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SOME COCAINE-LIKE ACTIONS OF 3-PHENOXYPROPYLGUANIDINE

BY

A. L. BARTLET*

From the Department of Pharmacological Research, Parke, Davis & Company, Hounslow, Middlesex

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In anaesthetized cats 3-phenoxypropylguanidine caused a contracture of the nictitating membrane, a dilatation of the pupil and a fall followed by a rise in the arterial blood pressure. In spinal preparations of cats the initial fall in blood pressure was usually absent and the rise in blood pressure subsided to a steady level, which was about 10 mm Hg above the initial pressure. The pressor action and the contracture of the nictitating membrane were inhibited by phenoxybenzamine and by previous treatment with reserpine, but were not abolished by adrenalectomy and bretylium. 3-Phenoxypropylguanidine potentiated the actions of adrenaline and noradrenaline, increased the blood glucose concentration of the rabbit and decreased the appetite of the cat. The action of tyramine on the cardiovascular system was inhibited by 3-phenoxypropylguanidine, but the stimulant action of tyramine on the nictitating membrane of the cat was not abolished by this substance. Although 3-phenoxypropylguanidine produced a local anaesthesia of long duration in guinea-pig skin, it failed to anaesthetize the rabbit cornea. The responses to stimulation of the preganglionic cervical sympathetic nerve of the cat and the great auricular nerve of the rabbit ear were not abolished by 3-phenoxypropylguanidine; neither did this substance abolish the nicotinic action of acetylcholine in atropinized cats. Contractions of the rat fundus to tryptamine and 5-hydroxytryptamine were antagonized by 3-phenoxypropylguanidine, but were potentiated by cocaine.

When reserpine is administered to a mouse, the eyes become closed. This may be due to the disappearance of catechol amines and 5-hydroxytryptamine from the mouse; since when these amines reappear, as after administration of their amino-acid precursors, the eyes are reopened (Carlsson, Lindqvist & Magnusson, 1957). The eyes of the reserpinized mouse are also reopened by the administration of 3-phenoxypropylguanidine (Chen, unpublished observation), and this suggested that this substance might potentiate the action of one of these amines. The experiments to be described showed that this was the case; adrenaline and noradrenaline were potentiated by 3-phenoxypropylguanidine, although 5-hydroxytryptamine was antagonized. The examination of 3-phenoxypropylguanidine in more detail revealed a most striking pharmacological resemblance to cocaine.

* Present address: Department of Veterinary Pharmacology, Royal (Dick) School of Veterinary Studies, University of Edinburgh.

METHODS

Rat seminal vesicle. After removal of the secretory gland the seminal vesicle was suspended in a 10 ml. bath of a modified Ringer-Locke solution (Garcia de Jalon, Bayo Bayo & Garcia de Jalon, 1945) at 30° C (9.0 g sodium chloride, 0.42 g potassium chloride, 0.06 g calcium chloride, 0.5 g sodium bicarbonate, 0.5 g glucose in 1 l. water) and gassed with 5% carbon dioxide in oxygen. Contractions were recorded with an isotonic frontal writing lever; the load on the seminal vesicle was 300 mg.

Rat fundus. Strips of rat fundus, prepared as described by Vane (1957), were suspended in a 10 ml. bath of Tyrode solution (Tyrode, 1910) at 35° C (8.0 g sodium chloride, 0.2 g potassium chloride, 0.2 g calcium chloride, 0.1 g magnesium chloride, 1.0 g sodium bicarbonate, 0.05 g sodium dihydrogen phosphate, 1.0 g glucose in 1 l. water) and gassed with 5% carbon dioxide in oxygen. Contractions were recorded with a pendulum auxotonic lever (Paton, 1957); the weight on the fundus was 1 g.

Intradermal test for local anaesthesia. A modification of the method of Bülbring & Wajda (1945) was used. Solutions of local anaesthetics made isotonic with sodium chloride were injected intradermally, six injections of 0.1 ml. being made on the depilated back of each guinea-pig. Injections were made into groups of pigs according to a design, so that each concentration of drug was injected once in each of the six injection positions in different pigs. The reactions of each weal to five pin-pricks were tested at 5, 15 and 30 min and 1, 2, 3 and 4 hr after injection. Next day the weals were examined for full recovery from anaesthesia and for possible toxic skin reactions.

Corneal test for local anaesthesia. Solutions of drugs made isotonic with sodium chloride were tested in rabbits. After clipping the eyelashes a solution was instilled into the conjunctiva and the corneal reflex was tested after intervals of 5, 10, 20 and 30 min.

Perfused rabbit ear. Rabbits with large ears were anaesthetized with ether. A polythene cannula was tied into the central artery of the ear, and the auricular nerve freed along about an inch of its length. The rabbit was killed and the ear cut off. The ear was fixed on to a perspex plate and perfused with Ringer-Locke solution (Locke & Rosenheim, 1907) at 35° C from a Marriotte bottle. The venous outflow was recorded by a phototransistor drop recorder as it dropped off the plate. Injections into the arterial cannula were made from micrometer syringes, the volume injected never exceeding 0.015 ml. The auricular nerve was laid across bipolar platinum electrodes and stimulated by a square-wave stimulator for 20 sec at a rate of 20 pulses/sec and a pulse duration of 0.3 msec. Periods of stimulation were separated by intervals of at least 20 min.

Cat blood pressure and nictitating membrane. Cats were anaesthetized with ether followed by chloralose (80 mg/kg injected intravenously) or were made spinal as described by Burn (1952). Blood pressure was recorded from the right carotid artery. Movements of the left nictitating membrane were recorded with an isotonic frontal writing lever; the load on the nictitating membrane was 2 g. Injections were made into a femoral vein. The left preganglionic cervical sympathetic nerve was separated from the vagus, severed and its peripheral end laid across bipolar platinum electrodes in a pool of paraffin. The nerve was stimulated supramaximally from a square-wave stimulator for 5 sec every 4 or 8 min at a rate of 50 pulses/sec and a pulse duration of 0.3 msec.

