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PHARMACOLOGICAL PROPERTIES OF PEMPIDINE (1:2:2:6:6-PENTAMETHYLPIPERIDINE), A NEW GANGLION-BLOCKING COMPOUND

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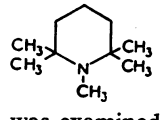
Pempidine (1:2:2:6:6-pentamethylpiperidine) is a long-acting ganglion-blocking compound which is effective by mouth. By intravenous injection it has a similar potency to hexamethonium on the preganglionically stimulated nictitating membrane of the cat. The compound blocks the effects of intravenous nicotine and of peripheral vagal stimulation on the blood pressure; it also causes dilatation of the pupil after removal of the sympathetic innervation. On the guinea-pig ileum, the predominant effect of the compound is to inhibit nicotine contractions. Pempidine is well absorbed from the gastro-intestinal tract as judged by (*a*) the low ratio (6.9) of oral to intravenous toxicities, (*b*) the rapid development of mydriasis in mice after oral administration of small doses, and (*c*) the rapid onset of hypotension when the compound is injected directly into the duodenum of anaesthetized cats. Other actions include neuromuscular paralysis of curare-like type when large doses of the compound are injected intravenously and central effects such as tremors which occur with near toxic doses. In cats with a low blood pressure, large intravenous doses have a slight pressor action.

Until recently ganglion-blocking compounds of high potency and specificity were thought to be confined to polymethylene bis-quaternary ammonium series, such as hexamethonium (Paton and Zaimis, 1951) and pentolinium (Mason and Wien, 1955). The discovery by Stone, Torchiana, Navarro, and Beyer (1956) that mecamylamine was a potent ganglion-blocking compound revealed that this property was not dependent on the presence of a quaternary nitrogen atom in the molecule. Furthermore, mecamylamine, a secondary amine, was found to be well absorbed from the gastro-intestinal tract and to have a prolonged action.

We have found that several secondary and tertiary amines have potent ganglion-blocking properties and this paper describes the properties of one of them, pempidine (1:2:2:6:6-pentamethylpiperidine).



This compound was examined as a result of an investigation into the structural requirements for orally effective ganglion-blocking compounds with a comparatively long duration of action (Lee, Wragg, Corne, Edge, and Reading, 1958), and it has been described independently by Spinks and Young (1958).

METHODS

Cats were anaesthetized with ether or thiopentone, which was followed by chloralose (80 mg./kg.).

Supramaximal rectangular pulses of 0.5 msec. duration were used to stimulate nerves. In addition to continuous stimulation at a frequency of 10/sec. of the cervical sympathetic nerve trunk, intermittent stimulation at frequencies of 20 or 50/sec. was also applied for 5 sec./min. The peripheral end of the cut right vagus nerve was stimulated at frequencies of 50/sec. The peripheral stump of the cut sciatic nerve of the cat was stimulated at frequencies of 10/min. and 50/sec.

The superior cervical ganglion was perfused by the method of Kibjakow (1933) as used by Emmelin and MacIntosh (1956) using double-dextrose Locke solution containing physostigmine salicylate (10^{-5}). The preganglionic nerve was stimulated at a frequency of 10/sec. for periods of 3 min. and the samples were assayed against acetylcholine chloride on the blood pressure of the eviscerated cat previously treated with mepyramine and physostigmine.

The responses of the pupil of the cat were determined after removal of the ipsilateral superior cervical ganglion (Mason and Wien, 1955).

The isolated guinea-pig ileum was set up in oxygenated Tyrode solution at 37°. Agonists were allowed to act for 30 sec. in a 3 min. cycle and the bath was washed out twice in each cycle. In other

experiments the method of Trendelenburg (1917) was employed.

Langendorff preparations of the rabbit heart were perfused with oxygenated double-dextrose Locke solution at 37°. Drugs were dissolved in the perfusate before injection into the arterial cannula.

The isolated rectus abdominis muscle of the frog was set up in a bath of oxygenated frog Ringer solution at room temperature.

Mydriatic responses were studied in groups of five albino mice (Edge, 1953). The compounds were administered orally.

The effect on nicotine-induced convulsions was studied in groups of 10 albino mice and graphical estimates of the ED50 obtained. Nicotine was injected intravenously 30 min. after an intraperitoneal injection of a ganglion-blocking agent.

