Br. J. Pharmac. (1970), **39**, 490–500.

Oxotremorine-induced hypertension in the anaesthetized rat

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Summary

1. Oxotremorine exerts an action on peripheral cholinoceptors in the anaesthetized rat. After the blockade of peripheral cholinoceptors with the quaternary atropine derivative, atropine methylbromide, oxotremorine produced a rise in arterial blood pressure and tachycardia.

2. The rise in arterial pressure and tachycardia was absent in rats that had been pithed or had undergone high spinal cord transection.

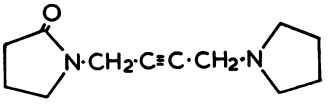
3. A non-quaternary atropine derivative antagonized these cardiovascular effects.

4. Drugs which inhibit activity of the sympathetic nervous system antagonized the oxotremorine-induced hypertension and tachycardia.

5. It is concluded that the rise in arterial pressure and tachycardia seen in the anaesthetized rat treated with a quaternary derivative of atropine is due to an action of oxotremorine on the central nervous system at a supraspinal level, resulting in an increase in activity of the efferent sympathetic nervous system.

Introduction

Oxotremorine [1-(2-oxopyrrolidino)-4-pyrrolidino-2-butyne] (I) is a substance with powerful peripheral muscarinic properties which penetrates into the central nervous system and produces tremor in mice and rats (Haslett & Jenden, 1961).



(I)

We have found that the administration of oxotremorine causes hypertension and tachycardia in anaesthetized rats in which the peripheral muscarinic actions are inhibited by a quaternary atropine derivative.

This paper describes these experiments and attempts to analyse the mechanism by which oxotremorine produces this effect. A preliminary account of the work was given to the British Pharmacological Society in January 1966.

Methods

Albino rats of either sex were anaesthetized by intraperitoneal or in some cases intraperitoneal and subcutaneous administration of a 25% w/v solution of urethane

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in water. In most experiments the dose was 1·8 g/kg. The trachea was cannulated with a short glass or polythene tube. Intravenous injections were made through a fine polythene tube tied into the left jugular vein. A polythene tube, filled with 200 u./ml of heparin in saline (0·9% w/v sodium chloride in glass distilled water) was tied into the right carotid artery and connected to a Consolidated Electrodynamics pressure transducer. The amplified output was displayed on a Devices M4 recorder. The electrocardiogram was detected using needle electrodes placed under the skin in the lead II position, but was not recorded on the polygraph. When monitored, the heart rate was recorded with a Neilson (direct reading) or Devices (indirectly reading) ratemeter. With either system the signal was derived from the electrocardiogram or the arterial blood pressure output.

In some experiments the spinal cord was transected between the first and second cervical vertebrae while the arterial blood pressure and heart rate were being recorded. The rats were in the prone position and the spinal cord was exposed. Transection was carried out with a thermo-cautery. Positive pressure ventilation of the lungs was commenced immediately after the lesion had been made. A miniature "Ideal" pump was adjusted to deliver room air in the manner described by Kleinman & Radford (1964).

Drugs were dissolved in saline. Care was taken to ensure that the maximum volume of drug solution and saline follower injected intravenously at one time was 0·2 ml. In some experiments, drugs were administered on 1 or more days before the experiment in the anaesthetized animal was commenced. All doses refer to the salts mentioned below.

Drugs used

Atropine methylbromide (McFarlan Smith Ltd.); atropine sulphate (British Drug Houses Ltd.); dimethylphenylpiperazinium iodide (Aldrich Chemical Co. Inc.); guanethidine sulphate (Ciba); phentolamine (Ciba); (−)-noradrenaline bitartrate (Koch-Light Laboratories Ltd.); reserpine (Ciba); α-methylparatyrosine (May & Baker Ltd.); α-methyldopa (Aldomet) (Merck, Sharp & Dohme); α-methylmetatyrosine (Koch-Light Laboratories); pentolinium tartrate (May & Baker Ltd.); oxotremorine was synthesized in May & Baker Research Laboratories. It was weighed as the free base, dissolved in 10% w/v hydrochloric acid and stored at 4° C until required.

Results

Cardiovascular effects

The administration of oxotremorine (4–8 μg/kg intravenously) into rats anaesthetized with urethane resulted in a typical transient fall in arterial blood pressure and bradycardia (Fig. 1). These effects were immediately followed by a sustained but small increase in heart rate and slight rise in arterial pressure. The initial hypotension and bradycardia produced by oxotremorine were completely prevented by administration of atropine methyl bromide (1 mg/kg given intravenously), but the subsequent increases in heart rate and arterial pressure were unchanged. Higher doses of oxotremorine in rats which had received atropine methyl bromide resulted in an abrupt and prolonged rise in arterial pressure usually accompanied by an increase in heart rate.

In all subsequent experiments to be described here, a single dose of atropine methyl bromide (0·5–1·0 mg/kg intravenously) was administered early in the experiment, usually 5–20 min before giving the initial dose of oxotremorine.

