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The central sympatho-inhibitory effect of 5,6-dihydroxy-2-dimethylaminotetralin (M7) is mediated by α_2-adrenoceptors

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Abstract

M7 (5,6-dihydroxy-2-dimethylaminotetralin) produces in anesthetized rats a hypotensive response previously attributed to peripheral dopaminergic mechanisms. We re-examined the effects of this drug on arterial blood pressure, heart rate and sympathetic nerve activity in anesthetized rats and dogs. M7 (1–100 μg/kg i.v.) produced in the rats transient dose-dependent pressor effects, with bradycardia and sympatho-inhibition, followed by long-lasting dose-dependent hypotension, bradycardia and sympatho-inhibition. The sympatho-inhibitory and hypotensive effects were comparable in baroreceptor-denervated rats and were reversed by idazoxan (0.1 mg/kg i.v.). The sympatho-inhibitory response induced by M7 (1–100 μg/kg) was prevented by treatment with the specific α_2-adrenoceptor antagonist, 2-methoxy-idazoxan (0.03 mg/kg i.v.). This central effect of M7 was not altered by treatment with the α_1-adrenoceptor antagonist, prazosin (0.1 mg/kg i.v.) and was reduced by treatment with the α_2-adrenoceptor antagonists, yohimbine (1 mg/kg i.v.) or idazoxan (0.3 mg/kg i.v.), and the dopaminergic antagonists, haloperidol (0.5 mg/kg i.v.) or sulpiride (3 mg/kg i.v.). Bilateral microinjections of M7 (0.3–3 nmol) into the rostroventral medulla in the rat produced dose-dependent hypotension, bradycardia and sympathetic nerve inhibition which were reversed and prevented by bilateral microinjection of 2-methoxy-idazoxan (1 nmol) into the same sites. Microinjections of 2-methoxy-idazoxan into the rostroventral medulla also inhibited the central effects of M7 at 0.03 mg/kg i.v. In anesthetized dogs, M7 administered into the cisterna magna (1–10 μg/kg) reduced arterial blood pressure, heart rate and sympathetic nerve activity; these effects were reversed by administration of 2-methoxy-idazoxan (0.03 mg/kg i.v.). In conclusion, M7, a rigid catecholamine, produces a potent central sympatho-inhibitory and hypotensive effect by activation of α_2-adrenoceptors.

Keywords: α_2-Adrenoceptor; Sympathetic nerve activity; Blood pressure; Ventrolateral medulla, rostral

1. Introduction

M7 (5,6-dihydroxy-2-dimethylaminotetralin) and its 4,7-dihydroisomer (TL 99), initially described as dopaminergic agonists (Cannon, 1975) are also potent peripheral pre- and postsynaptic α_2-adrenoceptor agonists (Hicks and Cannon, 1979; Drew, 1980). It has been reported that M7 and TL 99 can elicit hypotension and bradycardia in anesthetized and conscious rats (Cannon and Hicks, 1979), as well as in anesthetized cats and dogs (Kitzen et al., 1978). Also it has been claimed that M7 or TL 99 fail to activate central α_2-adrenoceptors to elicit a hypotensive response when the drug is administered intravenously, probably because M7 or TL 99 do not cross the blood-brain barrier (Kitzen et al., 1978; Clapham and Hamilton, 1982a). Two peripheral mechanisms have been proposed to explain the hypotensive and bradycardiac effects of the drug. Firstly, M7 stimulates presynaptic dopamine receptors on sympathetic nerves of the vasculature (Clapham and Hamilton, 1982a,b). Secondly, M7 also stimulates presynaptic α_2-adrenoceptors on cardiac sympathetic nerve endings (Hicks and Cannon, 1979; Clapham and Hamilton, 1982b). However, intracerebroventricular (i.c.v.) administration of M7 induces a dose-related hypotension and bradycardia in anesthetized rats. These cardiovascular responses are antagonized by i.c.v. pretreatment with yohim-

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bine or prazosin (Cannon and Hicks, 1979) or piperoxan (Clapham and Hamilton, 1982b). In addition, intravenous administration of M7 produces a bradycardia which is largely reduced by bilateral vagotomy (Cannon and Hicks, 1979). Excluding the results with prazosin, these data, published in abstract form, are compatible with a possible activation of central α_2-adrenoceptors.

The chemical structures required to reproduce the central sympatho-inhibitory effects of clonidine and clonidine-like substances include imidazoline derivatives, among which isosteric compounds such as B-HT 930, B-HT 920 or xylazine and 'open ring imidazolidines' such as guanabenz or guanfacine. M7 and TL 99, whose structure corresponds to the α and β rotameric forms of dopamine (Cannon, 1975), are semi-rigid dopamine congeners which may be regarded as the active moiety of apomorphine. M7 may be also considered as a rigid catecholamine structurally related to α-methyl-noradrenaline, a product of the metabolic conversion of α-methyldopa by catecholamine-containing neurones. This conversion is necessary for α-methyl-noradrenaline to exert its central sympatho-inhibitory effect through the stimulation of α_2-adrenoceptors.

We decided to re-examine the mechanism and the origin of the reduction (site of action) by M7 of blood pressure and heart rate in anesthetized rats and dogs when the drug is administered intravenously.