Blood glucose after adrenaline. The blood glucose concentration in rabbits was estimated by the method of Huggett & Nixon (1957) on 0.1 ml. samples of blood withdrawn from the marginal ear vein. Six rabbits were used in a cross-over experiment, the two halves of the experiment being separated by an interval of two weeks. Before each half of the experiment the rabbits were kept without food for 24 hr. All injections were made subcutaneously on the flank. In the first half of the experiment three rabbits were injected with 3-phenoxypropylguanidine (20 mg/kg), and three were given a control injection, 18 hr before withdrawing blood. In the second half of the experiments the previously treated rabbits served as controls and the previous controls were injected with 3-phenoxypropylguanidine. Estimations of blood glucose were made on two samples of blood before injecting 50 μg/kg adrenaline and on samples taken 1, 2, 3, 4 and 5 hr after the injection.

Drugs. Acetylcholine chloride, adrenaline bitartrate, atropine sulphate, bretylium tosylate, cocaine hydrochloride, hexahydro-1-azepine-propionamidoxime dihydrochloride (Su-4029), 5-hydroxytryptamine creatinine sulphate, lignocaine hydrochloride, (−)-noradrenaline bitartrate, 2-(octahydro-1-azocinyl)-ethyl guanidine sulphate (guanethidine), N-phenoxyisopropyl-N-benzyl-β-chlorethylamine hydrochloride (phenoxybenzamine), 3-phenoxypropylguanidine sulphate, phenylbiguanide hydrochloride, reserpine, tryptamine hydrochloride and tyramine hydrochloride were used. Doses have been expressed as weights of base, with the exception of atropine, bretylium and phenoxybenzamine, which refer to the corresponding salts. Reserpine was dissolved in 20% w/v aqueous ascorbic acid; other drugs were dissolved in 0.9% w/v aqueous sodium chloride.

The mean of the results of a group of observations is presented as the mean ± s.e. of mean (number of observations).

RESULTS

Effects of 3-phenoxypropylguanidine and of cocaine on the response of isolated tissues to catechol amines and tryptamines. The response of the rat seminal vesicle to adrenaline or noradrenaline was potentiated by 3-phenoxypropylguanidine. The potentiation was apparent when 2.5 μg 3-phenoxypropylguanidine had been in the bath for 2 min, and persisted for more than 30 min after washing out the drug

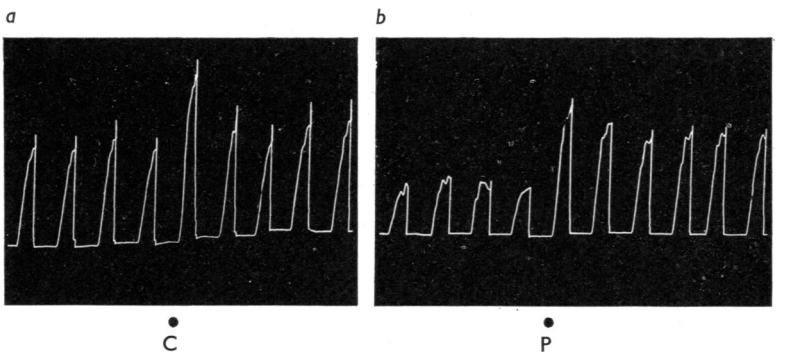

Fig. 1. Rat seminal vesicles. 10 ml. bath. Stimulated by 1.0 μg adrenaline at 5 min intervals. In (*a*) 2.5 μg cocaine was in the bath for 2 min at C. In (*b*) 2.5 μg 3-phenoxypropylguanidine was in the bath for 2 min at P. Cocaine and 3-phenoxypropylguanidine potentiated the response to adrenaline, but potentiation by 3-phenoxypropylguanidine was the more prolonged.

(Fig. 1b). Fig. 1a shows the effect of 2.5 μg of cocaine. Although cocaine potentiated the response of the seminal vesicle to adrenaline the effect soon disappeared when cocaine was washed from the bath.

The rat fundus was stimulated by alternating doses of tryptamine and 5-hydroxytryptamine. When the sensitivity of the preparation became steady, 3-phenoxypropylguanidine or cocaine was added to the Tyrode solution in concentrations ranging from 10^{-6} to 10^{-4}. 3-Phenoxypropylguanidine depressed responses to tryptamine and 5-hydroxytryptamine to the same extent. The effect of 3-phenoxypropylguanidine in a concentration of 10^{-6} is shown in Fig. 2a. Cocaine potentiated the actions of tryptamine and 5-hydroxytryptamine on the fundus. The effect of cocaine in a concentration of 10^{-4} is shown in Fig. 2b. After cocaine the actions of tryptamine and 5-hydroxytryptamine were potentiated to about the same degree.

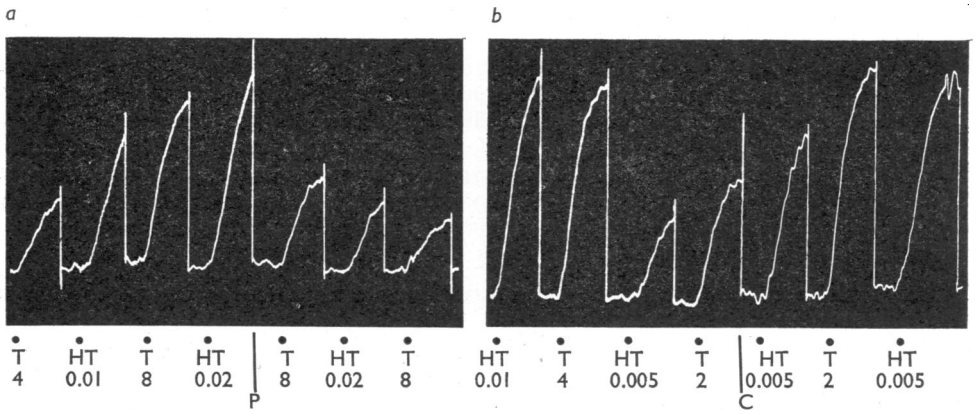

Fig. 2. Rat fundal strips. 10 ml. bath. Interval 5 min. Numerals represent μg of 5-hydroxytryptamine (HT) or tryptamine (T) added to bath. (*a*) At P, 3-phenoxypropylguanidine at a concentration of 10^{-6} was added to the Tyrode solution. Responses to tryptamine and 5-hydroxytryptamine were depressed by 3-phenoxypropylguanidine. (*b*) At C, cocaine at a concentration of 10^{-4} was added to the Tyrode solution. Responses to tryptamine and 5-hydroxytryptamine were potentiated by cocaine.