The rise in arterial pressure produced by oxotremorine was of long duration, a period of 30–60 min being required for the arterial pressure to return to normal when moderate doses of oxotremorine were used (40–80 μg/kg). Subsequent administrations of oxotremorine produced rises in arterial pressure comparable to the initial effect. In three experiments, in atropine methyl bromide treated rats to which 40 μg oxotremorine was administered at 45 min intervals, there was no significant tachyphylaxis to the pressor response.

Figure 2 shows the magnitude of the pressor response produced by different doses of oxotremorine. In this series of experiments, a single administration of oxotremorine was made in each animal 5 min after it had received 0·5 mg/kg atropine methyl bromide. In most subsequent experiments a dose of 80 μg/kg oxotremorine was used because this resulted in a large but submaximal response which was highly reproducible from animal to animal. The size of the pressor response to oxotremorine 80 μg/kg was independent of the resting blood pressures of individual rats when this fell between 65 and 85 mmHg (1 mmHg≡1·333 mbar).

Contribution of the adrenals

In a series of experiments, the result of isolating the adrenal glands from the systemic circulation on the size of oxotremorine hypertension was observed. Four sham operated rats exhibited a rise in arterial pressure of 49·5 ± 3·25 S.E. when 80 μg/kg oxotremorine was administered, whereas rats with the adrenals tied out of the circulation gave a 42 ± 8·2 S.E. mmHg rise in arterial pressure. These values

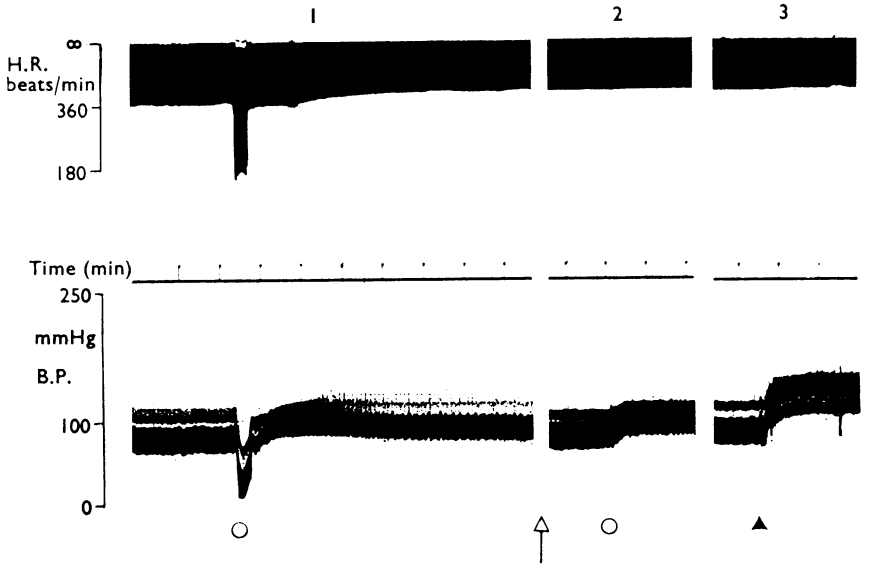

FIG. 1. Effects of oxotremorine on arterial blood pressure (B.P.) and heart rate (H.R.) of an anaesthetized 200 g rat before and after administration of atropine methyl bromide. ○, Oxotremorine 44 μg/kg; ▲, oxotremorine 40 μg/kg; ↑, atropine methyl bromide 1 mg/kg was administered between panels 1 and 2.

are not significantly different at the 5% probability level. Heart rate was not recorded in this series of experiments.

Effect of vagotomy

In two experiments, the rises in arterial pressure following oxotremorine (80 μg/kg) were compared before and after bilateral vagotomy. Section of the vagi did not alter the size of the hypertensive response.

Site of action of oxotremorine

Control responses to oxotremorine and noradrenaline (0·5 μg/kg) were obtained in four rats. The brains and spinal cords were then destroyed by pithing. None of these animals exhibited a hypertensive effect when oxotremorine was subsequently administered, but the response to noradrenaline was unaffected (Fig. 3).

In another series of six experiments, the spinal cord was sectioned between C_1 and C_2 during the hypertensive response to oxotremorine. There was an immediate fall in arterial pressure and heart rate to the original control values. Subsequent doses of oxotremorine were without effect although the animals responded normally to noradrenaline (Fig. 4).