The method of Haley and McCormick (1957) was used to study the effect of compounds injected intracerebroventricularly in mice, the drugs being dissolved in 0.9% w/v NaCl solution and injected in volumes of 0.01 to 0.05 ml.

Acute toxicity experiments were performed in groups of 10 albino mice and rats, and the parameters estimated graphically by the method of de Beer (1945).

Pempidine (1:2:2:6:6-pentamethylpiperidine) was used as the hydrochloride (or, in a few experiments, as the hydrogen tartrate). Mecamylamine was used as the hydrochloride, hexamethonium as the bromide, and pentolinium as the hydrogen tartrate. The doses of these four compounds and of adrenaline hydrochloride refer to the cation. The doses of the following compounds refer to their salts unless otherwise stated in the text: decamethonium iodide, tubocurarine and acetylcholine chloride, nicotine hydrogen tartrate, histamine acid phosphate, pilocarpine nitrate, atropine sulphate, mepyramine maleate, physostigmine salicylate, neostigmine methylsulphate, and phenoxybenzamine [benzyl-2-chloroethyl (1-methyl-2-phenoxyethyl) amine hydrochloride].

RESULTS

Action on the Superior Cervical Ganglion of the Cat

An intravenous injection of pempidine or of mecamylamine caused relaxation of the preganglionically stimulated nictitating membrane. This relaxation was slower in development than with hexamethonium (Fig. 1), and, having reached a maximum, recovery was usually very prolonged so that often there was only a very slight recovery over a period of 30 min. or more. In an attempt to follow the course of recovery after an injection, we applied intermittent stimulation to the nerve, but in general it was found that complete recovery of the response did not occur and that a period of rest was required before the response returned. In this respect the experiment illustrated in Fig. 1 is not typical since there was practically complete recovery of the response with no intervening rest period. In this experiment the time required for 50% recovery was, after pempidine, about eight times that for hexamethonium.

The duration of action of pempidine and mecamylamine on the superior cervical ganglion was also relatively prolonged when they were injected into the cannulated lingual artery during occlusion of the external carotid artery.

Having once given an effective intravenous dose of one of these long-acting compounds, a further dose of any ganglion-blocking compound given within a period of 2 to 4 hr. was markedly potentiated. It was therefore difficult to obtain valid graded responses. To determine the potency we first obtained graded responses to hexamethonium and then gave two injections of the long-acting compound separated by a period of at least 2 hr.

It is with these limitations that we assign, on the basis of 13 experiments, a potency to pempidine of 1.35 times that of hexamethonium. In a series of five similar experiments mecamylamine had a potency of 1.22.

Pempidine had no stimulant action on the relaxed nictitating membrane in doses up to 8 mg./kg.

Bennett, Tyler, and Zaimis (1957) found that, after a dose of mecamylamine sufficient to cause a partial block of transmission to the nictitating membrane, tetanic stimulation of the preganglionic

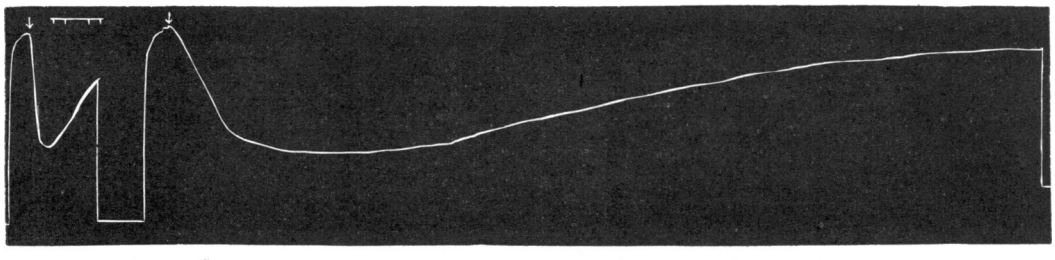


FIG. 1.—Cat, 3.40 kg., chloralose anaesthesia. Tracing of response of the preganglionically stimulated nictitating membrane. At first arrow, 56 μg./kg. hexamethonium intravenously. At second arrow, 51 μg./kg. pempidine intravenously. Time, 1 min.

