

A CENTRALLY MEDIATED PROLONGED HYPOTENSION PRODUCED BY OXOTREMORINE OR PILOCARPINE

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1 Oxotremorine, methyloxotremorine, pilocarpine or arecoline were given intravenously to anaesthetized cats, dogs or rats, and intraperitoneally to conscious normotensive and spontaneously hypertensive rats, pretreated with doses of methylatropine that completely blocked peripheral muscarinic receptors, to ascertain their effects on blood pressure and heart rate.

2 Oxotremorine but not methyloxotremorine produced a prolonged hypotension in cats and dogs but not in rats. Heart rate was not changed. Pilocarpine, although less potent, produced an identical effect, whereas the effect of arecoline was short by comparison. The hypotensive effect of these drugs was reversed by atropine.

3 In dogs, oxotremorine produced a prolonged hypotension with no change in heart rate or cardiac output.

4 A decrease in spontaneous sympathetic nerve activity accompanied the hypotension in cats. Both effects were reversed by atropine but could be reinvoked by large doses of oxotremorine.

5 The oxotremorine-induced hypotension in cats was not altered by decerebration but was abolished by high cervical spinal section.

6 The results indicate that the prolonged hypotension elicited by oxotremorine is mediated by an action at muscarinic receptors in the brain stem resulting in a decrease in sympathetic nerve activity and peripheral resistance but not heart rate or cardiac output.

Introduction

Oxotremorine was first reported by Cho, Haslett & Jenden (1962) to be a muscarinic agent equal in potency to acetylcholine but completely devoid of nicotinic activity. These authors found that a large intravenous dose of oxotremorine (0.05 $\mu\text{mol/kg}$), produced a prolonged hypotension and bradycardia in anaesthetized cats that was completely reversed by methylatropine, a quaternary derivative of atropine that does not readily penetrate the brain (Herz, Teschemacher, Hofstetter & Kurz, 1965). They attributed the hypotension, therefore, solely to an action of oxotremorine on postganglionic cholinergic receptors. M.J. Hosko observed, during an investigation of the central pharmacology of oxotremorine, that it produced a transient hypotension without a bradycardia in anaesthetized cats pretreated with methylatropine, suggesting an action other than on postganglionic

cholinergic receptors (unpublished results). The present experiments describe a prolonged hypotensive action of oxotremorine and pilocarpine that is not antagonized by methylatropine.

Methods

Blood pressure and heart rate

Mongrel cats (2.5 to 3.8 kg) and dogs (9.4 to 18.3 kg) of either sex were anaesthetized with sodium pentobarbitone (32 mg/kg, i.v.). Rats were anaesthetized with chloralose (60 mg/kg, i.p.) and urethane (600 mg/kg, i.p.). Spontaneously hypertensive rats refer to the Kyoto Wistar strain of Okamoto & Aoki (1963). Normotensive rats were also of the Wistar strain. Blood pressure was recorded with an arterial pressure transducer (Statham, P23AC) connected to a polyethylene cannula inserted into the abdominal aorta

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through the femoral artery. Heart rate was recorded with a tachograph preamplifier (Grass Instruments, 7P4D) triggered from the electrocardiogram.

Spontaneous sympathetic nerve activity

Cats weighing 2.6 to 2.9 kg were anaesthetized with urethane (250 mg/kg, i.v.) and chloralose (50 mg/kg, i.v.). Spontaneous sympathetic nerve activity was recorded from the lesser splanchnic nerve, with a bipolar recording electrode and a low level preamplifier (Tetronix, FM122). Nerve activity was monitored on an oscilloscope (Tetronix, Type 561) and recorded with a kymograph camera (Grass Instruments, C4).

Decerebrate cats

Six cats of either sex weighing between 2.2 and 3.4 kg were anaesthetized with chloralose (55 mg/kg, i.v.). The dorsal surface of the midbrain was exposed and decerebration was carried out by sectioning the brain between the superior and the inferior colliculi. These cats were artificially respired with a positive pressure respirator.

Spinal cats

Five cats of either sex weighing between 2.1 and 3.0 kg were anaesthetized with ether. Their spinal cords were transected at the level of the first cervical vertebra, and the cats were artificially respired with a positive pressure respirator. The cats were allowed sufficient time to recover from the ether anaesthesia before drug administration.