Local anaesthesia. In guinea-pig skin 3-phenoxypropylguanidine produced a local anaesthesia of long duration. The time course of the local anaesthesia produced by 0.5% solutions of 3-phenoxypropylguanidine, cocaine and lignocaine is shown in Fig. 3. The action of 3-phenoxypropylguanidine was slower in onset and of longer duration than that of cocaine or lignocaine. The maximum intensity of anaesthesia of cocaine or lignocaine was produced within 5 min, whereas the action of 3-phenoxypropylguanidine developed more slowly and was greatest when

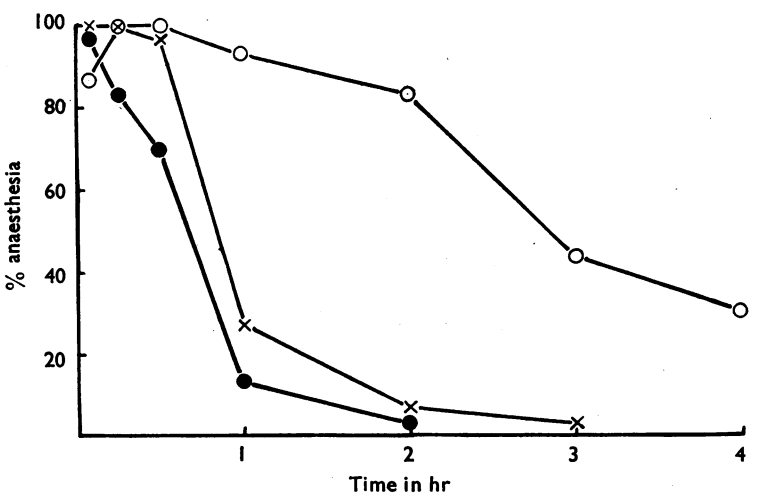

Fig. 3. Local anaesthesia in guinea-pig skin. Ordinate, mean % anaesthesia from six guinea-pigs. Abscissa, duration of anaesthesia (hr). ○——○, 0.5% 3-phenoxypropylguanidine. ●——●, 0.5% lignocaine. X——X, 0.5% cocaine.

measured after 15 or 30 min. The action of 3-phenoxypropylguanidine lasted more than twice that of cocaine or lignocaine, since the effect of 0.5% cocaine or lignocaine had nearly disappeared by 2 hr, while recovery from 0.5% 3-phenoxypropylguanidine was incomplete after 4 hr. No skin reactions were observed after the intradermal injection of 0.5% 3-phenoxypropylguanidine and skin sensation had returned by 30 hr. However, injections of a 2% solution led to the formation of petechiae.

Isotonic solutions containing 1% 3-phenoxypropylguanidine or 1% cocaine were tested on the corneal reflex of the rabbit. The effect of each solution was compared with that of an isotonic solution of sodium chloride in four rabbits. 3-Phenoxypropylguanidine failed to anaesthetize the rabbit cornea, since its apparent local anaesthetic action was no greater than that of the saline control; the local anaesthetic action of cocaine in the eye was observed.

Experiments with the perfused rabbit ear. The rabbit ear perfusion experiments were made in order to find if 3-phenoxypropylguanidine, like cocaine, antagonized certain actions of tyramine (Tainter & Chang, 1927); and whether, like bretylium, it inhibited the function of adrenergic nerves (Boura & Green, 1959).

Vasoconstriction was recorded after stimulation of the auricular nerve and after injections of tyramine and adrenaline. In two experiments 3-phenoxypropylguanidine at a concentration of 10^{-5} was added to the Locke solution and the actions of the vasoconstrictor agents re-examined. The tracing from one of these experiments is shown in Fig. 4. In both experiments doses of tyramine and adrenaline which

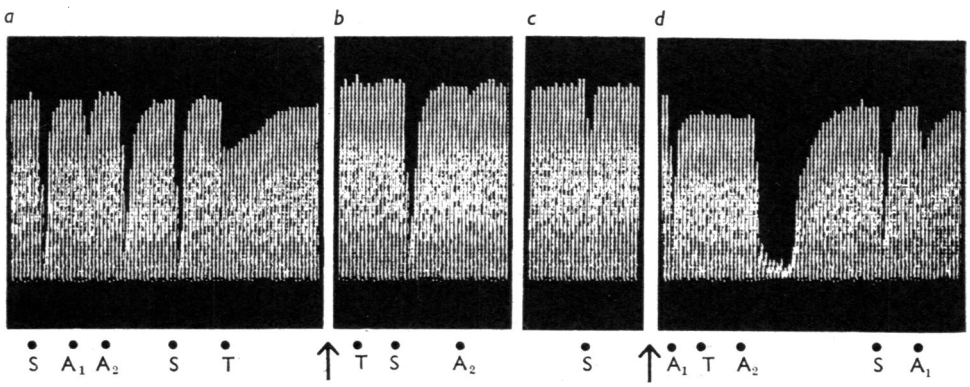

Fig. 4. Outflow from perfused rabbit ear. Drop counter record returned to base line at 30 sec intervals. Injections made into the arterial cannula were 0.01 μg adrenaline (A_1), 0.02 μg adrenaline (A_2), and 10 μg tyramine (T). At S the nerve was stimulated for 20 sec. 3-Phenoxypropylguanidine (10^{-5}) was present in the perfusion fluid between the arrows.