2. Materials and methods

2.1. General

Male Sprague-Dawley rats weighing 340–450 g were anesthetized with sodium pentobarbital (50 mg/kg i.p. as the initial dose followed by a continuous infusion at 12 mg/kg per h). The trachea was cannulated and artificial ventilation was provided with a Harvard rodent ventilator at a frequency of 70 cycles/min with a tidal volume of 3.5 ml. Body temperature was maintained at 38°C with a homeothermic blanket. Systemic arterial blood pressure was measured from the right common femoral artery via a Statham P10 EZ pressure transducer connected to a pressure recorder (Gould). Heart rate was measured with a Gould cardiotachometer triggered by the pressure pulse. The femoral vein was cannulated for the i.v. administration of drugs. A 30-min stabilization period was allowed before drug administration. Cumulative i.v. doses of M7 were administered when the maximal hypotensive response was reached, thus with 10- to 15-min intervals.

2.2. Recording of sympathetic nerve activity

Left splanchnic or renal nerve activity was recorded as previously described (Vayssettes-Courchay et al., 1990). The nerve was exposed by retroperitoneal dissection. The nerve was dissected free and placed on a bipolar stainless steel electrode. The nerve signal was amplified (DAM 60, WPI) with the band-pass filter set at 30 Hz and 1 kHz, visualised on an oscilloscope (Tektronix 5.115) and measured with a Gould integrator (μV/s). Absolute values were corrected by subtracting the residual electrical output recorded at the end of the experiment after application of xylocaine 5% on the nerve. Nerve activity, arterial pressure, heart rate and the output of the nerve traffic analyser were displayed on a Gould ES 1000 recorder. Baroreceptor responsiveness of the nerve preparation was tested by i.v. administration of phenylephrine (10 μg/kg i.v.). This dose of phenylephrine produced complete inhibition of sympathetic nerve activity. As the absolute value of the recorded traffic is dependent on the number of active fibers on the recording electrodes, the control value of nerve activity was taken as 100%.

2.3. Vagotomized baroreceptor-denervated rats

The aorta was denervated by section of the vago-sympathetic trunks, the recurrent laryngeal and the superior laryngeal nerves. The carotid sinuses were then denervated by transection of the carotid sinus nerves. In addition, the carotid bifurcations were painted with 10% phenol in ethanol. The success of the sinoaortic denervation procedure was verified from the inhibition of the reflex decrease in renal or splanchnic nerve activity in response to phenylephrine (5 μg/kg i.v.).

2.4. Microinjections in the rat

The rats were placed in a stereotaxic frame. The basioccipital bone was removed, creating a window over the ventral surface of the medulla oblongata. The rostroventral medulla was reached by a ventral approach, 0.5–1 mm rostral to the cranial rootlet of the hypoglossal nerve, 1.7–2.2 lateral to the midline and 0.45–0.8 mm beneath the ventral surface. The microinjections were made from a glass micropipette (tip diameter 10 μm) held in a micromanipulator, using a WPI nanopump (Vayssettes-Courchay et al., 1993). The drugs were dissolved in artificial cerebrospinal fluid: L-glutamate 3 nmol into 15 nl, M7 0.3, 1 and 3 nmol into 40–60 nl and 2-methoxy-idazoxan 1 nmol into 40 nl. In each experiment a microinjection of L-glutamate was performed 30 min before microinjection of M7 or 2-methoxy-idazoxan.

2.5. Intracisternal administration in the dog

The study was performed on 4 mongrel dogs of either sex weighing 15–25 kg, anesthetized with sodium pentobarbital (Sanofi) 30 mg/kg i.v. into the cephalic vein. A needle, Unicath 22G, was inserted in the cephalic vein for infusion of 1 ml/h of sodium pentobarbital 6% in order to maintain a continuous level of anesthesia. The trachea was

cannulated and ventilation was monitored with a Bird markVII respirator. The respiratory frequency was between 11 and 14.5 cycles/min, and the volume between 2.4 and 4.4 l/min with an inspiration pressure of 10 cmH_2O. Arterial P_{CO_2}, P_{O_2} and pH were measured and adjusted: P_{CO_2} between 33 and 38 mmHg, P_{O_2} between 99 and 105 mmHg and pH between 7.33 and 7.36 (Radiometer ABL3). Arterial blood pressure, heart rate and renal nerve activity were recorded and analysed as described above for the rats. M7 was injected in 0.3 ml into the cisterna magna through a metallic cannula (diameter 1.6 mm) inserted into the cisterna magna through the neck muscles.

2.6. Analysis

Arterial blood pressure, heart rate and nerve activity were recorded on magnetic tape (Teac, TX 310) and displayed on a Gould ES 1000 recorder. The data were analyzed with a Compaq 486 computer coupled with a CED 1401 laboratory interface system unit and SPIKE 2 software (CED). Mean blood pressure was expressed in mmHg, heart rate in beats per minute (beats/min). Nerve activity was rectified, integrated, averaged and is given as percentage of the control value, after subtraction of noise. Changes in nerve activity and heart rate and the hypotensive effects were measured versus the basal values after stabilization; the short-lasting hypertensive effect was measured versus the arterial pressure before each dose. The data are expressed as means ± S.E.M. Student's *t*-test for paired and unpaired comparisons was used to assess the significance of the results; one-way analysis of variance (ANOVA) with a complementary Dunnett test was used to assess the significance of the effect when increasing doses were used.