Cardiac output

Six dogs of either sex weighing between 9.4 and 18.2 kg were anaesthetized with sodium pentobarbitone (32 mg/kg, i.v.) and artificially respired with a positive pressure respirator. The heart was exposed through a midline thoracic incision and supported by a pericardial cradle. An electromagnetic flow probe was placed around the root of the ascending aorta. Cardiac output less coronary blood flow was recorded as blood flow in the ascending aorta, by means of a Biotronex BL610 flowmeter.

Drugs

All drugs were dissolved in 0.9% w/v NaCl solution (saline) and administered intravenously via a polyethylene cannula inserted into the inferior vena cava via the femoral vein. In experiments where methylatropine pretreatment was required, a dose of 3.5 $\mu\text{mol/kg}$ was given and peripheral muscarinic receptor blockade was deemed adequate when a dose of 10 $\mu\text{mol/kg}$

of methacholine did not produce a change in blood pressure.

Drugs employed were methacholine chloride, oxotremorine oxalate, oxotremorine methiodide, atropine methylnitrate, pilocarpine hydrochloride, arecoline hydrochloride and atropine sulphate.

Statistics

Statistical analysis of the data was performed according to Snedecor & Cochran (1967). Comparisons of paired samples were made by the *t* test. Significance was taken as $P < 0.05$.

Results

Cardiovascular effects of oxotremorine, arecoline and pilocarpine in cats

Cardiovascular responses produced in anaesthetized, vagotomized, non-pretreated cats by intravenous injections of methacholine or oxotremorine are illustrated in Figure 1. A typical brief vasodrepressor response was observed following both 0.01 and 0.05 $\mu\text{mol/kg}$ doses of these drugs. However, after the 0.05 $\mu\text{mol/kg}$ dose of oxotremorine, the initial vasodrepressor response was followed by a prolonged hypotension. A prolonged hypotension was also observed with this dose of oxotremorine after the administration of methylatropine, but it was no longer associated with a bradycardia. By contrast, both the hypotension and tachycardia elicited by methacholine were completely inhibited by methylatropine.

Increasing the dose of oxotremorine in increments from 0.05 to 0.4 $\mu\text{mol/kg}$ resulted in a progressively greater hypotension in six cats pretreated with methylatropine, as is shown in Figure 2. The hypotension following each dose of oxotremorine was sustained for the entire 60 min interval between doses. Atropine (0.4 $\mu\text{mol/kg}$) administered 60 min after the last dose of oxotremorine completely reversed the hypotension within 15 min.

As had been observed with oxotremorine, both pilocarpine (1.2 to 4.8 $\mu\text{mol/kg}$) and arecoline (0.2 to 0.6 $\mu\text{mol/kg}$) decreased mean blood pressure in cats pretreated with methylatropine (3.5 $\mu\text{mol/kg}$) (Table 1). However, the hypotensive response produced by arecoline was less intense and short lasting (2 to 5 min). In addition, increasing the dose of arecoline to 1.9 $\mu\text{mol/kg}$, in two cats did not produce any additional blood pressure decrease, suggesting a much weaker hypotensive activity compared to oxotremorine. On the other hand, the hypotensive response produced by pilocarpine was long lasting (> 60 min) and qualitatively similar to that produced by oxotremorine. The hypotensive response produced by both

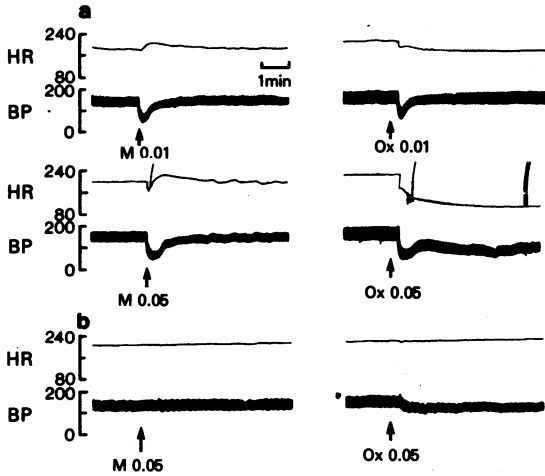


Figure 1 Effects of methacholine (M) and oxotremorine (Ox) on heart rate and blood pressure in an anaesthetized cat before (a) and after (b) an intravenous injection of methylatropine. In each panel the upper recording is heart rate (HR) in beats/min; and the lower panel is blood pressure (BP) in mmHg. Drugs were given by intravenous injection. The drug injected as well as the dose ($\mu\text{mol/kg}$) is indicated at each arrow.

drugs was reversed by atropine (0.3 to 0.8 $\mu\text{mol/kg}$, i.v.).