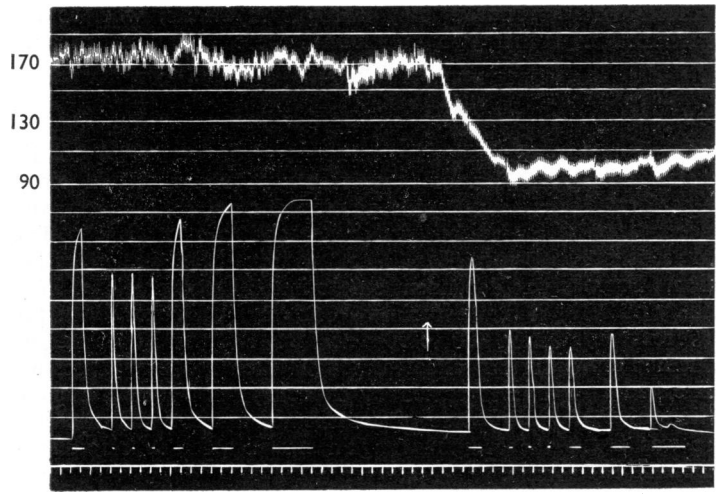


FIG. 2.—Cat, 2.70 kg., thiopentone sodium (10 mg./kg.) followed by chloralose (60 mg./kg.) anaesthesia. Upper tracing, blood pressure; middle tracing, contractions of nictitating membrane stimulated preganglionically for 5, 30, 60, and 120 sec. as indicated. Lowest tracing, time in 30 sec. intervals. At arrow, 0.1 mg./kg. pempidine intravenously. Vertical scale, blood pressure (mm. Hg).

nerve caused a reduced but fully maintained contraction. We have failed to confirm this observation with either mecamylamine or pempidine although various doses and time intervals after injection have been tested. In our experiments with mecamylamine and pempidine, the contractions of the nictitating membrane waned rapidly as with hexamethonium (Fig. 2).

Site of Action.—Pempidine was found to act specifically at the ganglion and not on the nictitating membrane itself, nor on the postganglionic nerve. Thus an intravenous dose of 8 mg./kg. caused complete relaxation of the nictitating membrane stimulated preganglionically whereas there was no diminution of the response to adrenaline injected intravenously nor to stimulation of the postganglionic nerve (Fig. 3).

Effect on Release of Acetylcholine.—There was evidence that large doses of pempidine reduced the output of acetylcholine from the perfused superior cervical ganglion during preganglionic stimulation. The results of one experiment are shown graphically in Fig. 4. During periods with no stimulation there was no detectable acetylcholine present in the samples (less than 3 ng./ml.). The activity in the first two samples collected during stimulation was equivalent to 13 and 15 ng./ml. respectively (totally 57 and 54 ng.). After the injection of 10 mg. of pempidine into the arterial cannula, the two samples collected during stimulation, which failed to cause a contraction of the nictitating membrane, each contained 10 ng./ml. (totally 32 and 31 ng.). However, this is still a

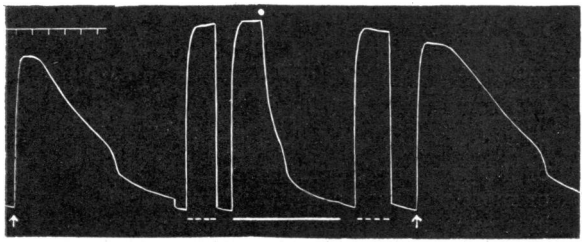


FIG. 3.—Cat, 2.25 kg., chloralose anaesthesia. Contractions of nictitating membrane. At arrows, 100 μg. adrenaline was given intravenously; the solid line indicates preganglionic stimulation at 10/sec.; the broken line indicates postganglionic stimulation at 10/sec.; at the white dot, 8 mg./kg. pempidine was administered intravenously. Time, 30 sec.