2.7. Drugs

The drugs used in these experiments were: phenylephrine-HCl (Sigma), idazoxan hydrochloride (RBI), yohimbine hydrochloride (Sigma), 2-methoxy-idazoxan free base (RX821002, synthesized by Dr. Cordi, Servier Research Institute), haloperidol (Haldol, Janssen), sulpiride (Sigma-Aldrich), L-glutamate (monosodium L-glutamate), tertatolol (S2395; Servier) and M7 (5,6-dihydroxy-2-dimethylaminotetralin, synthesized by Dr. Regnier, Servier Research Institute). The quantities used refer to the free bases. The doses of the antagonists were chosen for their ability to block α_2-adrenoceptors or dopaminergic receptors. Idazoxan, M7, phenylephrine, haloperidol and tertatolol were dissolved in saline; yohimbine was dissolved in isotonic glucose solution; sulpiride was dissolved in glucose solution with 0.5% acetic acid; 2-methoxy-idazoxan was dissolved in 5% HCl N/10, 94% saline and 1% NaOH N/10 (pH 7.5).

3. Results

3.1. Effects of increasing i.v. doses of M7 on arterial blood pressure, heart rate and renal sympathetic nerve activity in intact anesthetized rats

Intravenous administration of M7 produced a biphasic effect on arterial blood pressure: a transient hypertensive response followed by a prolonged hypotensive phase. The results are illustrated in Fig. 1 ($n = 6$).

M7 administered in cumulative doses (1–100 μg/kg i.v.) produced dose-dependent increases in arterial blood pressure. At the high dose (100 μg/kg i.v.), the mean blood pressure increased by 69 ± 6 mmHg from a basal value of 124 ± 7 mmHg. The duration of this hypertensive phase was also dose dependent and the arterial blood pressure returned to near its control value within 1–3 min.

During the hypertensive responses, the heart rate and

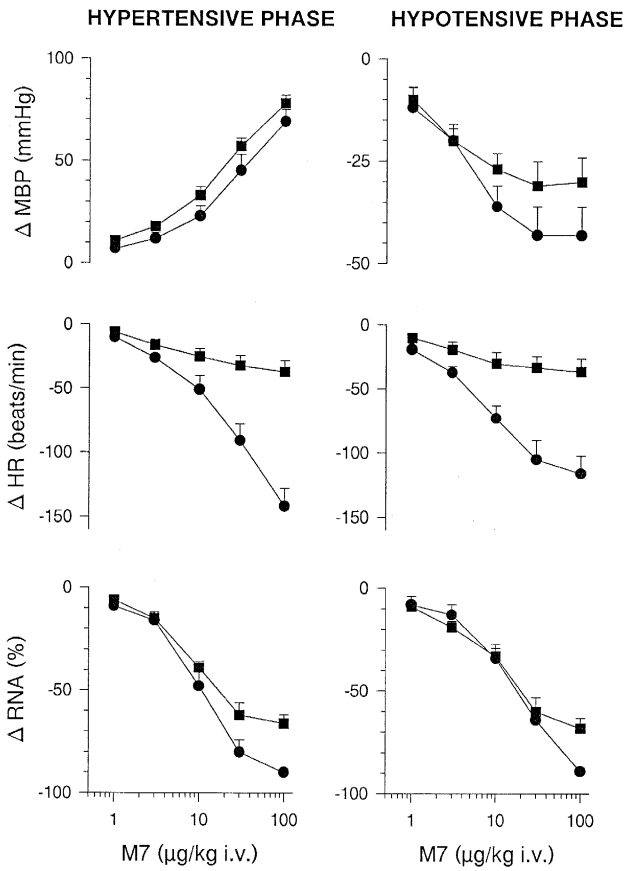

Fig. 1. Effects of M7 on mean blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RNA) in intact (●) and vagotomized baroreceptor-denervated rats (■). The two phases of the response to M7 are shown: hypertensive phase (left) and hypotensive phase (right). Baseline values: BP 124 ± 7 and 123 ± 2 mmHg; HR: 400 ± 18 and 337 ± 12 bpm in intact (6 experiments) and denervated (6 experiments) animals, respectively. The effects of M7 on heart rate were significanltly reduced in baroreceptor-denervated rats (one-way analysis of variance with a complementary Dunnett test).

renal sympathetic nerve activity were markedly reduced by 142 ± 14 beats/min from a basal value of 400 ± 18 beats/min and by $90 \pm 2\%$, respectively.

The second phase was characterized by hypotension, bradycardia and sympatho-inhibition. After cumulative doses of M7 (up to 100 μg/kg i.v.), mean blood pressure decreased by 43 ± 7 mmHg, heart rate was reduced (-116 ± 14 beats/min) and renal sympathetic nerve activity was decreased by $89 \pm 2\%$. These effects were dose related and the results at maximal effect are reported in Fig. 1.

3.2. Effects of M7 on renal sympathetic nerve activity in vagotomized baroreceptor-denervated rats

In order to check the influence of baroreceptor and vagal afferents on the cardiovascular effects of M7, experiments were performed in deafferented animals.

As illustrated in Fig. 1, M7 produced dose-dependent increases in mean arterial blood pressure in denervated rats. For the last dose (100 μg/kg i.v.), mean arterial blood pressure increased by 78 ± 4 mmHg from a basal value of 123 ± 2 mmHg. Heart rate was reduced by -37 ± 9 beats/min; this value was significantly less than that recorded in intact animals (Section 2.1) (-142 ± 14 beats/min). In addition, renal sympathetic nerve activity was rapidly and dose dependently reduced during the hypertensive phase in baroreceptor-denervated animals in contrast to the response elicited by phenylephrine (5–10 μg/kg i.v.) which failed to alter the electrical activity of the nerve despite the hypertension in the denervated rats.