Cardiovascular effects of oxotremorine in dogs and rats

The fall in blood pressure produced by oxotremorine following methylatropine pretreatment could be

Table 1 Comparison of the hypotensive responses produced by pilocarpine, arecoline and oxotremorine in cats pretreated with methylatropine (3.5 $\mu\text{mol/kg}$, i.v.)

Drug	Dose ($\mu\text{mol/kg}$, i.v.)	n	Mean blood pressure (mmHg)	
			Control	Response
Pilocarpine	4.80	5	125 \pm 13	-18 \pm 4
Arecoline	0.06	6	145 \pm 10	-2 \pm 2
	0.20		147 \pm 8	-7 \pm 1*
	0.60		148 \pm 10	-11 \pm 1*
Oxotremorine	0.05	6	132 \pm 7	-11 \pm 3
	0.10		121 \pm 9	-15 \pm 3

Values are mean \pm s.e. mean. *The peak response to arecoline occurred approximately 2 min after administration with complete recovery within 5 min. By contrast, the hypotensive effects of both pilocarpine and oxotremorine lasted longer than the 60 min interval between doses.

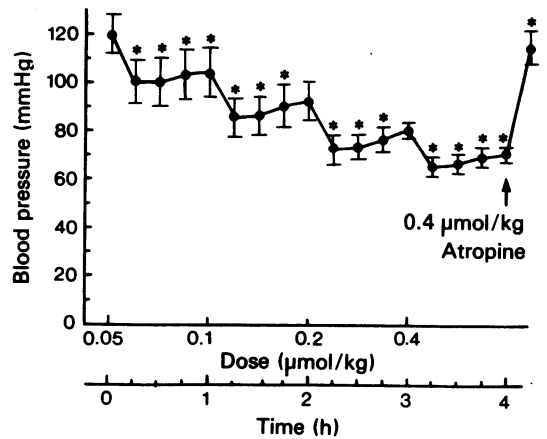


Figure 2 Prolonged hypotension produced by the sequential administration of increasing doses of oxotremorine (i.v.) in 6 anaesthetized cats pretreated with methylatropine (3.5 $\mu\text{mol/kg}$, i.v.). Each point represents average mean blood pressure; vertical bars indicate s.e. means. Asterisk indicates a significant ($P < 0.05$) change from the respective baseline just before each dose of drug. Note that both doses of oxotremorine and elapsed time following the initial administration of oxotremorine are represented on the abscissae.

demonstrated in dogs (Table 2) but not in rats. In dogs, the fall in blood pressure was not accompanied by a significant change in either heart rate or cardiac output (Table 2) and can be ascribed to a decrease in total peripheral resistance (Table 2). This effect of oxotremorine on blood pressure and total peripheral resistance was reversed following a single (0.44 $\mu\text{mol/kg}$) dose of atropine. In two anaesthetized normotensive rats pretreated with methylatropine, no fall in blood pressure was noted following intravenous doses of oxotremorine ranging from 0.05 to 5 $\mu\text{mol/kg}$. Instead, a temporary rise in blood pressure (10 to 43 mmHg) was observed after doses of 0.15 and 5 $\mu\text{mol/kg}$. In conscious normotensive rats ($n = 6$) or spontaneously hypertensive rats ($n = 6$) pretreated with methylatropine, oxotremorine given intraperitoneally in doses of 0.05, 0.15, 0.50 and 1.5 $\mu\text{mol/kg}$, did not change blood pressure, although tremors were observed at the high dose.

Oxotremorine and methyloxotremorine

The administration of 0.5 $\mu\text{mol/kg}$ of methyloxotremorine, a quaternary derivative of oxotremorine, did not have any effect on blood pressure in 6 anaesthetized vagotomized cats pretreated with methylatropine (3.5 $\mu\text{mol/kg}$) as shown in Figure 3. Larger doses of methyloxotremorine, namely 1.0 and 2.0 $\mu\text{mol/kg}$, given to two additional cats also did not affect blood

pressure. In contrast, oxotremorine (0.05 to 0.1 $\mu\text{mol/kg}$) produced the expected hypotensive response in these experiments (Figure 3). The oxotremorine-induced hypotension was not greatly influenced by a supplemental 1.8 $\mu\text{mol/kg}$ dose of methylatropine but was completely reversed by a 0.18 $\mu\text{mol/kg}$ dose of atropine.