initially produced vasoconstriction became inactive, but the vasoconstrictor action of nerve stimulation was at first little affected and later reduced. When 3-phenoxypropylguanidine had been included in the perfusion fluid for more than 1 hr it was discontinued and the ear perfused with fresh Locke solution. The vasoconstrictor action of adrenaline then returned to the ear, and was potentiated when compared with the response to the initial injections of this substance. The response

to nerve stimulation also increased, but showed little potentiation when compared with the initial response to stimulation of the nerve. The vasoconstrictor action of tyramine remained antagonized and did not return in either experiment, although the period of perfusion with fresh Locke solution extended for over 1 hr.

In two other experiments cocaine at a concentration of 10^{-5} was added to the perfusion fluid after recording the actions of adrenaline, tyramine and nerve

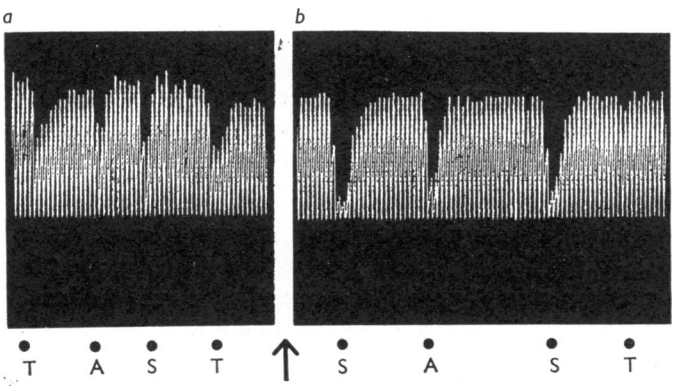

Fig. 5. Outflow from perfused rabbit ear. Drop counter record returned to base line at 30 sec intervals. Injections made into the arterial cannula were 0.02 μg adrenaline (A) and 15.0 μg tyramine (T). At S the nerve was stimulated for 20 sec. The perfusion fluid contained cocaine (10^{-5}) from the arrow to the end of the tracing.

stimulation. The tracing from one of these experiments is shown in Fig. 5. In both experiments cocaine antagonized the constrictor action of tyramine, but potentiated the actions of adrenaline and nerve stimulation.

Effect of 3-phenoxypropylguanidine on the actions of noradrenaline, tyramine and on cervical sympathetic stimulation in spinal cats. An antityramine action of 3-phenoxypropylguanidine was observed on the blood pressure of the spinal cat. Fig. 6 shows tracings from an experiment in which responses of the blood pressure and the nictitating membrane to noradrenaline, tyramine and supramaximal stimulation of the cervical sympathetic nerve were recorded both before and after the intravenous injection of 1 mg/kg 3-phenoxypropylguanidine. 3-Phenoxypropylguanidine itself produced a rise in blood pressure and a prolonged contracture of the nictitating membrane. In most experiments the blood pressure returned within 30 min of making an injection of 3-phenoxypropylguanidine to a new level, about 10 mm Hg above the initial blood pressure, but the contracture of the nictitating membrane persisted to the end of the experiment. 3-Phenoxypropylguanidine potentiated the pressor action of noradrenaline and inhibited the pressor action of tyramine, but the stimulant action of tyramine on the nictitating membrane was never abolished by this substance. After 3-phenoxypropylguanidine, supramaximal stimulation of the cervical sympathetic nerve still produced contractions of the nictitating membrane, but these were sometimes obliterated by the contracture caused by 3-phenoxypropylguanidine itself, for example, in Fig. 6 the response of the nictitating membrane to supramaximal stimulation of the cervical sympathetic nerve may be seen superimposed on the contracture produced by the first injection

of 3-phenoxypropylguanidine ; however, the second injection of 3-phenoxypropylguanidine produced a maximal contracture of the nictitating membrane so that subsequent responses to stimulation of the nerve were obliterated. A tachyphylaxis of the pressor action of 3-phenoxypropylguanidine occurred when two or more injections of this substance were made into a cat ; thus, while a second dose of 3-phenoxypropylguanidine increased the contracture of the nictitating membrane, it produced a fall in blood pressure (Fig. 6d).

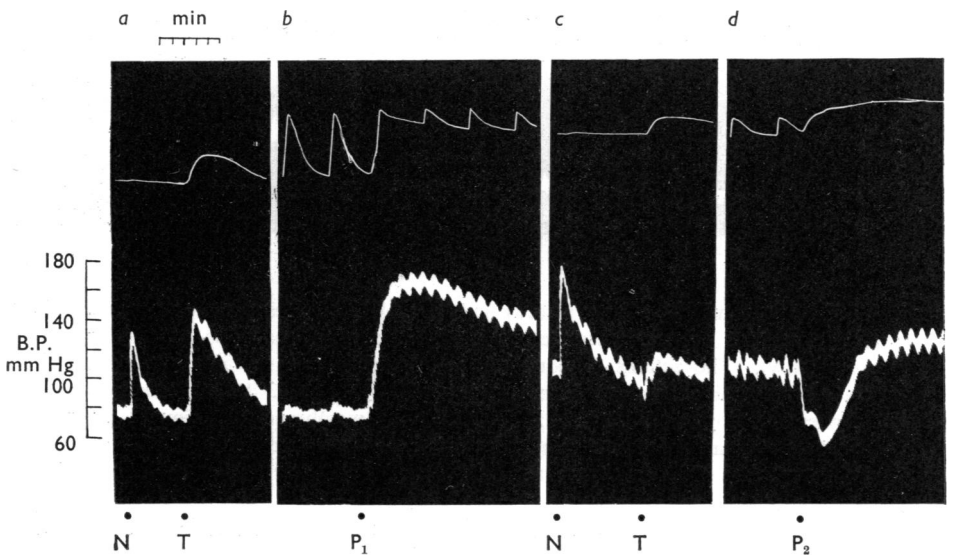

Fig. 6. Nictitating membrane (upper record) and arterial blood pressure (lower record) of a spinal cat. In (*b*) and (*d*) the nictitating membrane was stimulated supramaximally for 5 sec in every 4 min. The subscripts mark intravenous injections of noradrenaline 1.0 μg (N), tyramine 250 μg (T), 3-phenoxypropylguanidine 1 mg/kg (at P_1) and 2 mg/kg (at P_2).