As in intact anesthetized rats, M7 elicited hypotension, bradycardia and sympatho-inhibition about 10–15 min after its injection ($n = 6$). At the highest cumulative dose used (100 μg/kg i.v.), mean blood pressure decreased by -30 ± 6 mmHg from a basal value of 123 ± 2 mmHg, heart rate was reduced by 36 ± 10 beats/min and renal sympathetic nerve activity was inhibited by $-68 \pm 5\%$. The reduction of mean arterial blood pressure and renal sympathetic nerve activity was similar in intact and in vagotomized baroreceptor-denervated rats. In contrast, the heart rate was significantly less reduced in denervated animals.

3.3. Effects of M7 on splanchnic nerve activity in vagotomized baroreceptor-denervated rats

M7, administered in cumulative doses (1–100 μg/kg i.v.) to vagotomized baroreceptor-denervated rats, produced a dose-dependent decrease in splanchnic nerve activity (-3 ± 4, -12 ± 5, -21 ± 8, -40 ± 10 and $-45 \pm 10\%$) which was not significantly different from the decrease in renal nerve activity (5 experiments).

3.4. Effect of dopamine receptor and α-adrenoceptor antagonists

These experiments were performed in bivagotomized baroreceptor-denervated rats. Pretreatment with the dopaminergic antagonists, haloperidol (0.5 mg/kg i.v. 20 min before M7, $n = 8$) or sulpiride (3 mg/kg i.v. 10 min before i.v. M7, $n = 6$), induced decreases in mean blood pressure (90 ± 5 and 109 ± 7 mmHg versus 123 ± 2 mmHg) without changes in heart rate or renal sympathetic nerve activity. Subsequent i.v. administration of cumulative doses of M7 (1–100 μg/kg) elicited dose-dependent hypertensive responses similar to those recorded in intact

Table 1
Effects of M7 in cumulative doses (1–100 μg/kg i.v.) on mean blood pressure (BP) and renal sympathetic nerve activity (RNA) 10–15 min after administration (second phase) in 3 groups of baroreceptor denervated rats: the control group ($n = 6$) and after treatment with the dopaminergic antagonists sulpiride (3 mg/kg i.v., $n = 6$) or haloperidol (0.5 mg/kg i.v., $n = 8$)

		Control	After sulpiride	After haloperidol
Mean blood pressure (mmHg)				
Before		123 ± 2	109 ± 7	90 ± 5
M7:	1	-10 ± 3	2 ± 2	2 ± 4
	3	-20 ± 3	-5 ± 3	0 ± 5
	10	-27 ± 4	-11 ± 4	-4 ± 3
	30	-31 ± 6	-18 ± 3	-9 ± 2
	100 μg/kg i.v.	-30 ± 6	-25 ± 4	-13 ± 3
Renal nerve activity (%)				
Before		100	100	100
M7:	1	-9 ± 5	-8 ± 3	-4 ± 4
	3	-19 ± 3	-15 ± 4	-11 ± 5
	10	-33 ± 4	-28 ± 2	-17 ± 6
	30	-60 ± 7	-56 ± 7	-41 ± 7
	100 μg/kg i.v.	-68 ± 5	-76 ± 8	-72 ± 7

The dose effects of M7 after treatments were not significantly different from the control (one-way analysis of variance).

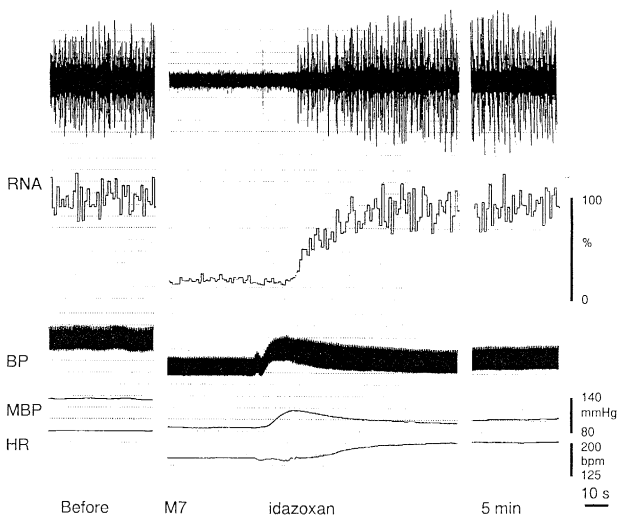

Fig. 2. Effects of idazoxan (0.1 mg/kg i.v.) on the hypotensive, bradycardiac and renal sympatho-inhibitory effects of M7. Idazoxan was administered during the maximal hypotensive phase obtained with the highest dose of M7 (100 μg/kg i.v.) after cumulative administration (see Section 2).

animals (+75 ± 6 mmHg after haloperidol and +77 ± 9 mmHg after sulpiride at the highest dose of M7). In contrast, the hypotensive responses were attenuated, likely due to a lower basal mean arterial blood pressure in rats pretreated with haloperidol or sulpiride (Table 1). After haloperidol or sulpiride, the renal sympatho-inhibitory effect of M7 was slightly reduced but was not different from the effect reported in Section 2.2 for untreated rats (Table 1).