Sympathetic nerve activity

To ascertain if the hypotensive effect of oxotremorine was associated with a reduction in sympathetic activity, splanchnic sympathetic nerve activity and blood pressure were monitored concomitantly, in three cats. Sympathetic nerve activity decreased concurrently with mean blood pressure following the administration of oxotremorine after pretreatment with methylatropine (3.5 $\mu\text{mol/kg}$, i.v.) as illustrated by the experiment shown in Figure 4. Both sympathetic nerve activity and mean blood pressure decreased progressively following the sequential administration of 0.05 and 0.15 $\mu\text{mol/kg}$ doses of oxotremorine, 13 min apart. The decrease in mean blood pressure was 48 mmHg following the cumulative dose of 0.2 $\mu\text{mol/kg}$ of oxotremorine. Both the fall in blood pressure and the reduction in spontaneous sympathetic nerve activity produced by oxotremorine were progressively reversed by the administration of three individual 0.17 $\mu\text{mol/kg}$ doses of atropine, 10 min apart. The reversal of the depressed sympathetic nerve activity was complete after 0.34 $\mu\text{mol/kg}$ of atropine. However, mean blood pressure was still 23 mmHg below baseline, representing a 48% reversal. An additional 0.17 $\mu\text{mol/kg}$ dose of atropine brought about an additional 20% reversion in the blood pressure without any detectable change in sympathetic nerve activity; hence, after a cumulative dose of atropine amounting to 0.51 $\mu\text{mol/kg}$, mean blood pressure had been reversed to 18 mmHg below baseline representing a

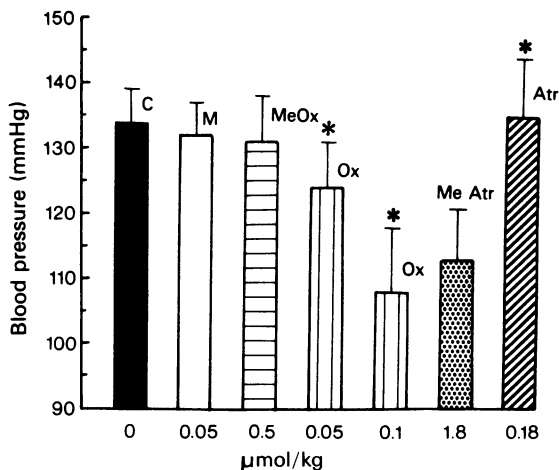


Figure 3 A comparison of the blood pressure decrease from control (C) elicited by methacholine (M), methyl-oxotremorine (MeOx) and oxotremorine (Ox) in 6 anaesthetized cats pretreated with methylatropine (3.5 $\mu\text{mol/kg}$, i.v.) and the reversal of the oxotremorine-induced hypotension by atropine (Atr) but not by methylatropine (MeAtr). Each drug was given by intravenous injection. Asterisk indicates a significant ($P < 0.05$) change in blood pressure from respective baseline value. Each column represents average mean blood pressure of 6 experiments, and the vertical lines represent the s.e. means.

68% reversal. In an attempt to override the atropine antagonism, 3 additional doses of oxotremorine (0.15, 0.45 and 0.60 $\mu\text{mol/kg}$) were given, 12 min apart. The initial 0.15 $\mu\text{mol/kg}$ dose of oxotremorine, at this point, was without effect (Figure 4); however, the subsequent 0.45 and 0.60 $\mu\text{mol/kg}$ of oxotremorine again reduced both blood pressure and sympathetic nerve activity.