The effects on blood pressure and nictitating membrane of noradrenaline, tyramine and preganglionic stimulation of the cervical sympathetic nerve were examined in three cats after previous treatment with 3-phenoxypropylguanidine. The cats were given 20 mg/kg 3-phenoxypropylguanidine subcutaneously, one day (two cats) or three days (one cat) before testing. Fig. 7 shows tracings from two of these cats made spinal one day (a), and three days (b), after previous treatment with 3-phenoxypropylguanidine. Contractions of the nictitating membrane produced by stimulation of the nerve were prolonged when a preparation was used one day after treatment with 3-phenoxypropylguanidine. Relaxation of the nictitating membrane took 8 min in these preparations, whereas it relaxed fully within 4 min in untreated cats and in the preparation tested 3 days after administration of 3-phenoxypropylguanidine. These preparations were also very sensitive to noradrenaline, especially one day after 3-phenoxypropylguanidine, when 0.2 μg noradrenaline produced small contractions of the nictitating membrane and pressor responses were produced by as little as 5 ng noradrenaline. The inhibition of the pressor action of tyramine was of long duration, as one day after 3-phenoxypropylguanidine 1 mg tyramine had

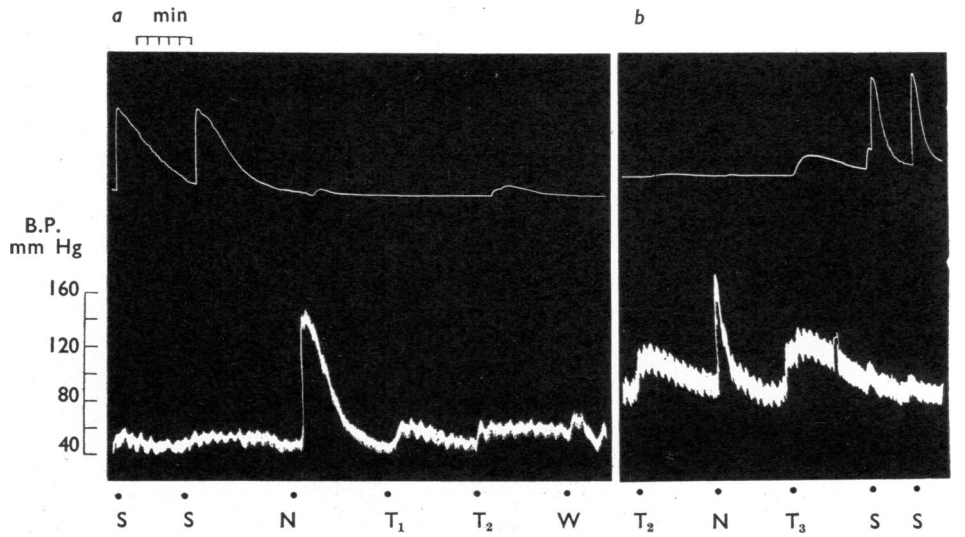

Fig. 7. Nictitating membrane (upper record) and arterial blood pressure (lower record) of two spinal preparations of cats pretreated with 3-phenoxypropylguanidine (20 mg/kg injected subcutaneously). The actions of noradrenaline, tyramine and supramaximal preganglionic stimulation of the nictitating membrane are shown 1 day (*a*) and 3 days (*b*) after pretreatment with 3-phenoxypropylguanidine. At S, the nictitating membrane was stimulated for 5 sec. Other subscripts mark intravenous injections of 0.2 μg noradrenaline (N), 250 μg tyramine (T_1), 1 mg tyramine (T_2), 5 mg tyramine (T_3) and 1 ml. 0.9% sodium chloride solution (W).

no more effect on blood pressure than an injection of saline, but its pressor action had partly returned after three days. In contrast 3-phenoxypropylguanidine did not abolish the effect of tyramine on the nictitating membrane.

A dilatation of the pupil was observed after subcutaneous injection of 20 mg/kg 3-phenoxypropylguanidine; the light reflex was not abolished, however, and the nictitating membrane did not relax. After 3-phenoxypropylguanidine (20 mg/kg), cats became sedated and refused to take food or drink, but when roused they moved quite normally. Two further cats died after receiving a second daily injection of 20 mg/kg of this substance.

The tyramine inhibiting action of 3-phenoxypropylguanidine was compared with that of several other substances (Table 1). Spinal cats were prepared and rested for 1 hr before the commencement of an experiment. Intravenous submaximal doses of noradrenaline and tyramine were injected alternately, the dose of tyramine being selected to produce a rise in blood pressure of between 40 and 80 mm Hg. A substance inhibiting the action of tyramine was then injected intravenously; usually a single dose of one drug was tested in each cat, as these substances had a long duration of action. After an injection of 3-phenoxypropylguanidine, guanethidine or hexahydro-1-azepine-propionamidoxime preparations were rested 30 min to allow the blood pressure to reach a steady level before re-testing tyramine. The inhibition of tyramine by these compounds was fully developed by 30 min, and the percentage inhibition was calculated from measurements of the rise in

blood pressure produced by the same dose of tyramine before and 30 min after an antityramine substance. However, the inhibition of tyramine by intravenous cocaine was more transient, and the percentage inhibition of tyramine by cocaine was calculated from the response to the same dose of tyramine initially and 5 min after cocaine. In all experiments the response to noradrenaline was potentiated or unaltered after an antityramine substance.