The blocking effects of α_2-adrenoceptor antagonists were evaluated in rats pretreated with idazoxan (0.3 mg/kg i.v. 10 min before M7), yohimbine (1 mg/kg i.v. 10 min before) or 2-methoxy-idazoxan (0.03 mg/kg i.v. 10 min before). Idazoxan (6 experiments) produced a small but non-significant increase in mean blood pressure or heart rate 10 min after administration. No changes were recorded after yohimbine (5 experiments). 2-Methoxy-idazoxan (5 experiments) did not change mean blood pressure but induced increases in heart rate (from 305 ± 13 to 354 ± 16 beats/min) and renal sympathetic nerve activity (+47 ± 5%). After i.v. administration of idazoxan, yohimbine or 2-methoxy-idazoxan, M7 (at cumulative doses; Fig. 2) produced dose-dependent increases in mean blood pressure which were attenuated when compared with the results reported in Section 2.2. The hypotensive effect of M7 was significantly reduced after idazoxan and 2-methoxy-idazoxan, the renal sympatho-inhibitory effect of M7 was reduced and the dose-response curves were shifted to the right after idazoxan, yohimbine and 2-methoxy-idazoxan.

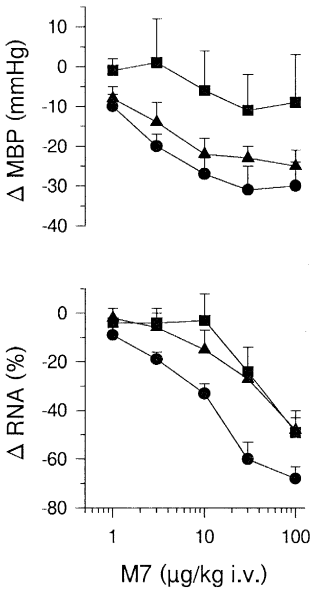

Fig. 3. Effects of 2-methoxy-idazoxan (30 μg/kg i.v., $n = 5$) and yohimbine (1 mg/kg i.v.) on the hypotensive responses and the sympatho-inhibition elicited by M7 in baroreceptor-denervated rats (●) without pretreatment; (■) after 2-methoxy-idazoxan; (▲) after yohimbine. The effect induced by M7 after treatment with 2-methoxy-idazoxan was significantly reduced as compared with the control (one-way analysis of variance with a complementary Dunnett test).

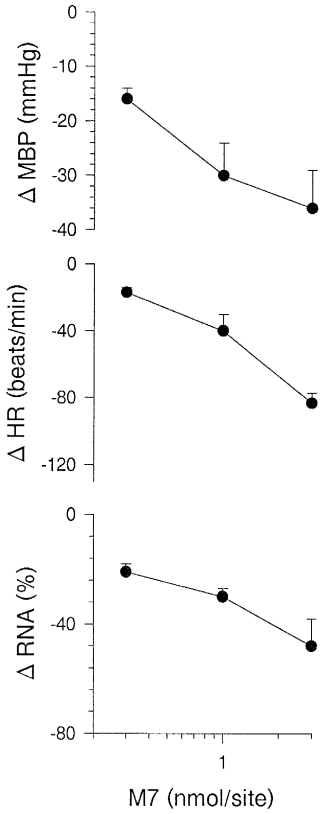

Fig. 4. Effects of bilateral microinjections of M7 into the rostral ventrolateral medulla on mean blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RNA) in anesthetized rats. The results are given at maximal effect. Basal values: 0.3 nmol/site, $n = 4$, 105 ± 13 mmHg, 372 ± 21 beats/min; 1 nmol/site, $n = 6$, 117 ± 6 mmHg, 411 ± 20 beats/min; 3 nmol/site, $n = 4$, 107 ± 15 mmHg, 382 ± 13 beats/min. The dose effects of M7 were significant (one-way analysis of variance with a complementary Dunnett test).

The difference was significant after 2-methoxy-idazoxan; the results are reported in Fig. 3.

After administration of 2-methoxy-idazoxan, M7 at the lowest doses (1 and 3 μg/kg) induced a short-lasting hypotensive effect (−18 ± 4 and −17 ± 5 mmHg) which was reduced (−9 ± 5 and +4 ± 4 mmHg, $n = 4$) in rats pretreated with 2-methoxy-idazoxan and tertatolol 30 μg/kg.

In the intact and bivagotomized baroreceptor-denervated rats reported on in Sections 2.1 and 2.2, the α_2-adrenoceptor antagonist, idazoxan, was administered when the maximal hypotensive, bradycardiac and renal sympatho-inhibitory effects of M7 were reached, about 15 min after the last cumulative dose of M7: 100 μg/kg i.v. As illustrated in Fig. 2, idazoxan (0.1 mg/kg i.v.) produced a rapid reversal of the hypotensive, bradycardiac and sympatho-inhibitory effect of M7, 86 ± 32, 53 ± 9 and 74 ± 13% recovery, respectively, in intact rats; 92 ± 28, 128 ± 44 and 132 ± 18% in baroreceptor-denervated rats.

To determine whether blockade of α_1-adrenoceptors could modify the central sympatho-inhibitory effect of M7, rats were pretreated with prazosin (0.1 mg/kg 10 min

before M7). Intravenous administration of prazosin (0.1 mg/kg i.v.) in vagotomized baroreceptor-denervated rats elicited a rapid fall in mean blood pressure from 98 ± 5 to 66 ± 3 mmHg 10 min after administration (7 experiments). Heart rate and renal sympathetic nerve activity were not significantly altered. Subsequent i.v. administration of M7 elicited dose-dependent hypertensive responses similar to those recorded in untreated rats. Similarly, the reduction in renal sympathetic nerve activity produced by M7 was also not altered by prazosin. At the highest dose used (100 μg/kg i.v. M7) the mean blood pressure increased by 78 ± 4 mmHg in untreated rats and by 67 ± 6 mmHg in prazosin-pretreated animals. Also, the renal nerve activity recorded 10 min after administration of M7 (second phase) was reduced by −68 ± 5% in untreated rats (Section 2.2) and by −79 ± 6% in rats pretreated with prazosin.