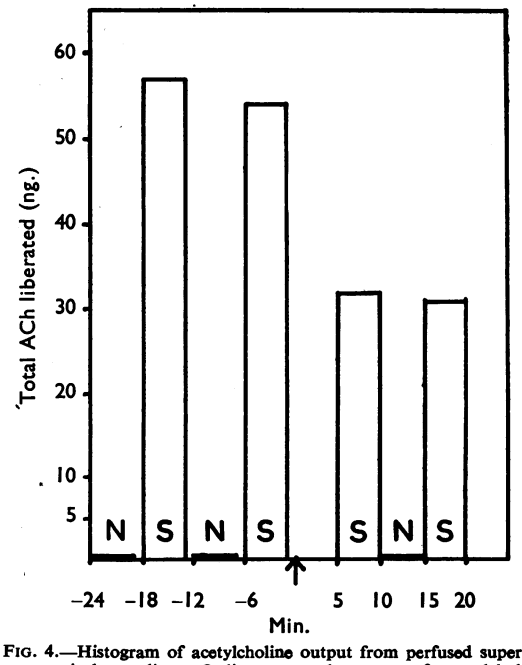


FIG. 4.—Histogram of acetylcholine output from perfused superior cervical ganglion. Ordinates, total output of acetylcholine during 5 min. collection periods. Abscissae, time in min. before and after the injection of 10 mg. pempidine at the arrow. N, no stimulation; S, preganglionic nerve stimulated for 3 min. at 10/sec.

high output of acetylcholine and could not account for the complete block in transmission which was observed.

Effect on the Ciliary Ganglion

Mydriasis in Anaesthetized Cats.—Experiments to compare the effect of pempidine and hexamethonium on pupillary diameter were performed in cats. As with the nictitating membrane preparation it was difficult to obtain graded responses to pempidine due to its prolonged action. From five experiments pempidine had an activity of 2.0 to 4.0 and a duration of action (50% recovery time) of 1.5 to 10.0 times that of hexamethonium. In two other experiments mecamylamine had an activity of 1.5 and a duration of action of 5.0 times that of hexamethonium. There was thus no indication that either of these compounds had a useful differential action on parasympathetic ganglia.

Mydriasis in Mice.—When pempidine or mecamylamine were administered orally to mice, mydriasis was maximal at 10 to 20 min. and persisted for up to 4 hr. Fig. 5 shows the results of two experiments, in the first of which 4 mg./kg. of mecamylamine was compared with 56 mg./kg. of hexamethonium, and in the second experiment the effects of 2 and 4 mg./kg. of pempidine were compared. The large dose of hexamethonium produced only a slight mydriasis, whereas the other two compounds were highly effective in much smaller doses.

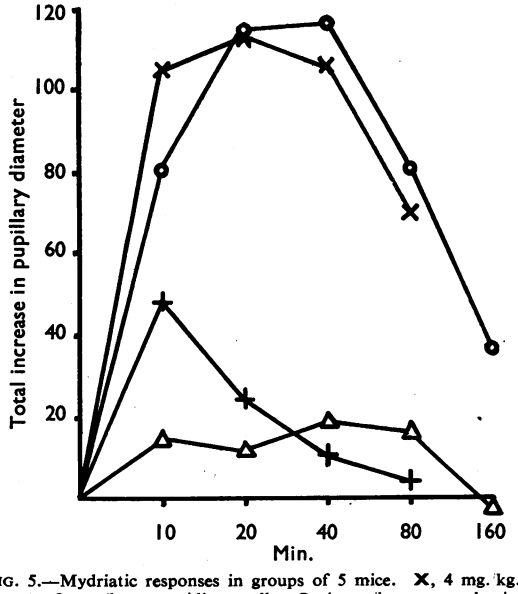


FIG. 5.—Mydriatic responses in groups of 5 mice. X, 4 mg./kg., +, 2 mg./kg. pempidine orally; O, 4 mg./kg. mecamylamine orally; Δ, 56 mg./kg. hexamethonium orally. Ordinates, total increase in pupillary diameter (arbitrary units); abscissae, time in min. after dosing.

Action on the Cardiovascular System

In vivo *Experiments.*—Doses of 0.05 to 1.0 mg./kg. of pempidine produced a fall in blood pressure which developed more slowly than with hexamethonium and reached a maximum in 3 to 10 min. (Fig. 2). As with hexamethonium the fall was dependent on the initial blood pressure. Thus in one experiment 0.25 mg./kg. produced a fall from 200 to 90 mm. Hg. A second dose had no further effect although when the blood pressure was increased by inducing anoxia with a paralysing dose of decamethonium the same dose of the compound again caused a fall in blood pressure.