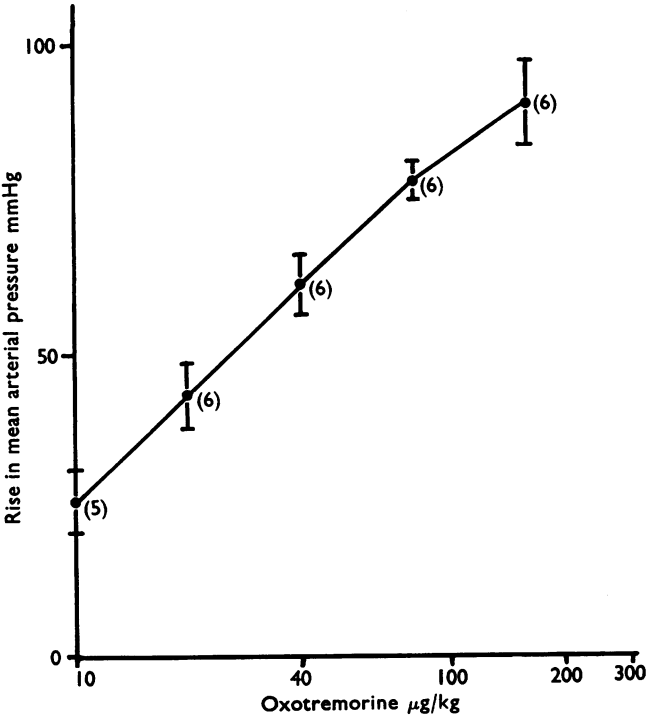

FIG. 2. Effects of different doses of oxotremorine on the mean arterial pressure of the anaesthetized rat after atropine methyl bromide. Figures in parentheses indicate the number of individual values from which the mean was calculated, and vertical bars the standard errors of the means.

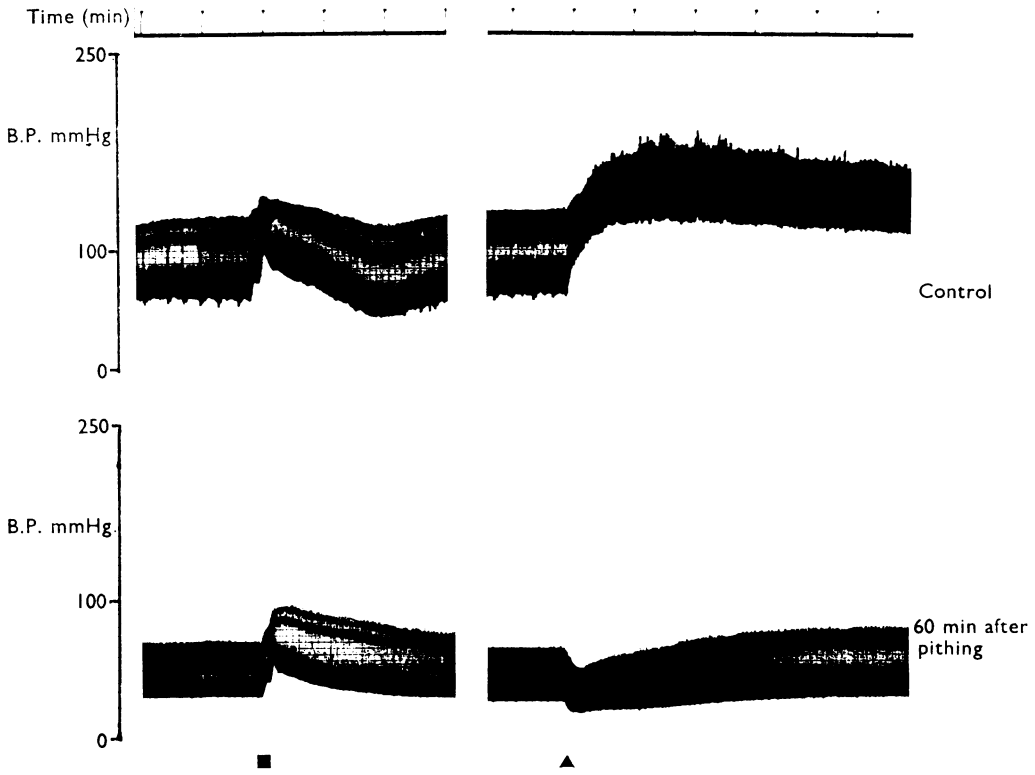

FIG. 3. Influence of pithing on the effect of noradrenaline 0·5 μg/kg and of oxotremorine 80 μg/kg on the blood pressure (B.P.) of a 200 g rat anaesthetized with urethane and previously treated with atropine methyl bromide 1 mg/kg. After pithing the animal was artificially respired. ■, Noradrenaline 0·5 μg/kg ; ▲, oxotremorine 80 μg/kg.

