mm Hg (495 ± 9 bts min^{-1}) and for vehicle, M-7, piperoxan, M-7 and piperoxan groups respectively, 212 ± 5 mm Hg (494 ± 9 bts min^{-1}); 231 ± 6 mm Hg (487 ± 2 bts min^{-1}); 235 ± 6 mm Hg (489 ± 4 bts min^{-1}) and 230 ± 6 mm Hg (485 ± 12 bts min^{-1}). Abscissa scale shows time (h). *indicates *P* values <0·05.

Effect of M-7 on stimulation-evoked pressor responses and tachycardia in pithed SHR

Following pithing, the basal blood pressure in rats which received M-7 (1 mg kg^{-1}) was significantly higher (60 ± 8 mm Hg) than that in rats receiving vehicle alone (43 ± 3 mm Hg).

Pretreatment with M-7 (1 mg kg^{-1} s.c.) significantly inhibited stimulation-evoked pressor responses and tachycardia at low (0·25 to $2H_z$), but not high (4 and $8H_z$), frequencies of stimulation (Fig. 2). Angiotensin II (100 ng kg^{-1} i.v.) pressor responses (64 ± 4 mm Hg) in M-7 pretreated rats were not

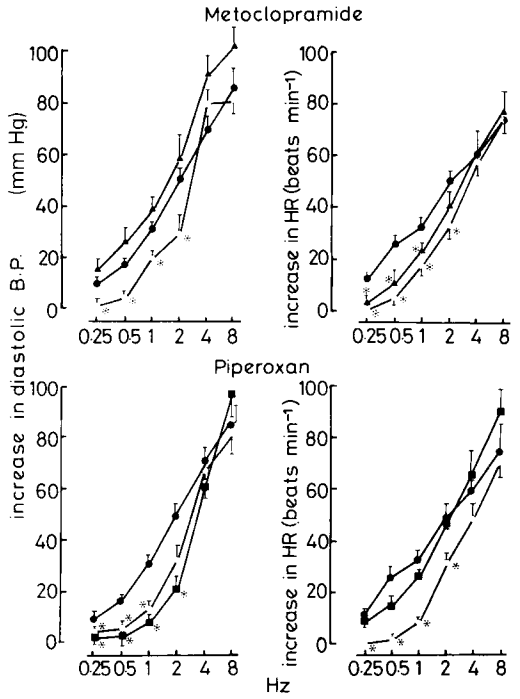

FIG. 2. Effect of treatment 2 h previously with M-7, M-7 and metoclopramide or M-7 and piperoxan on stimulation-evoked pressor responses and tachycardia in pithed SHR. (●——●) combined vehicle control; (○——○) M-7 (1 mg kg^{-1} s.c.); (▲——▲) M-7 (1 mg kg^{-1} s.c.) and metoclopramide (30 mg kg^{-1} i.p.); (■——■) M-7 (1 mg kg^{-1} s.c.) and piperoxan (5 mg kg^{-1} i.p.). Except for the combined vehicle control where n = 12, n = 6 rats per group. Values are means ± s.e. mean. Abscissa scale shows frequency of stimulation (H_z). * indicates P value <0·05.

different from those responses (66 ± 4 mm Hg) in control rats indicating that higher basal blood pressure was not an important factor in accounting for the inhibitory effect of M-7.

Metoclopramide (30 mg kg^{-1} i.p.), but not piperoxan (5 mg kg^{-1} i.p.), prevented the inhibition of stimulation-evoked pressor responses elicited by pretreatment with M-7 (Fig. 2). By contrast, the inhibitory effect of M-7 on stimulation-evoked tachycardia was not significantly reduced by metoclopramide but was significantly attenuated by piperoxan (Fig. 2). Neither metoclopramide nor piperoxan themselves had any effect on stimulation-evoked pressor responses and tachycardia, or on pressor responses to angiotensin II. Pressor responses to injected noradrenaline (200 ng kg^{-1} i.v.) and the high basal blood pressure in rats pretreated with M-7 were reduced to control levels by piperoxan but were unaffected by metoclopramide.

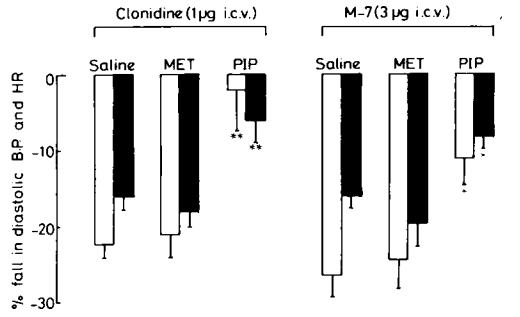

FIG. 3. Effect of i.p. treatment 2 h previously with metoclopramide (MET) (30 mg kg^{-1}) or piperoxan (PIP) (5 mg kg^{-1}) on cardiovascular responses elicited by i.c.v. administration of clonidine or M-7 in anaesthetized rats. Open and solid bars represent maximum % falls in blood pressure and heart rate respectively. n = 7–8 rats per group. * indicates P value <0·05 and ** indicates P value <0·02.

Effect of metoclopramide and piperoxan on responses to i.c.v. administration of clonidine or M-7 in anaesthetized rats

In anaesthetized rats the maximum falls in blood pressure and heart rate elicited by i.c.v. administration of either clonidine (1 μg) or M-7 (3 μg) were unaffected by pretreatment with metoclopramide (30 mg kg^{-1} i.p.) but were significantly reduced by pretreatment with piperoxan (5 mg kg^{-1} i.p.). Neither of the pretreatments affected the basal levels of blood pressure or heart rate when compared with values for saline pretreated animals.