TABLE 1

INHIBITION OF PRESSOR ACTION OF TYRAMINE BY SEVERAL SUBSTANCES IN SPINAL PREPARATIONS OF CATS

Compound	Dose (mg/kg)	% inhibition of pressor action of tyramine. Mean±s.e. (no. of observations)
3-Phenoxypropyl-guanidine	0·25	41·0± 6·3 (4)
	0·5	68·3±13·5 (3)
	1·0	87·0± 1·9 (3)
Cocaine	0·5	29·0± 6·5 (3)
	1·0	50·3± 2·9 (3)
	2·0	75·3± 6·8 (3)
Guanethidine	4·0	34·3± 2·9 (3)
	8·0	54·3± 9·6 (3)
	16·0	87·7± 4·3 (3)
Hexahydro-1-azepine-propionamidoxime	8·0	36·3± 4·9 (3)
	16·0	53·7± 1·7 (3)

Effect on pressor action of guanethidine. Small doses of guanethidine have a marked pressor action in spinal preparations of cats (Bartlet, 1962). In 5 experiments 2 mg/kg guanethidine produced a mean rise in blood pressure of 123.8± 9.5 mm Hg. In two other cats 3-phenoxypropylguanidine (5 mg/kg) reversed the pressor action of guanethidine; in these experiments guanethidine (2 mg/kg) reduced the blood pressure by 24 and 9 mm Hg respectively.

Effect on actions of acetylcholine. In a chloralosed cat the vasodepressor response to acetylcholine was unaltered after 5 mg/kg 3-phenoxypropylguanidine. Nicotinic actions of acetylcholine were studied in six spinal preparations of cats injected intravenously with 2 mg/kg atropine sulphate. Two cats were adrenalectomized and in two other preparations the adrenal artery and vein were ligated. In all six cats a large dose of acetylcholine (2 to 4 mg) produced a rise in blood pressure. The pressor response to acetylcholine was not abolished by 5 mg/kg 3-phenoxypropylguanidine, but it is impossible to say whether it was reduced, since the blood pressure was raised permanently after 3-phenoxypropylguanidine and the response to acetylcholine became biphasic, a fall followed by a rise in blood pressure (Fig. 8). A pressor component in the response to acetylcholine always persisted after 3-phenoxypropylguanidine.

Effect of reserpine, bretylium, phenoxybenzamine, adrenalectomy and vagotomy on the responses of blood pressure and nictitating membrane to 3-phenoxypropylguanidine. A small dose of 3-phenoxypropylguanidine (50 μg/kg) produced a sharp fall in the arterial blood pressure of the chloralosed cat. This was not caused by

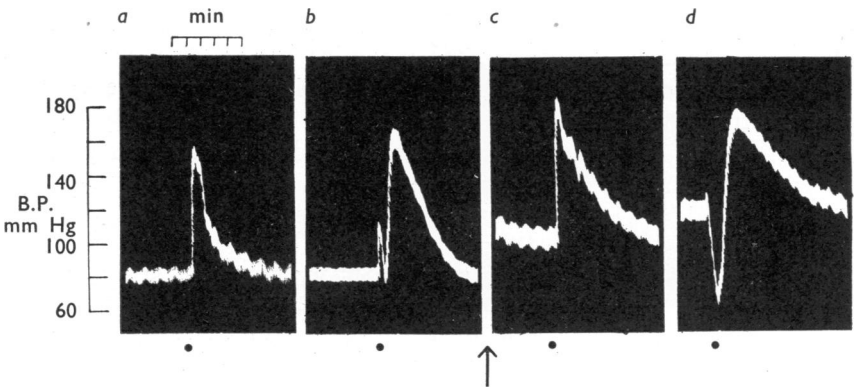

Fig. 8. Arterial blood pressure of spinal cat injected with atropine sulphate (2 mg/kg) and with adrenals ligated. The responses to 1.0 μg noradrenaline are shown in (*a*) and (*c*), and to 4.0 mg acetylcholine in (*b*) and (*d*). At the arrow between (*b*) and (*c*) the cat was injected with 3-phenoxypropylguanidine (5 mg/kg).

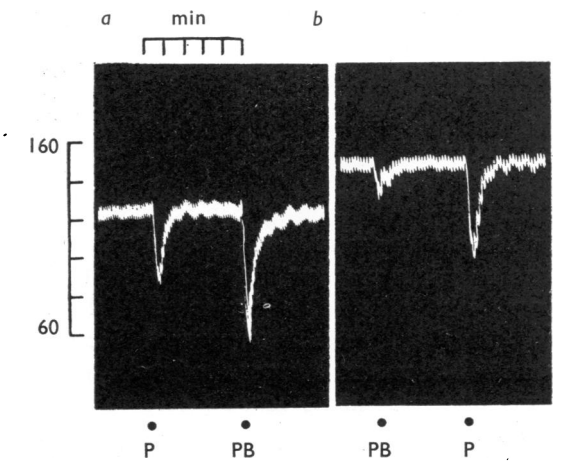

Fig. 9. Arterial blood pressure of cat anaesthetized with chloralose. The subscripts mark intravenous injections of 100 μg 3-phenoxypropylguanidine (P) and 50 μg phenylbiguanide (PB). The vagi were severed between (*a*) and (*b*).

a reflex stimulation of the vagus, as it remained after severing the vagi (Fig. 9), and so was unlike the fall in blood pressure produced by phenylbiguanide (Dawes & Mott, 1950).