It was difficult to produce a fall in blood pressure in anaesthetized cats when the compound was administered orally. However, when injected directly into the duodenum the subsequent fall in blood pressure became maximal at 30 min. In four cats and one dog 0.5 mg./kg. of pempidine, administered in this way, produced falls in blood pressure from 100–160 to 70–100 mm. Hg. In another cat 0.2 mg./kg. produced a fall from 180 to 90 mm. Hg, and in all these experiments the effect was maintained for at least 5 hr.

Up to 10 mg./kg. of pempidine injected intravenously was without effect on the depressor actions of acetylcholine and histamine and, as with other ganglion-blocking compounds, the pressor response to adrenaline was potentiated. The nicotine pressor effect was abolished by a dose of 0.1 mg./kg. and in another experiment a dose of 0.025 mg./kg. caused a 47% reduction in the response produced by 75 μg./kg. of nicotine injected every 15 min. This reduction in the nicotine response was prolonged, 50% recovery occurring only after 2 hr.

In fully atropinized cats, doses up to 64 mg./kg. caused no delayed depressor response, thus indicating the absence of histamine releasing properties. On the contrary when the blood pressure was initially low or had already been depressed to below 80 to 100 mm. Hg by a previous dose of the compound, intravenous doses of 4 mg./kg. or more produced a pressor response which commenced 5 to 10 sec. after injection and having reached a peak within 10 to 60 sec. declined over a period of 5 to 15 min. Fig. 6*a* shows an experiment in which the initial blood pressure was 80 mm. Hg. A dose of 16 mg./kg. of pempidine produced a rise of 50 mm. Hg which then declined

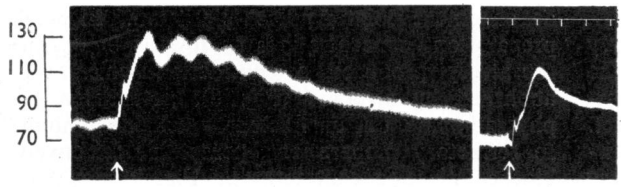


FIG. 6.—Cat, 2.50 kg., chloralose anaesthesia. Blood pressure (*a*), before; (*b*), after acute bilateral adrenalectomy. At arrows, 16 mg./kg. pempidine. Vertical scale, blood pressure (mm. Hg). Time, 1 min.

over a period of 15 min. This pressor effect contrasts with the effect of large doses of mecamylamine (Payne and Rowe, 1957), which invariably caused a further transient fall in blood pressure. In a few experiments the pressor response to large doses of pempidine was preceded by a short-lasting fall in blood pressure similar to that seen with mecamylamine.

The pressor action of pempidine could not be attributed to the release of adrenaline from the adrenal medulla, since it was very rapid in onset, was not abolished by phenoxybenzamine, nor by bilateral adrenalectomy (Fig. 6*b*). After bilateral vagotomy, and in spinal or pithed preparations, the effect was still present although very slight and evisceration of the animal did not abolish the response.

A dose of 0.4 mg./kg. abolished the bradycardia and depressor effect of peripheral vagal stimulation, although much larger doses did not prevent the effects of an injection of acetylcholine.