DISCUSSION

The results of this study in SHR provide evidence that the antihypertensive effect of M-7 may be due, in part, to impairment of sympathetic neurotransmission to the vasculature. Both the antihypertensive effect and inhibition of stimulation-evoked pressor responses caused by M-7 appear to be mediated by stimulation of presynaptic dopamine receptors, and not α_2-adrenoceptors, as judged by preferential antagonism of these effects by metoclopramide, but not piperoxan. Further evidence suggesting the involvement of peripheral dopamine receptors in the blood pressure lowering activity of the dopamine agonists lergotrile and pergolide has been provided by others (Yen et al 1979; Lefèvre-Borg & Cavero 1980; Sved & Fernstrom 1980; Hahn & Farrell 1981).

The bradycardia induced by M-7 probably results from activation of presynaptic α_2-adrenoceptors on cardiac sympathetic nerve endings, as judged by preferential antagonism of this effect by piperoxan but not metoclopramide. Moreover the antihypertensive effect of M-7 in conscious SHR cannot be a

consequence of bradycardia alone since (1) heart rate was maximally reduced before the antihypertensive response was established, (2) the bradycardia and inhibition of stimulation-evoked tachycardia produced by M-7 were still present after abolition, by metoclopramide, of the M-7 induced antihypertensive effect and inhibition of stimulation-evoked pressor responses and (3) the antihypertensive effect and inhibition of stimulation-evoked pressor responses produced by M-7 persisted despite reduction of M-7 induced bradycardia and inhibition of stimulation-evoked tachycardia by piperoxan.

Cannon & Hicks (1980) have previously reported that i.c.v. administration of M-7 to anaesthetized rats elicits a hypotensive response and bradycardia which are antagonized by yohimbine (and possibly prazosin), but not by the dopamine antagonists, haloperidol and fluphenazine, indicating an α_2-adrenoceptor-mediated response. Peripheral administration of piperoxan has been shown to antagonize the anti-hypertensive response to clonidine in conscious doca/saline hypertensive rats (Finch et al 1975) indicating that piperoxan readily crosses the blood brain barrier to antagonize stimulation of central α_2-adrenoceptors by clonidine. However, piperoxan did not antagonize the antihypertensive response to peripheral administration of M-7 in conscious SHR in our study indicating that, following peripheral administration, M-7 does not activate central α_2-adrenoceptors to elicit a hypotensive response. Further evidence for a peripheral site in the mode of action of M-7 was provided by the fact that metoclopramide, at the dose used, did not antagonize the hypotensive response and bradycardia to i.c.v. administration of either clonidine or M-7 and that piperoxan reduced the hypotensive response to i.c.v., but not peripheral, administration of M-7. Some evidence exists suggesting that metoclopramide blocks α_2-adrenoceptors in rat isolated vas deferens (Spedding 1980) and displaces, with similar affinity to M-7, [^{3}H]yohimbine in rat cortical slices (D. Howlett, personal communication). However, in our study, metoclopramide, in contrast to piperoxan, had no effect on the pressor responses due to noradrenaline or on the raised blood pressure in pithed rats pretreated with M-7. Antagonism by piperoxan confirms that the pressor responses produced by noradrenaline and by M-7 are due to stimulation of postsynaptic vascular α_2-adrenoceptors (Drew & Whiting 1979; Drew 1980) whilst the lack of effect of metoclopramide on these responses indicates that metoclopramide does not block vascular α_2-adrenoceptors. Despite its vasoconstrictor properties M-7 lowers blood pressure in conscious SHR suggesting that, by the subcutaneous route, stimulation of postsynaptic vascular α_2-adrenoceptors by M-7 does not influence the overall antihypertensive effect of M-7 in conscious SHR. Presumably, in the pithed rat, as opposed to conscious SHR, the effects of stimulation of postsynaptic vascular α_2-adrenoceptors by M-7 become apparent due to removal of sympathetic tone in this model.

These experiments suggest that in SHR the antihypertensive effect of M-7 was in part due to stimulation of presynaptic dopamine receptors located on sympathetic nerve endings in the blood vessels; the bradycardia induced by M-7, probably mediated by stimulation of cardiac presynaptic α_2-adrenoceptors, was independent of the antihypertensive effect of M-7 in conscious SHR.

REFERENCES

Brown, R. A., Brown, R. C., O'Connor, S. E., Solca, A. M. (1979) Br. J. Pharmacol. 67: 420P

Cannon, J. G., Hicks, P. E. (1980) Ibid. 68: 188P

Clapham, J. C., Hamilton, T. C. (1981) Ibid. 73: 278P

Drew, G. M. (1980) Eur. J. Pharmacol. 65: 85–87

Drew, G. M., Whiting, S. B. (1979) Br. J. Pharmacol., 67: 207–215

Finch, L., Buckingham, R. E., Moore, R. A., Bucher, J. J. (1975) J. Pharm. Pharmacol., 27: 181–186

Hahn, R. A., Farrell, S. K. (1981) Life Sci. 28: 2497–2524

Hayden, I. F., Johnson, R. L., Maickel, R. P. (1966) Ibid. 5: 1509–1575

Hicks, P. E., Cannon, J. G. (1979) J. Pharm. Pharmacol. 31: 494–496

Ilhan, M., Long, J. P., Cannon, J. G. (1976) Arch. Int. Pharmacodyn. 222: 70–80

Lefèvre-Borg, F., Cavero, I. (1980) Clin. Sci. 59: 291s–294s

Long, J. P., Heintz, S. G., Cannon, J. G., Kim, J. (1975) J. Pharm. Exp. Ther. 192: 336–342

Spedding, M. (1980) Br. J. Pharmacol. 71: 113–120

Sved, A. F., Fernstrom, J. D. (1980) Life Sci. 27: 349–354

Yen, T. T., Stamm, N. B., Clemens, J. A. (1979) Ibid. 25: 209–216