When a larger dose of 3-phenoxypropylguanidine (3 mg/kg) was given the fall in blood pressure was succeeded by a rise in blood pressure and the nictitating membrane went into a contracture (Fig. 10a). The rise in blood pressure and the contracture of the nictitating membrane were antagonized by 2 mg/kg phenoxybenzamine (Fig. 10b). In two cats, bilateral adrenalectomy and blockade of adrenergic nerve fibres by previous intravenous injection of 15 mg/kg bretylium (Boura & Green, 1959) failed to abolish the rise in blood pressure and contracture of the nictitating membrane produced by 3 mg/kg 3-phenoxypropylguanidine (Fig.

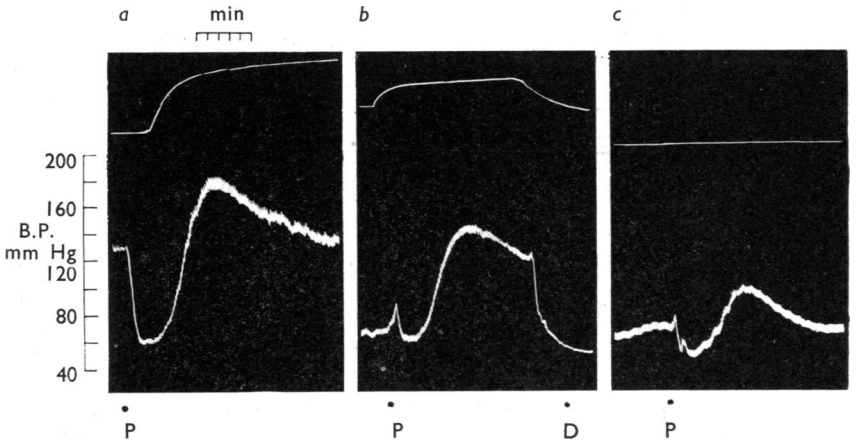

Fig. 10. Effect of 3 mg/kg 3-phenoxypropylguanidine (P) on arterial blood pressure (lower record) and nictitating membrane (upper record) of (*a*) a chloralosed cat, (*b*) an adrenalectomized chloralosed cat pretreated with bretylium (15 mg/kg), and (*c*) a chloralosed cat pretreated with reserpine. In (*b*), the actions of 3-phenoxypropylguanidine were reversed by 2 mg/kg phenoxybenzamine (D).

10b). However, in two other cats after daily subcutaneous injections of reserpine (2 mg/kg) for two days before use, 3-phenoxypropylguanidine (3 mg/kg) had very little action on the blood pressure and did not produce a contracture of the nictitating membrane (Fig. 10c).

Effect of 3-phenoxypropylguanidine on the blood glucose concentration and on the hyperglycaemic action of adrenaline. 3-Phenoxypropylguanidine (20 mg/kg) raised the blood glucose concentration of the rabbit by about 23% (Table 2).

TABLE 2

EFFECT OF 3-PHENOXYPROPYLGUANIDINE ON BLOOD GLUCOSE CONCENTRATION

Rabbit	Blood glucose (mg/100 ml.) 18 hr after subcutaneous injection of 0.9% sodium chloride solution	Blood glucose (mg/100 ml.) 18 hr after subcutaneous injection of 3-Phenoxypropyl-guanidine (20 mg/kg)	Increase in blood glucose after 3-phenoxy-propyl-guanidine (mg/100 ml.)	Mean increase	*P* value of mean increase
1	79·0	91·5	12·5		
2	47·0	70·0	23·0		
3	53·5	69·0	15·5	13·7	<0·01
4	50·5	55·5	5·0		
5	66·5	80·5	14·0		
6	64·0	76·0	12·0		

The hyperglycaemic action of adrenaline in the rabbit was potentiated by 3-phenoxypropylguanidine, the rise in blood glucose produced by 50 μg/kg adrenaline being greater after administration of this substance (Table 3). This enhancement of hyperglycaemia was found 1, 2 and 3 hr after adrenaline ($P<0.05$), but was not found at a significant level after 4 and 5 hr ($P>0.1$). Results were obtained from only 5 animals 3, 4 and 5 hr after adrenaline, since in the second part of the experiment one rabbit died in convulsions. This rabbit was very sensitive

TABLE 3

EFFECT OF 3-PHENOXYPROPYLGUANIDINE ON THE HYPERGLYCAEMIC RESPONSE TO 50 μG/KG ADRENALINE

Time (hr after adrenaline)	Mean rise in blood glucose (mg/100 ml. ± s.e.) after adrenaline		Mean increase in adrenaline hyperglycaemia after 3-phenoxypropylguanidine	P value of mean increase
	Control	18 hr after 20 mg/kg 3-phenoxypropylguanidine		
1	45·3± 9·3 (6)	59·9±10·7 (6)	14·6± 8·0 (6)	<0·05
2	77·4±17·3 (6)	116·2±15·1 (6)	38·8±12·0 (6)	<0·025
3	63·8±17·5 (5)	106·9±21·7 (5)	43·1±12·2 (5)	<0·05
4	45·0±15·0 (5)	71·2±15·5 (5)	26·2± 8·1 (5)	>0·1
5	35·0±10·9 (5)	38·3±10·3 (5)	3·3± 8·2 (5)	>0·25

to adrenaline, its blood glucose rising from a fasting level of 69 mg/100 ml. to 244 mg/100 ml. just before death. None of the other rabbits showed signs of discomfort after adrenaline.

DISCUSSION

3-Phenoxypropylguanidine potentiated the actions of adrenaline and noradrenaline on the isolated rat seminal vesicle, the perfused rabbit ear, the blood pressure and the nictitating membrane of the cat. Potentiation of adrenaline by 3-phenoxypropylguanidine was not confined to smooth muscle, since adrenaline hyperglycaemia was increased by this substance.

The potentiation of catechol amines by 3-phenoxypropylguanidine was only one way in which it resembled cocaine. In addition it reduced the appetite of the cat, produced a local anaesthesia of long duration in guinea-pig skin, and it inhibited the pressor action of tyramine in the cat and the vasoconstrictor action of tyramine in the rabbit ear. However, 3-phenoxypropylguanidine did not anaesthetize the rabbit cornea or abolish the stimulant action of tyramine on the nictitating membrane.