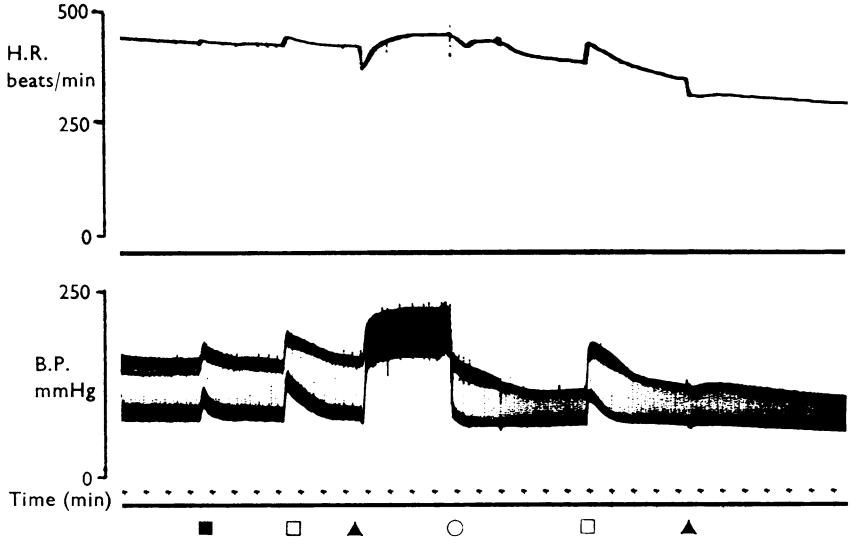

FIG. 4. Effect of section of the spinal cord between C_1 and C_2 on the blood pressure (B.P.) and heart rate (H.R.) responses to oxotremorine and noradrenaline in a 250 g rat anaesthetized with urethane and previously given 1 mg/kg atropine methyl bromide. ■, Noradrenaline 1 μg/kg ; □, noradrenaline 15 μg/kg ; ▲, oxotremorine 80 μg/kg ; ○, section of spinal cord between C_1 and C_2.

Atropine sulphate (0·5–1·0 mg/kg) antagonized the rise in arterial pressure produced by oxotremorine (Fig. 5). Very large doses of oxotremorine (300 μg/kg) in these rats resulted in a small rise in arterial pressure, but this could be antagonized by increasing the dose of atropine sulphate two to three-fold.

Antagonism of oxotremorine-induced hypertension

Two types of experiment have been carried out to study the effects of drugs on the magnitude of the rise in arterial pressure produced by oxotremorine. In some experiments, a drug was administered intravenously after obtaining a control response to oxotremorine and the effect on subsequent administration of the vasopressor agent was studied. In other experiments, drugs were administered either intraperitoneally or orally to conscious rats. Some hours later the animals were anaesthetized and their reactivity to oxotremorine was assessed. The effectiveness of oxotremorine in the pretreated rats was compared with that in untreated controls on the same day using the same solutions. The results of both types of experiment are shown in Table 1.

Both guanethidine (2 mg/kg intravenously), which prevents the release of noradrenaline from sympathetic nerve endings, and the sympathetic α-adrenoceptor blocking agent phentolamine (0·5–2·0 mg/kg intravenously) were found to be effective antagonists when used acutely (Figs. 6 and 7). The ganglion blocking agent pentolinium (10 mg/kg intravenously) almost completely abolished the hypertensive response to oxotremorine.

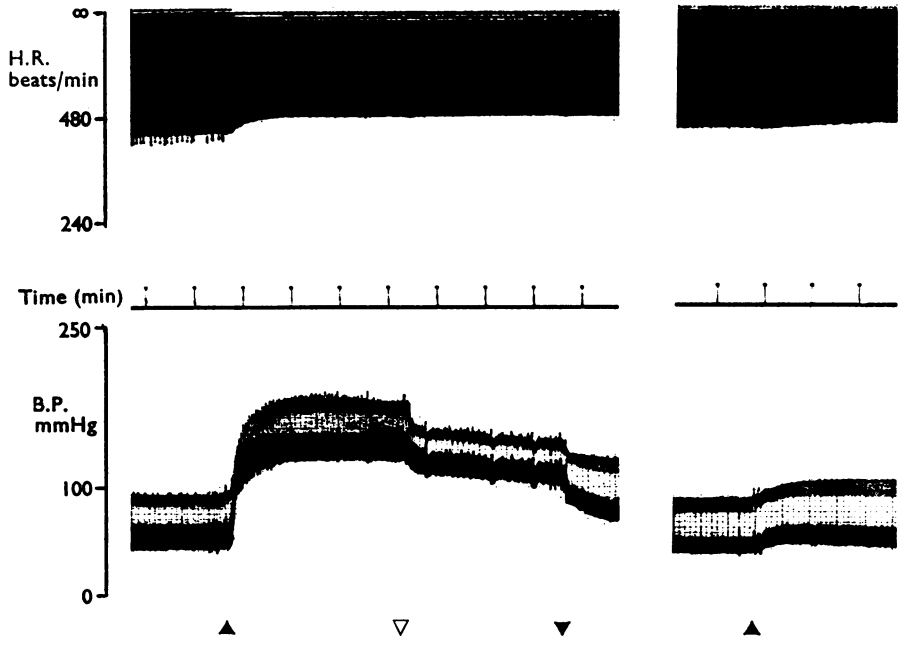

FIG. 5. Influence of atropine sulphate on the oxotremorine induced rise in blood pressure (B.P.) and increase in heart rate (H.R.) of a 250 g rat anaesthetized with urethane and treated with atropine methyl bromide 1 mg/kg intravenously. ▲, Oxotremorine 80 μg/kg ; ▽, atropine sulphate 0·5 mg/kg ; ▼, atropine sulphate 1·0 mg/kg.