Table 2 Cardiovascular effects of oxotremorine in 6 vagotomized dogs pretreated with methylatropine (3.5 $\mu\text{mol/kg}$)

Dose ($\mu\text{mol/kg}$ i.v.)	Mean blood pressure (mmHg)		Heart rate (beats/min)		Cardiac output (ml/min)		Total peripheral resistance (dyne $\text{s}^{-1} \text{cm}^{-5}$)	
	Control	% Δ	Control	% Δ	Control	% Δ	Control	% Δ
0.02	116 \pm 5	-7 \pm 1*	149 \pm 2	+1 \pm 1	1,093 \pm 123	+1 \pm 4	8,848 \pm 753	-6 \pm 4
0.05	107 \pm 7	-18 \pm 4*	147 \pm 5	+1 \pm 2	1,103 \pm 155	+2 \pm 4	8,357 \pm 993	-19 \pm 3*
0.15	96 \pm 8	-25 \pm 4*	150 \pm 2	+1 \pm 3	1,017 \pm 121	-2 \pm 3	7,949 \pm 702	-23 \pm 6*
0.30	85 \pm 9	-23 \pm 4*	146 \pm 4	-2 \pm 2	927 \pm 104	+3 \pm 8	7,448 \pm 488	-24 \pm 7*

Values are mean \pm s.e. mean.
Significant change: * $P < 0.05$.

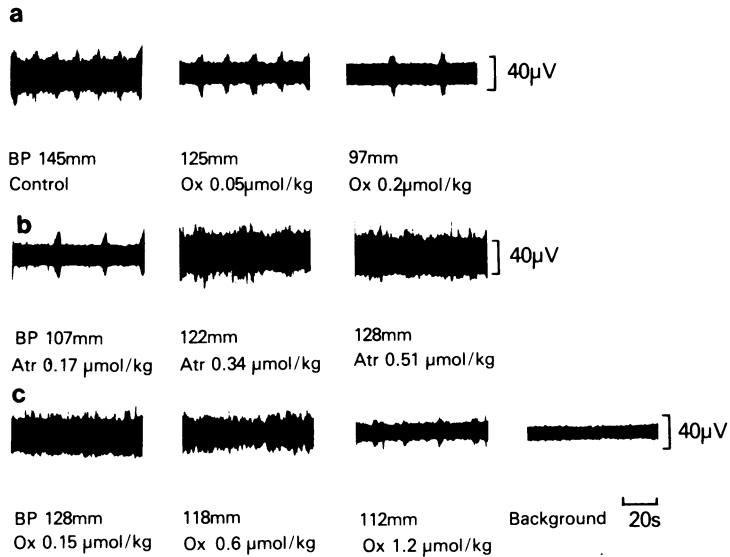


Figure 4 Concomitant decreases from control in sympathetic nerve activity and mean blood pressure induced by oxotremorine (Ox) administration in an anaesthetized cat pretreated with methylatropine (3.5 μmol/kg, i.v.) (a). The reversal of these effects of oxotremorine by the administration of atropine (Atr) is shown in (b), and their reinduction by larger doses of oxotremorine in (c). Panels were taken at the point of maximum effect of each dose of drug. Drugs were given by intravenous injection. The mean blood pressure (BP, mmHg) as well as the drug and the cumulative dose injected are indicated below each panel.

Decerebrate and spinal cats

Mean blood pressure decreased maximally following a 0.05 μmol/kg dose of oxotremorine in 6 decerebrate cats pretreated with methylatropine (Table 3). This effect was readily and completely reversed by a single 0.44 μmol/kg dose of atropine. By contrast, in 5 spinal cats pretreated with methylatropine, mean blood pressure did not change following doses of oxotremorine as large as 0.20 μmol/kg (Table 3).

Discussion

The hypotension elicited by doses of oxotremorine 0.05 μmol/kg and larger consisted of an immediate, brief vasodepressor response and a secondary long lasting hypotension. The immediate vasodepressor response resulted from an action on peripheral muscarinic receptors since it is effectively inhibited by methylatropine (Cho *et al.*, 1962). The secondary, long lasting hypotension seemed to result from an action in

Table 3 Hypotension elicited by oxotremorine in decerebrate cats but not in spinal cats and its reversal by atropine^a

Cat	Drug	Dose (μmol/kg, i.v.)	Mean blood pressure (mmHg)	
			Control	Response
Decerebrate (6)	Oxotremorine	0.05	80 ± 8	-12 ± 1**
		0.10	69 ± 8	+1 ± 1
		0.20	68 ± 8	+4 ± 2
	Atropine	0.44	74 ± 9	+12 ± 1**
Spinal (5)	Oxotremorine	0.05	57 ± 5	+2 ± 4
		0.10	61 ± 4	-2 ± 2
		0.20	59 ± 4	+1 ± 1
	Atropine	0.44	59 ± 4	+4 ± 3