The inhibition of tyramine by 3-phenoxypropylguanidine was compared with the antityramine actions of cocaine (Tainter & Chang, 1927), guanethidine (Maxwell, Plummer, Povalski & Schneider, 1960) and hexahydro-1-azepine-propionamidoxime (Maxwell, Plummer, Daniel, Schneider & Povalski, 1958). Doses of these drugs producing a 50% inhibition of the pressor action of tyramine in spinal preparations of cats were calculated to be: 3-phenoxypropylguanidine 0.3 mg/kg, cocaine 1.0 mg/kg, guanethidine 7.2 mg/kg, and hexahydro-1-azepine-propionamidoxime 15.0 mg/kg. Cocaine was the only substance to approach the potency of 3-phenoxypropylguanidine as an inhibitor of tyramine. Furthermore, experiments with the rat seminal vesicle, the guinea-pig skin and the spinal cat showed that the actions of 3-phenoxypropylguanidine were more prolonged than those of cocaine.

Recent work has thrown some light on the way in which cocaine potentiates catechol amines and inhibits tyramine. Cocaine retards the rate of disappearance of noradrenaline from the plasma (Trendelenburg, 1959; Whitby, Hertting & Axelrod, 1960), and inhibits its fixation to tissues such as heart and spleen (Whitby, Hertting & Axelrod, 1960; Muscholl, 1961). Muscholl (1961) found a direct

relationship between the potentiation of the pressor action of noradrenaline and the inhibition of the uptake of noradrenaline by the myocardium after cocaine. This suggested that tissue fixation contributed substantially towards the pharmacological inactivation of the catechol amines, the fixed amines being unable to stimulate receptors. Tyramine produces its actions indirectly by releasing adrenaline and noradrenaline into plasma, and the release of catechol amines by tyramine is abolished by cocaine (Lockett & Eakins, 1960a and b). In displacing tissue-bound catechol amines, tyramine must itself become fixed to the tissues. Since cocaine inhibits the uptake of catechol amines by the tissues, it is not unlikely that it would inhibit an uptake of tyramine by the tissues. If this is so it explains how cocaine potentiates catechol amines and inhibits tyramine. Cocaine would potentiate catechol amines, since an inhibition of their fixation to tissue would prolong their free pharmacologically active life, but cocaine would inhibit tyramine since an inhibition of the tissue fixation of tyramine would decrease its ability to release inactive tissue-bound catechol amines. The effect of 3-phenoxypropylguanidine on the pressor and vasoconstrictor actions of noradrenaline and tyramine can be explained in this way if one assumes that it also inhibits the tissue fixation of the sympathomimetic amines. In addition, many of the direct actions of 3-phenoxypropylguanidine may be explained if one accepts this hypothetical mode of action.

3-Phenoxypropylguanidine did not abolish the nicotinic action of acetylcholine in the atropinized spinal cat, even after exclusion of the adrenal glands from the circulation. Furthermore, stimulation of the great auricular nerve in the rabbit ear or the preganglionic cervical sympathetic nerve in the cat still produced a vasoconstriction in the rabbit ear or a contraction of the nictitating membrane in the cat when the cardiovascular actions of tyramine had been abolished in these preparations by 3-phenoxypropylguanidine. As 3-phenoxypropylguanidine did not prevent the sympathomimetic response to acetylcholine and to stimulation of sympathetic nerves, it was not surprising to find that it produced sympathomimetic actions itself. These would be the outcome of the inhibited binding of the endogenously released catechol amines.

The pressor action of 3-phenoxypropylguanidine and its stimulant action on the nictitating membrane of the cat were antagonized by phenoxybenzamine and were diminished in reserpinized animals. These actions of 3-phenoxypropylguanidine were peripheral in origin, since they occurred in spinal preparations. Further, they were independent of functional adrenergic nerves and adrenals, since bilateral adrenalectomy and blockade of adrenergic nerve fibres with bretylium did not abolish them. It was concluded that the sympathomimetic actions of 3-phenoxypropylguanidine were produced either by a potentiation of the actions of circulating catechol amines or by a release of catechol amines from a peripheral site other than in nerve or adrenals. Neither of these explanations can be excluded, but the analogy with cocaine favours the former possibility. The opening of the eyes of the reserpinized mouse, the dilatation of the pupil of the cat and the increase in the blood glucose concentration of the rabbit may also be explained in this way.

According to McCubbin, Kaneko & Page (1961) guanethidine raises blood pressure through the release of endogenous catechol amines, but other experiments with

spinal cats suggest that when the intravenous dose of guanethidine exceeds 4 mg/kg additional factors influence the pressor response (Bartlet, 1962). In the present experiments the pressor action of 2 mg/kg guanethidine was reversed by 3-phenoxypropylguanidine, which suggests that the action on blood pressure of a small dose of guanethidine is similar to that of tyramine.

The antagonism of tryptamine and 5-hydroxytryptamine by 3-phenoxypropylguanidine on the rat fundus showed that this compound was not cocaine-like in all respects, since cocaine potentiated the actions of tryptamine and 5-hydroxytryptamine. However, the potentiation of tryptamine and 5-hydroxytryptamine by cocaine remains unexplained. It does not appear to be due to inhibition of monoamine oxidase by cocaine (Philpot, 1940), since inhibition of this enzyme potentiates tryptamine but not 5-hydroxytryptamine (Vane, 1959), whereas cocaine potentiated both amines to the same extent.

The prolonged hypersensitivity to catechol amines produced by 3-phenoxypropylguanidine may be of use in the biological estimation of adrenaline and noradrenaline, especially in the estimation of a catechol amine in the presence of a sympathomimetic amine with an indirect action (Burn & Rand, 1958), since tyramine was inhibited by 3-phenoxypropylguanidine.

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