TABLE 1. *Effect of antagonists of sympathetic nerve function on oxotremorine-induced hypertension in anaesthetized rats treated with atropine methyl bromide*

Drug	Dose mg/kg	Route of administration	Time between drug administration and testing	No. of animals in each group	Hypertensive response to oxotremorine (80 μg/kg)		% Reduction in hypertensive response = $\frac{C-T}{C}\times 100$	% Reduction in oxotremorine induced tachycardia
					Untreated rats (C) mmHg	Treated rats (T) mmHg		
Guanethidine	2×40	p.o.	18 & 2 h	3	74·0±5·5	22·6±3·2	69*	66
Reserpine	3×2·5	i.p.	75, 48 & 24 h	2	64·5±5·3	17±9·3	75*	—
Phentolamine	2	i.v.	15 min	2	50·0±7·1	13·5±3·2	73*	100
Pentolinium	10	i.v.	10 min	3	74·0±10·0	3·5±2·4	95*	—
Propranolol	1	i.v.	10 min	2	61·0±4·2	53·5±4·5	14	100
	10	i.p.	†	3	67·3±1·7	56·6±10·9	16	100
α-Methyldopa	2×100	i.p.	18 & 2 h	2	57·5±5·3	37·5±1·8	35	100
	2×100	i.p.	18 & 2 h	3	40·0±2·3	20·0±5·8	50*	100
	2×400	i.p.	18 & 2 h	3	69·6±1·7	34·0±2·0	51*	100
	2×800	i.p.	18 & 2 h	3	51·6±2·0	20·0±2·3	61*	100
	2×400	p.o.	18 & 2 h	3	59·0±6·5	34·0±3·3	42*	100
α-Methylparatyrosine	2×75	i.p.	18 & 2 h	4	53·0±4·3	54·0±7·4	0	—
	2×100	i.p.	18 & 2 h	2	67·5±1·8	30·0±0	55*	—
	2×200	p.o.	18 & 2 h	3	62·3±3·3	61·0±4·8	0	0
	2×400	p.o.	18 & 2 h	3	87·3±3·8	85·6±7·9	0	0
α-Methylmetatyrosine	2×400	i.p.	18 & 2 h	4	57·4±9·5	45·0±3·9	25*	—

Rats were pretreated with drugs as indicated and the responses to oxotremorine (80 μg/kg intravenously) following atropine methyl bromide (1 mg/kg intravenously) were compared with those in untreated animals on the same day. The results are mean values±S.E. The significance of the difference in hypertensive response between treated and untreated animals was determined in each case by Student's *t* test. *Indicates a significant difference ($P<0·05$). †Propranolol (10 mg/kg intraperitoneally) was given daily for 7 days. The last dose was administered 18 h before testing.

The administration of the noradrenaline depleting agent, reserpine (3 × 2·5 mg/kg intraperitoneally 75, 48 and 24 h before test), abolished the oxotremorine-induced rise in blood pressure. Propranolol (10 mg/kg intraperitoneally) a potent sympathetic β-adrenoceptor blocking agent, completely inhibited the tachycardia produced by oxotremorine but did not significantly reduce the rise in blood pressure.

α-Methyldopa, α-methylparatyrosine and α-methylmetatyrosine modify sympathetic nerve function by interfering with the synthesis of noradrenaline and/or