3.5. Microinjections of M7 into the rostral ventrolateral medulla

Bilateral microinjections of M7 (0.3–3 nmol in 40 nl) at L-glutamate hypertensive sites into the rostral ventrolateral medulla produced dose-dependent hypotension, bradycardia and inhibition of renal sympathetic nerve activity. At the highest dose used (3 nmol), the mean blood pressure decreased by −36 ± 7 mmHg from a control value of 107 ± 15 mmHg. Heart rate was reduced by 83 ± 6 beats/min from a baseline value of 382 ± 13 beats/min and renal nerve activity was inhibited by 48 ± 10% ($n = 4$), when the maximal effect was reached, about 5–6 min after the microinjections. The results are presented in Fig. 4.

In the experiments described above, the α_2-adrenoceptor antagonist, 2-methoxy-idazoxan, was administered at the same site when the central sympatho-inhibitory effect of M7 was fully established. Bilateral microinjections of 2-methoxy-idazoxan (1 nmol in 40 nl) produced a rapid and complete recovery (2–3 min, data not shown) of mean blood pressure, heart rate and renal sympathetic nerve activity for the three doses of M7 used (0.3, 1 and 3 nmol).

Table 2
Effects of M7 (30 μg/kg i.v.) on mean blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RNA) 15 min after administration (second phase)

	BP (mmHg)	HR (beats/min)	RNA (%)
Group I			
Before ($n = 5$)	132 ± 3	396 ± 17	100
After i.v. M7	91 ± 4 [a]	281 ± 7 [a]	27 ± 4 [a]
Group II			
Before ($n = 5$)	126 ± 7	350 ± 19	114 ± 5
After 2-methoxy-idazoxan into the RVLM	131 ± 9	375 ± 22	100
After i.v. M7	123 ± 10 [b]	330 ± 16 [b]	106 ± 10 [b]

The protective effects of bilateral micro-injections of 2-methoxy-idazoxan (1 nmol/site) into the rostral ventrolateral medulla are illustrated by group II. The results are given at maximal effect. [a] $P < 0.05$ compared with before; [b] $P < 0.05$ compared with group I. RVLM: rostral ventrolateral medulla.

Table 3
Effects of M7 (10 μg/kg) injected into the cisterna magna on mean blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RNA) in anesthetized dogs, and reversal of the effect by 2-methoxy-idazoxan (30 μg/kg i.v.)

	BP (mmHg)	HR (beats/min)	RNA (%)
Before ($n = 4$)	118 ± 9	123 ± 9	100
After M7 into the cisterna magna	99 ± 7 [a]	100 ± 3 [a]	23 ± 6 [a]
After i.v. 2-methoxy-idazoxan	99 ± 8 [a]	122 ± 8	102 ± 19

The results are given at maximal effect. [a] $P < 0.05$ compared with before.

3.6. Blockade of the effects of M7 i.v. by microinjection of 2-methoxy-idazoxan into the rostral ventrolateral medulla

To establish that the rostral ventolateral medulla is the major site for the central sympatho-inhibitory effect of M7, bilateral microinjections of 2-methoxy-idazoxan were administered into the rostral ventrolateral medulla before intravenous administration of M7.

M7 30 μg/kg i.v. in a control group of 5 rats induced a transient hypertensive effect (+26 ± 2 mmHg) associated with bradycardia and a decrease in sympathetic nerve activity (−46 ± 1 beats/min and −75 ± 5%). The long-lasting hypotensive, bradycardiac and sympatho-inhibitory effect is shown in Table 2.

In additional experiments, two bilateral microinjections of 2-methoxy-idazoxan were given into the rostrolateral and mediocaudal parts of the rostroventral medulla. The microinjections of 2-methoxy-idazoxan (1 nmol in 40 nl) did not change either mean blood pressure, heart rate or renal sympathetic activity. Subsequent i.v. administration of M7 (30 μg/kg i.v, 5 experiments) produced a transient hypertensive response (+35 ± 6 mmHg). This hypertensive effect was similar to the hypertension recorded in intact animals. However, the second phase, i.e., hypotension, bradycardia and renal sympatho-inhibition was largely inhibited as compared to that in the control group. The results are reported in Table 2.

3.7. M7 into the cisterna magna in anesthetized dogs

Cumulative doses of M7 (1–10 μg/kg, 4 experiments) injected into the cisterna magna in anesthetized dogs induced a decrease in mean blood pressure, heart rate and renal sympathetic nerve activity. The maximal effects were reached about 10–15 min after administration of the drug. Subsequent i.v. administration of 2-methoxy-idazoxan (30 μg/kg i.v.) produced a rapid recovery in heart rate and sympathetic nerve activity to near the control values without changes in mean blood pressure. The results recorded

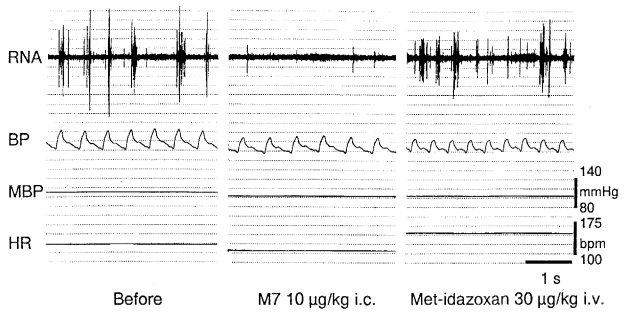

Fig. 5. Effects of 2-methoxy-idazoxan (30 μg/kg i.v.) on the hypotensive, bradycardiac and sympatho-inhibitory effect of M7 administered into the cisterna magna (10 μg/kg) in the anesthetized dog.