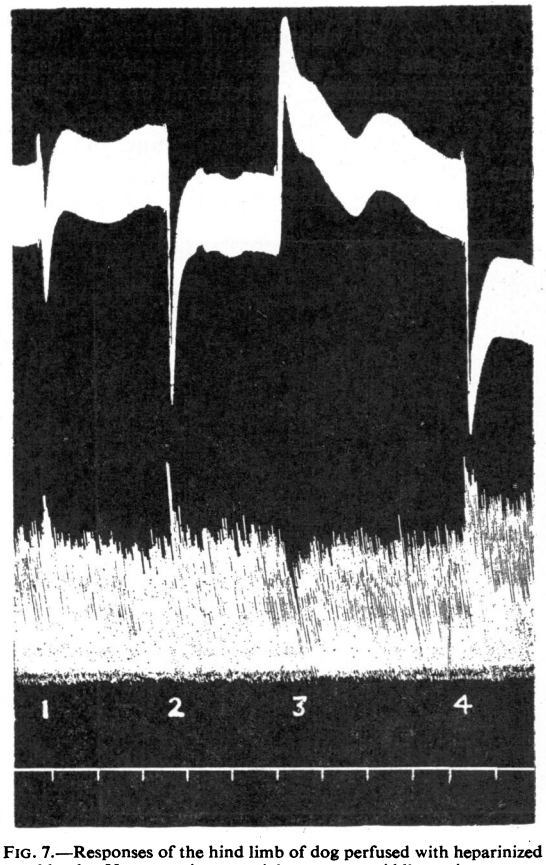


FIG. 7.—Responses of the hind limb of dog perfused with heparinized blood. Upper tracing, arterial pressure; middle tracing, venous outflow. Lowest tracing, time in 1 min. intervals. At **1**, 32 mg. of pempidine; **2**, 1 μg. of acetylcholine; **3**, 1 μg. of adrenaline; **4**, 8 mg. of mecamylamine.

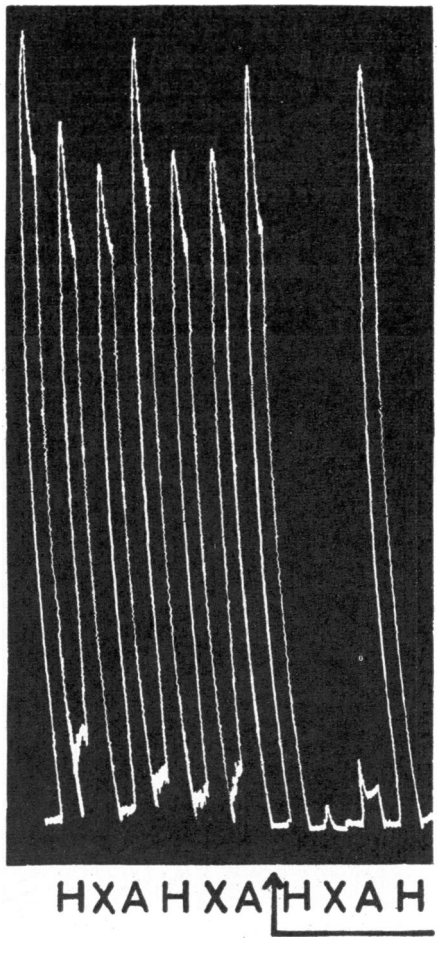


FIG. 8.—Contractions of isolated guinea-pig ileum in a 2 ml. bath. At **H**, 0.05 μg./ml. histamine; **X**, 8.0 μg./ml. pempidine; **A**, 0.25 μg./ml. acetylcholine. The arrow indicates the presence of 0.1 μg./ml. atropine in the bath fluid.

In vitro *Experiments.*—On the isolated rabbit heart about 16 mg. of pempidine injected directly into the aortic cannula was required to arrest the heart for about 30 sec. About 8 mg. of mecamylamine was required to produce a similar effect. Smaller doses of either compound caused a slowing and decrease in amplitude of the beat. These effects have been described for mecamylamine by Bennett *et al.* (1957), using the isolated cat heart which was arrested by 1 mg. of the drug. Spinks and Young (1958) found that a concentration of 0.01 mg./ml. of pempidine in the perfusion fluid caused a decrease in the coronary flow.

Perfused Hind Limb of Dog.—In view of the pressor action seen with large doses of pempidine in *in vivo* experiments, the effect of the compound on the vessels of the hind limb of the dog perfused with heparinized blood was determined. Doses of less than about 8 mg. injected into the arterial cannula were without effect. Larger doses invariably produced vasodilatation (Fig. 7). Vasoconstrictor effects were never observed. Mecamylamine was found to have a considerably greater vasodilator action than pempidine (Fig. 7).