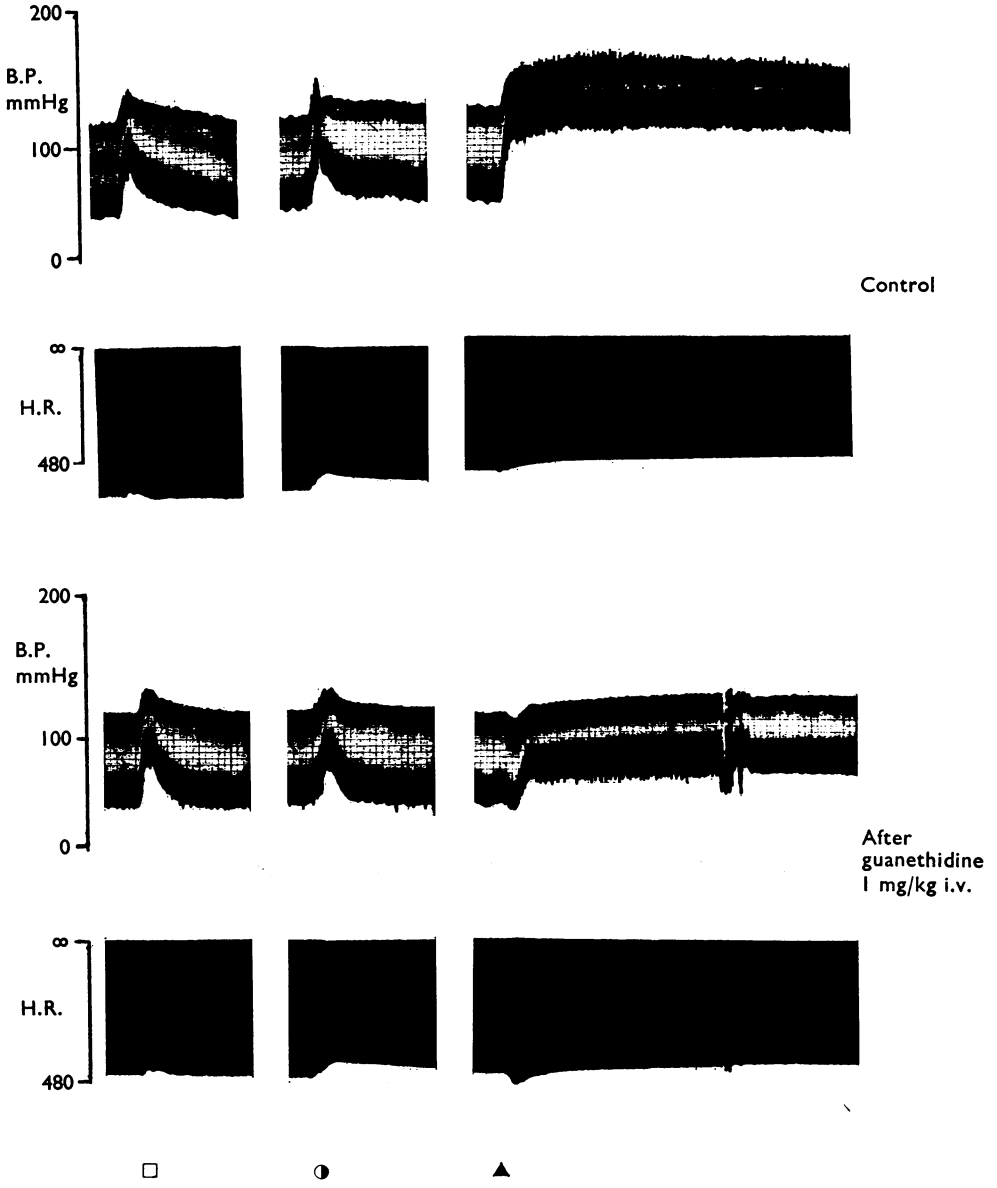

FIG. 6. Influence of guanethidine 1 mg/kg on the changes in blood pressure (B.P.) and heart rate (H.R.) produced by the intravenous administration of noradrenaline 1 μg/kg, dimethylphenylpiperazinium (DMPP) 150 μg/kg and oxotremorine 80 μg/kg in a 250 g rat anaesthetized with urethane and previously given 1 mg/kg atropine methyl bromide. □, Noradrenaline 1 μg/kg ; ◑, DMPP 150 μg/kg ; ▲, oxotremorine 80 μg/kg.

replacing the latter by less potent false transmitter substances (Carlsson & Lindquist, 1962 ; Thoenen, Haefely, Gey & Hürlimann, 1966 ; Udenfriend, Zaltzman-Nirenberg, Gordon & Spector, 1966). α-Methyldopa in high doses (2 × 100–2 × 400 mg/kg intraperitoneally) produced a significant reduction in the oxotremorine-induced hypertension and tachycardia when administered orally or intraperitoneally. α-Methylparatyrosine reduced the hypertensive response by 55% at 2 × 100 mg/kg intraperitoneally, but no reduction was obtained when this drug was administered orally at four times the intraperitoneal dose. The related compound, α-methylmetatyrosine in a dose 2 × 400 mg/kg intraperitoneally, produced a slight (25%) but significant reduction in the size of the hypertensive response to oxotremorine.