^a All cats were pretreated with methylatropine (3.5 μmol/kg, i.v.). Values are mean ± s.e. mean; *n* is given in parentheses. Significant change ***P* < 0.001.

the central nervous system since it was not prevented by methylatropine, a muscarinic receptor blocking agent that does not readily penetrate the brain (Herz *et al.*, 1965), but it was readily reversed by atropine. This was substantiated when a similar hypotension was not observed with methyloxotremorine, a potent quaternary analogue of oxotremorine that also does not readily penetrate into the central nervous system (Hanin, Jenden & Cho, 1966).

The persistence of the hypotensive action of oxotremorine following decerebration and its absence in spinal cats suggests a site of action in the brain stem. This is consistent with the observations of Guertzenstein (1973) and Edery & Guertzenstein (1974) that carbachol decreased blood pressure when applied locally to a discrete area on the ventral surface of the brain stem.

The lack of effect of oxotremorine in the spinal cat, a preparation with a low degree of sympathetic tone, suggests the importance of the sympathetic nervous system in the hypotensive action of oxotremorine. Schmitt, Schmitt & Fenard (1972) reported decreases in both blood pressure and sympathetic nerve activity in cats after oxotremorine, but these 2 actions did not appear to be associated. The hypotension was reversed by methylatropine suggesting a peripheral action, whereas the decreased spontaneous sympathetic nerve activity was reversed by atropine but not methylatropine suggesting a central action. The concomitant decrease in both blood pressure and spontaneous sympathetic nerve activity in the present experiments following methylatropine and their reversal by atropine suggest both effects result from an action of oxotremorine in the brain stem. In atropinized animals, the re-establishment of both effects by large doses of oxotremorine suggests a central muscarinic mechanism that may be competitive.

The importance of such a system in blood pressure control is not known. However, the lack of effect of atropine alone on blood pressure suggests that the inhibitory system stimulated by oxotremorine is not tonically active.

It is interesting that both clonidine (see van Zwieten, 1975) and oxotremorine produce identical effects on blood pressure and sympathetic nerve activity by actions at different receptors but do not affect heart rate in the same way; the bradycardia reported for clonidine (Magnus & Long, 1968) was not observed with oxotremorine suggesting an action at different neurones in separate pathways within the brain. Clonidine has also been reported to decrease cardiac output (Hoefke & Kobinger, 1966) but oxo-

tremorine does not. The hypotensive action of oxotremorine appears to result solely from a decrease in peripheral resistance.

The hypotensive activity of oxotremorine was not unique to this molecule but could be elicited by pilocarpine as well. The weak central hypotensive activity of arecoline is in accordance with the relatively short central action of this drug (Haubrich & Reid, 1972). Thus, the activities of oxotremorine in reducing blood pressure and sympathetic activity seem characteristic of drugs with muscarinic properties.

The lack of a hypotensive effect with oxotremorine in methylatropine pretreated rats is consistent with the observations of Brezenoff & Jenden (1969) and Brezenoff & Wirecki (1970) that the transient hypotension following micro-injections of oxotremorine into the posterior hypothalamus of rats was prevented by systemically administered methylatropine. Likewise, the rise in blood pressure observed in anaesthetized rats is consistent with the observations of Walker & Weetman (1970). The lack of effect of oxotremorine on blood pressure in normotensive rats or spontaneously hypertensive rats that were conscious suggests an influence of anaesthesia on the oxotremorine response. The production of a pressor rather than a hypotensive response in conscious dogs by intracerebroventricular injections of cholinomimetic drugs as reported by Lang & Rush (1973) is in agreement with this. Thus, rats respond differently to oxotremorine from cats or dogs, and anaesthesia seems to influence the response.

It was concluded that oxotremorine and pilocarpine can produce a prolonged hypotension in cats and dogs but not in rats following methylatropine pretreatment. The doses required for this action are larger than those producing vasodilatation by an action on peripheral muscarinic receptors in the non-pretreated animal. The prolonged hypotensive action of these drugs is mediated by an action in the brain stem resulting in a decrease in spontaneous sympathetic nerve activity and peripheral resistance. Cardiac output and heart rate are not involved in the central hypotensive action of oxotremorine, setting this action apart from that of clonidine.

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