Effects on Isolated Guinea-pig Ileum

In most preparations, pempidine in concentrations up to 800 μg./ml. had little or no stimulant action on the isolated guinea-pig ileum. In other preparations concentrations as low as 8 μg./ml. repeatedly produced a contraction which was abolished by atropine (Fig. 8), potentiated by physostigmine, but unaffected by hexamethonium. In refractory preparations, pempidine selectively blocked nicotine contractions. Fig. 9 shows an experiment in which 0.8 μg./ml. of pempidine was present in the bath for 17.5 min. The response to nicotine was inhibited, leaving contractions to histamine, pilocarpine, and acetylcholine unaffected. After pempidine had been removed from the bath the nicotine response was slow to recover, so that even in *in vitro* preparations the compound has a prolonged action. A positive correlation between concentration and recovery of the nicotine response was observed (Table I).

When the concentration of pempidine was increased, the responses to other agonists were reduced. Thus in an experiment in which 80 μg./ml. of pempidine was present for 14.5 min., the responses to nicotine and histamine were abolished and the responses to pilocarpine and acetylcholine reduced. On removal of the antagonist, the responses to the latter two compounds rapidly returned but those to nicotine and histamine remained depressed for several hours. Spinks and Young (1958) have also reported that

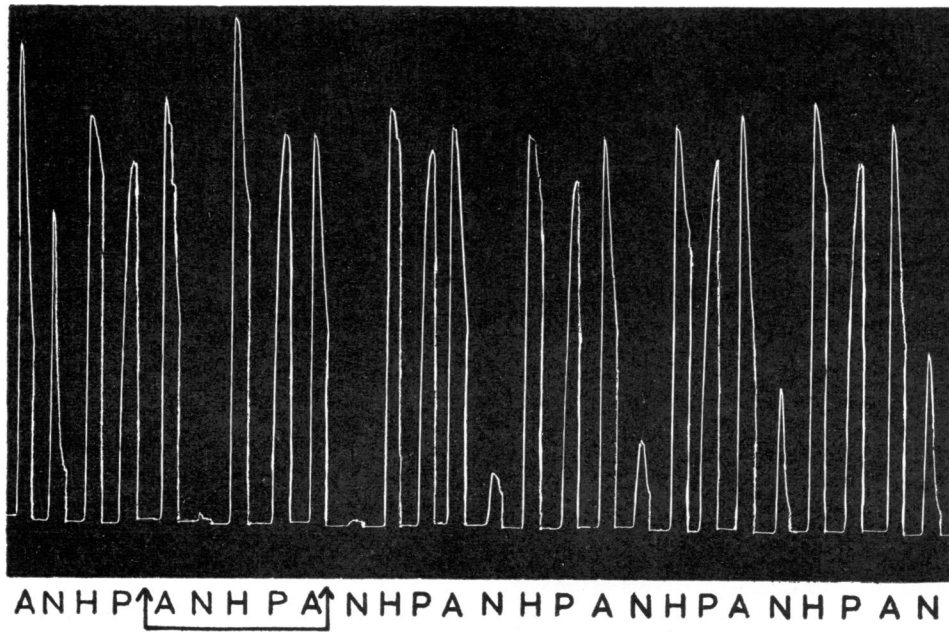


FIG. 9.—Contractions of isolated guinea-pig ileum in a 10 ml. bath. At A, 0.05 μg./ml. of acetylcholine; N, 5.0 μg./ml. of nicotine; H, 0.1 μg./ml. of histamine; P, 1.0 μg./ml. of pilocarpine. Between arrows, 0.8 μg./ml. of pempidine was present.

high concentrations of pempidine inhibit responses to 5-hydroxytryptamine.

In Trendelenburg preparations of the isolated guinea-pig ileum, pempidine (1 μg./ml.) inhibited peristaltic waves without preventing contraction of the longitudinal muscle.

Effect of Nicotine on Histamine Responses.—In the experiments in which the duration of action of pempidine on nicotine responses was studied (Table I) nicotine was placed in the bath after

TABLE I

RELATIONSHIP BETWEEN CONCENTRATION OF PEMPIDINE AND TIME FOR 50% RECOVERY OF NICOTINE RESPONSES OF GUINEA-PIG ILEUM

The contact time for pempidine was 14.5 min. Each numeral is the mean of 3 experiments and ± standard deviation is shown.