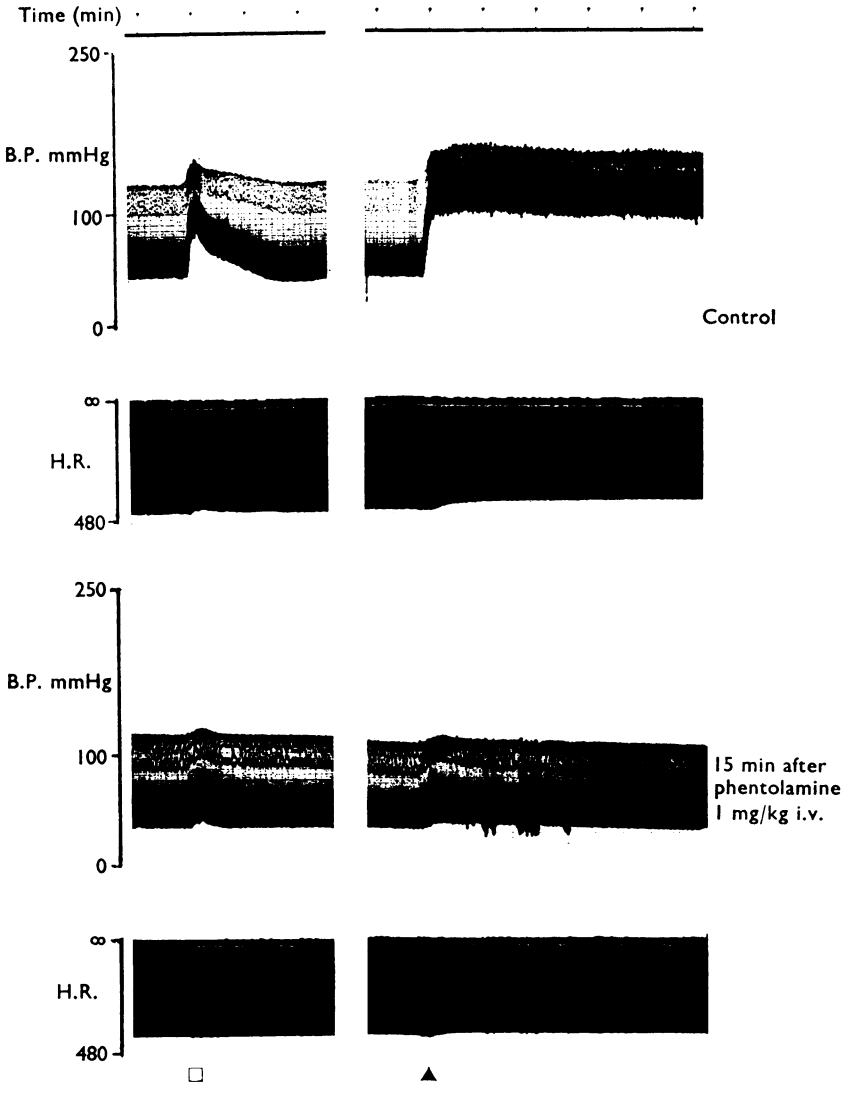

FIG. 7. Influence of phentolamine 1 mg/kg on the changes in blood pressure (B.P.) and heart rate (H.R.) produced by noradrenaline 1 μg/kg and oxotremorine 80 μg/kg in a 250 g rat anaesthetized with urethane and previously given 1 mg/kg atropine methyl bromide. □, Noradrenaline 1 μg/kg ; ▲, oxotremorine 80 μg/kg.

Discussion

Administration of oxotremorine to anaesthetized rats is known to produce hypotension and bradycardia (Cho, Haslett & Jenden, 1962). If, however, rats are pretreated with atropine methyl bromide, which blocks peripheral cholinoceptors (Bebbington, Brimblecombe & Shakeshaft, 1966 ; Leslie, 1965), these effects are abolished, revealing a second action of oxotremorine. This consists of a rise in arterial pressure accompanied by tachycardia. The hypertension produced is of long duration, is non-tachyphylactic and dose dependent.

These effects of oxotremorine could be due to an action on sensory pathways or to an action on the central nervous system. An action on afferent fibres included within the vagi was eliminated when it was shown that vagotomy did not affect the response to oxotremorine in any way, but this does not exclude a possible action on other sensory routes to the brain stem. However, destruction of the brain and spinal cord by use of a pithing rod completely abolished the effects of oxotremorine. The finding that section of the spinal cord between the first and second cervical vertebrae also abolished oxotremorine induced hypertension, placed the site of action rostral to this point. In rats, where this lesion of the cord had been made, responses to noradrenaline were normal, showing that the peripheral vascular system was unimpaired, but subsequent doses of oxotremorine failed to elicit hypertension. Because of the emergent pathway from the spinal cord, it was thought that oxotremorine might be producing its effects by increasing activity in the efferent sympathetic nervous system. This was confirmed by testing the effects of pretreatment with a wide range of drugs known to impair the function of the latter system. Whether this was due to a direct blocking of ganglionic transmission (pentolinium) or α-adrenoceptors (phentolamine) or to interference with the release (guanethidine), storage (reserpine) and/or synthesis (α-methyldopa, α-methylparatyrosine, α-methylmetatyrosine) of noradrenaline, there was a significant inhibition of the response to subsequently administered oxotremorine in all cases. The one exception was that where propranolol was administered, there was no significant difference in the hypertensive response of treated and control rats, although the increase in heart rate was completely absent in the former group. This finding indicated that the tachycardia produced by administration of oxotremorine in atropine methyl bromide treated rats does not significantly contribute to the observed rise in arterial blood pressure. This surmise was supported by the fact that adrenalectomy does not impair the hypertensive response to oxotremorine although liberation of adrenaline from the adrenals and consequent tachycardia would be an expected result of a general increase in activity of the sympathetic nervous system. Oxotremorine has no nicotinic action (Haslett & Jenden, 1961), which was confirmed in the experiments with atropine sulphate.