10 min after 10 μg/kg M7 are presented in Table 3 and illustrated in Fig. 5.

4. Discussion

The major findings of the present study can be summarized as follows: (1) The cardiovascular effects of M7 (hypotension, bradycardia) originally attributed to activation of peripheral presynaptic dopaminergic receptors of sympathetic nerves of the vasculature and presynaptic cardiac α_2-adrenoceptors, are due to stimulation of central α_2-adrenoceptors. (2) These central effects of M7 are due mainly to activation of α_2-adrenoceptors located in the rostral ventrolateral medulla, which is recognized as the site of action of clonidine and related substances. (3) M7, a rigid cathecholamine which does not contain an imidazoli(di)ne structure, has a clonidine-like action, suggesting that the imidazoli(di)ne moiety is not a prerequisite for central modulation of cardiovascular function.

M7 is a potent central dopamine receptor agonistic agent. Indeed, this substance dose dependently decreases spontaneous motor activity in mice (Costall et al., 1981) and stimulates motor activity following bilateral injections into the nucleus accumbens in conscious rats (Woodruff et al., 1977; Costall et al., 1977). The drug causes turning behavior in rats unilaterally lesioned with 6-hydroxydopamine in the nigro-striatal system (Cheng et al., 1976). Also, M7 induces picking in pigeons (Cheng et al., 1976), inhibition of release of prolactin (Cannon et al., 1972; Rusterholz et al., 1974) and produces emesis in cats and dogs (Burkman, 1973). All these effects are antagonized by haloperidol or spiroperidol.

Peripherally, M7 has been shown to inhibit the tachycardia elicited by cardiac nerve stimulation in the cat and the dog (Long et al., 1975). In these species, this effect is thought to be due to modulation of transmitter release by an action of M7 at presynaptic cardiac dopamine receptors, since haloperidol, chlorpromazine or pimozide were preferential antagonists of these effects. Such presynaptic cardiac dopamine receptors do not seem to be present in rats. In this species, M7 produces inhibition of noradrenaline release only by stimulation of presynaptic α_2-adrenoceptors (Hicks and Cannon, 1979). However, presynaptic dopamine receptors are present on terminals of sympathetic nerves of the vasculature in rats, since M7 inhibits the pressor response evoked by spinal cord stimulation at low frequencies. These effects are prevented by metoclopramide but not by piperoxan (Clapham and Hamilton, 1982b).

M7 is also a potent post- and presynaptic α_2-adrenoceptor agonist. The sympathetic innervation of the canine saphenous vein involves both α_1- and α_2-adrenoceptors. In fact, the contractile responses produced by sympathetic stimulation or by tyramine are antagonized more effectively by a combination of prazosin plus rauwolcine than by either blocker given alone (Flavahan et al., 1984). M7 produces dose-dependent contractile responses of the canine vein that are relatively resistant to prazosin but antagonized by rauwolcine or yohimbine (Shepperson and Langer, 1981; Cavero et al., 1983; Flavahan et al., 1984; Fowler et al., 1984). These data indicate that the contractions of the canine saphenous vein produced by M7 are mainly mediated through the stimulation of α_2-adrenoceptors.

Results of in vivo experiments also indicate that M7 activates postsynaptic α_2-adrenoceptors. In pithed rats, M7 produces dose-dependent increases of arterial blood pressure through its preferential α_2-adrenoceptor agonist activity (Drew, 1980; Cavero et al., 1983). However, some experiments show that, in the pithed rat, a significant participation of postjunctional α_1-adrenoceptors in the pressor response can be demonstrated at high doses (Timmermans et al., 1983). Also, in vitro experiments illustrate that M7 causes contraction of the rabbit pulmonary artery at high concentrations, indicating α_1-agonist activity (Shepperson and Langer, 1981).

Results of various experiments indicate that M7 also inhibits noradrenaline or acetylcholine release from terminals of sympathetic and parasympathetic postganglionic fibers by activation of presynaptic α_2-adrenoceptors. Indeed, M7 inhibits field stimulation-induced contraction of the isolated guinea pig ileum and this effect is antagonized by phentolamine (Maixmer et al., 1981). Also, M7 inhibits the twich response of the rat vas deferens to electrical stimulation in a concentration-dependent manner. This inhibition is identical to that produced by the α_2-adrenoceptor agonist, clonidine. The inhibition produced by both agonists is completely reversed by yohimbine (Shepperson and Langer, 1981). Similarly, in rabbit hypothalamus slices, M7 inhibits in a concentration-dependent manner the electrically evoked release of [^{3}H]norepinephrine without affecting the spontaneous outflow of radioactivity. This effect is antagonized by yohimbine (Garcin and Langer, 1985). The inhibition of noradrenaline release is also apparent in in vivo experiments. Indeed, M7 inhibits the tachycardia elicited by spinal cord stimulation in the rat;

pretreatment with yohimbine causes a progressive parallel shift of the cumulative dose-response curves to the right (Hicks and Cannon, 1979).