Atropine sulphate, in contrast to atropine methyl bromide, readily penetrates into the central nervous system and produces blockade of all muscarinic cholinoceptors. In rats treated with atropine sulphate, no hypertensive response to oxotremorine could be elicited. This indicated that oxotremorine exerts an action on central cholinoceptors, either directly or by an indirect mechanism. Anticholinesterase drugs, such as eserine, have been shown to produce effects similar to those caused by oxotremorine in atropine methyl bromide treated rats, presumably by increasing the availability of acetylcholine within the brain (Della Bella, Gandini & Preti,

1964 ; Dirnhuber & Cullumbine, 1955 ; Lalanne, Schmitt & Schmitt, 1966 ; Lesić & Varagić, 1961 ; McEwen, 1968 ; Varagić, 1955 ; Varagić & Krstić, 1966 ; Varagić & Vojvodić, 1962). Oxotremorine, however, has no significant anticholinesterase properties (Haslett & Jenden, 1961) and so cannot be acting by this mechanism. It could exert its effects by causing a release of acetylcholine from endogenous stores. Cox & Potkonjak (1969) found that oxotremorine does, in fact, cause an increase in brain acetylcholine in rats but there is a 15 min time lag between dosing and the observed rise in acetylcholine. As the hypertensive response to oxotremorine is almost immediate, it does not therefore seem likely that this effect is due to the latter mechanism of action, although it is possible that acetylcholine could be redistributed, thus increasing its availability at muscarinic receptors, with no immediate change in total brain content. It would seem more probable that oxotremorine has a direct action on cholinoceptors within the brain.

Although the detailed action of oxotremorine on the brain remains obscure, the overall effects are best described as an increase in activity of the efferent sympathetic nervous system.

REFERENCES

Bebbington, A., Brimblecombe, R. W. & Shakeshaft, D. (1966). The central and peripheral activity of acetylenic amines related to oxotremorine. *Br. J. Pharmac. Chemother.*, **26**, 56–67.

Carlsson, A. & Lindquist, M. (1962). *In vivo* decarboxylation of α-methyldopa and α-methylmetatyrosine. *Acta physiol. scand.*, **54**, 87–94.

Cho, A. K., Haslett, W. L. & Jenden, D. J. (1962). The peripheral actions of oxotremorine, a metabolite of tremorine. *J. Pharmac. exp. Ther.*, **138**, 249–257.

Cox, B. & Potkonjak, D. (1969). The relationship between tremor and change in brain acetylcholine concentration produced by injection of tremorine or oxotremorine in the rat. *Br. J. Pharmac.*, **35**, 295–303.

Della Bella, D., Gandini, A. & Preti, M. (1964). The mechanism of the pressor responses to physostigmine in the rat and their modification by mebutamate and amylobarbitone. *Br. J. Pharmac. Chemother.*, **23**, 540–551.

Dirnhuber, P. & Cullumbine, H. (1955). The effects of anti-cholinesterase agents on the rat's blood pressure. *Br. J. Pharmac. Chemother.*, **10**, 12–15.

Haslett, W. L. & Jenden, D. J. (1961). Pharmacological properties of oxotremorine, an active metabolite of tremorine. *Pharmacologist*, **3**, 2.

Kleinman, L. I. & Radford, E. P., Jr. (1964). Ventilation standards for small mammals. *J. appl. Physiol.*, **19**, 360–362.

Lalanne, J. L., Schmitt, H. & Schmitt, H. (1966). Sur le mécanisme de l'hypertension provoquée par l'eserine chez le rat. *Archs int. Pharmacodyn. Thér.*, **163**, 444–464.

Lesić, R. & Varagić, V. (1961). Factors influencing the hypertensive effect of eserine in the rat. *Br. J. Pharmac. Chemother.*, **16**, 99–107.

Leslie, G. B. (1965). Central stimulant properties of compounds with peripheral muscarinic properties. *Nature, Lond.*, **208**, 1291–1293.

McEwen, J. E. (1968). The pressor effects of large doses of physostigmine on the pithed rat. *Br. J. Pharmac. Chemother.*, **33**, 199–201.

Thoenen, H., Haefely, W., Gey, K. F. & Hürlimann, A. (1966). The effect of α-methylparatyrosine on peripheral sympathetic transmission. *Life Sci, Oxford*, **5**, 723–730.

Udenfriend, S., Zaltzman-Nirenberg, P., Gordon, R. & Spector, S. (1966). Evaluation of the biochemical effects produced *in vivo* by inhibitors of the three enzymes involved in norepinephrine synthesis. *Mol. Pharmac.*, **2**, 95–105.

Varagić, V. (1955). The action of eserine on the blood pressure of the rat. *Br. J. Pharmac. Chemother.*, **10**, 349–353.

Varagić, V. & Krstić, M. (1966). Adrenergic activation by anticholinesterases. *Pharmac. Rev.*, **18**, 799–800.

Varagić, V. & Vojvodić, N. (1962). Effect of guanethidine, hemicholinium and mebutamate on the hypertensive response to eserine and catecholamines. *Br. J. Pharmac. Chemother.*, **19**, 451–457.

(*Received April* 1, 1970)