As mentioned in Section 1, the hypotensive and bradycardiac action of M7 has been explained by peripheral vascular dopaminergic mechanisms. The present experiments provide evidence that the rigid catecholaminergic compound, M7, structurally related to α-methyl-noradrenaline, produced hypotension, bradycardia and renal nerve inhibition through activation of central α_2-adrenoceptors. A possible inhibitory effect of M7 on sympathetic ganglionic neurotransmission is unlikely because the decrease in preganglionic splanchnic nerve activity was comparable to the decrease in postganglionic renal nerve activity. Intravenous administration of the drug produced dose-dependent decreases in arterial blood pressure, heart rate and sympathetic nerve activity in both intact or deafferented anesthetized rats. Our results indicate that M7, administered intravenously, crosses the blood-brain barrier to produce central sympatho-inhibition. The bradycardia was reduced in vagotomized baroreceptor-denervated rats but the hypotensive and sympatho-inhibitory effect of M7 was similar. Experiments were performed to compare these decreases in blood pressure and sympathetic nerve activity after administration of either dopamine receptor or adrenoceptor antagonists. The α_1-adrenoceptor antagonist, prazosin, failed to alter the sympatho-inhibitory effect of M7. The dopamine receptor antagonists, haloperidol or sulpiride, and the α_2-adrenoceptor antagonists, yohimbine or idazoxan, likely because of their lack of specificity at peripheral receptors, partially prevented the central sympatho-inhibitory and hypotensive effect of M7, making the results confusing. Yohimbine and idazoxan have been shown to centrally increase sympathetic nerve activity in the cat. Such effects have not been reported for the rat and in the present experiments these compounds did not significantly alter either blood pressure or sympathetic activity. In the dog, 2-methoxy-idazoxan administered i.v. reversed the bradycardiac and sympatho-inhibitory effect but not the hypotensive effect of M7 administered i.c. It is not known how 2-methoxy-idazoxan acts at other receptors peripherally in the dog. In the rat, some α_2-adrenoceptor antagonists also have an effect at α_1-adrenoceptors which may alter their action at α_2-adrenoceptors (Vayssettes-Courchay et al., 1996). However, in the present experiments, the α_2-adrenoceptor antagonist, 2-methoxy-idazoxan, prevented the central effects of M7. Taken together with the reversal of the effect of idazoxan, these data are not consistent with the conclusion that the hypotensive effect of M7 was mediated by dopaminergic mechanisms and suggest that central α_2-adrenoceptors are involved in this sympatho-inhibitory and hypotensive effect of M7. These results fit in well with previous findings indicating that i.c.v. administration of M7 produces hypotension and bradycardia which can be prevented by pretreatment with i.c.v. yohimbine or piperoxan (Cannon and Hicks, 1979; Clapham and Hamilton, 1982b). Further experiments now presented, that used microinjections of M7 in the medulla, confirmed this hypothesis and the effects of M7 administered i.c. in the dog provide evidence that the central action of M7 is not confined to a particular species.

In deafferented rats pretreated with the α_2-adrenoceptor antagonist, 2-methoxy-idazoxan, M7 at low doses (1 and 3 μg/kg i.v.) produced hypotensive responses. These effects were not apparent when the β-adrenoceptor antagonist, tertatolol, 30 μg/kg was administered in addition to 2-methoxy-idazoxan. Similarly, M7 has been reported to produce a dose-dependent depressor effect in phentolamine-treated pithed rats whose diastolic pressure was raised by infusion of vasopressin. This depressor effect of M7 is inhibited by the selective β_2-adrenoceptor antagonist, ICI 118,551 (Timmermans et al., 1983), indicating that M7 acts as a stimulant of vascular β_2-adrenoceptors to produce vasodilatation.

Some α_2-adrenoceptors agonists have been suggested to act also at imidazoline-binding sites to produce their central hypotensive effect. A possible action of M7 on these imidazoline binding sites had to be considered. However, our results are not consistent with this suggestion. Indeed 2-methoxy-idazoxan, which has no affinity for imidazoline sites, is more efficient to reverse the effects of M7 than idazoxan, which has affinity for both α_2-adrenoceptor and imidazoline sites.

It is well known that the sites of action of clonidine or related compounds are located mainly in the medulla oblongata and that the rostral ventrolateral medulla, an area containing bulbo-spinal sympatho-excitatory neurons, is a major site for the sympatho-inhibitory effect of the drug. In the present experiments, bilateral microinjections of M7 into this area produced dose-dependent decreases in arterial blood pressure, heart rate and renal sympathetic nerve activity. These effects were reversed when the α_2-adrenoceptor antagonist, 2-methoxy-idazoxan, was injected in this area after M7. In addition, bilateral microinjections of 2-methoxy-idazoxan into the rostral ventrolateral medulla prevented the sympatho-inhibitory effects of M7 administered intravenously. These results indicate that the site of action of M7 in the medulla oblongata is identical with that of clonidine and that the central sympatho-inhibitory effect of M7 is mediated by activation of α_2-adrenoceptors located in the rostral ventrolateral medulla.

In conclusion, the chemical structures required for a central sympatho-inhibitory effect mediated by activation of α_2-adrenoceptors are at this time confined to imidazoli(di)ne derivatives and isosteric compounds. The results of the present study indicate that an imidazoline ring is not a prerequisite for a central sympatho-inhibitory effect and that a compound like M7, structurally related to α-methyl-noradrenaline, which crosses the blood-brain barrier, produces a potent central sympatho-inhibitory effect by activation of α_2-adrenoceptors located in the rostral ventrolateral medulla.

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