Conc. μg./ml. ..	0·8	2·6	8·0
Time for 50% recovery (min.) ..	12·5±3·8	58·3±3·6	110·5±48·0

three consecutive histamine responses. Following a nicotine response there was often some reduction in the succeeding one or two responses to histamine and therefore each nicotine response was expressed as % of the immediately preceding histamine response. In some preparations the effect of nicotine on the histamine response was sufficiently great to make the preparation unsuitable for further use, and even small doses of nicotine, which themselves produced no contraction, reduced the succeeding response to histamine. Nicotine was not observed to have this effect on acetylcholine responses.

Effect on Neuromuscular Transmission

Effect on Sciatic Nerve-Tibialis Muscle Preparation in the Cat.—Intravenous doses of 10 to 40 mg./kg. of pempidine were required to block the response of the indirectly stimulated tibialis muscle. The block resembled that due to tubocurarine since tetanic stimulation failed to produce a sustained contraction, was followed by marked potentiation of the twitch tension and neostigmine reversed the block. Pempidine resembled mecamylamine potentiating the effect of tubocurarine and antagonizing the effect of decamethonium (Bennett *et al.*, 1957).

Effect on Rectus Abdominis Muscle of the Frog.—On this preparation pempidine showed no stimulant properties in concentrations up to 0.8 mg./ml. although this concentration inhibited acetylcholine-induced contractures.

Anticholinesterase Activity.—This was determined by the method of Hobbiger (1951) using washed erythrocytes obtained from fresh defibrinated horse blood as a source of true acetylcholinesterase free from pseudo-esterase activity. Under similar conditions, pempidine was found to be about 10^6 times less active than neostigmine in inhibiting this enzyme system. Thus 50% inhibition was produced by a concentration of 3.6×10^{-8} M-neostigmine whereas a concentration of 2.1×10^{-2} M-pempidine was required to produce the same result.

Effect on Nicotine Convulsions in Mice

Several ganglion-blocking compounds were examined for their ability to protect mice from convulsions produced by the intravenous injection of 1 mg./kg. of nicotine base. Table II shows the

TABLE II

THE INHIBITORY EFFECT OF GANGLION-BLOCKING COMPOUNDS ON NICOTINE INDUCED CONVULSIONS IN MICE

Compound	ED50 (mg. cation/kg.)	Comparative Activity Against Nicotine Convulsions		Comparative Activity on Cat Nictitating Membrane	
		Hexamethonium =1	Mecamylamine =1	Hexamethonium =1	Mecamylamine =1
Hexamethonium	1·5	1	0·072	1·0	0·82
Pentolinium ..	0·25	6	0·43	4·0	3·3
Mecamylamine	0·108	13·9	1·0	1·22	1·0
Pempidine ..	0·09	16·7	1·2	1·35	1·11

results obtained using two quaternary ammonium compounds and two non-quaternary compounds. Whereas there is a good correlation within each class with activity obtained on the cat nictitating membrane, between the two classes of compound there is no such correlation, hexamethonium and pentolinium being considerably less effective than mecamylamine and pempidine.

Effects on the Central Nervous System

Effects of Intracerebroventricular Injections in Mice.—The effects produced by pempidine were compared with those produced by mecamylamine and hexamethonium. Animals which received physiological saline alone became motionless for about 1 min. immediately after the injection and then resumed normal activity. Following an injection of 10 μg. of hexamethonium the mice became motionless in a hunched up posture. After about 3 min. generalized tremors were seen in some animals. 50 μg. produced dyspnoea, generalized tremors and convulsions lasting for about ½ to 1 hr. Still higher doses increased the severity and duration of these symptoms. These signs are similar to those observed by Haley and McCormick (1957).

Following an injection of 100 μg. of pempidine the animals became motionless, assuming a hunched up posture. No tremors were observed although after a dose of 200 μg. slight tremors were seen in most of the mice. Mecamylamine had a similar effect in the same doses.

All the mice used in these experiments appeared normal after 24 hr. and in no instance did the non-quaternary compounds produce effects approaching the severity of those seen with hexamethonium.

Effect on Knee-jerk and Flexor Reflexes in the Cat.—In cats anaesthetized with chloralose, pempidine had no effect on the knee-jerk or flexor reflex except in doses which also reduced the twitch tension of the contralateral tibialis muscle stimulated peripherally by the sciatic nerve.

Toxicity

A 5% saline solution of the hydrochloride of pempidine injected intracutaneously in guinea-pigs produced no local reaction whereas 2% and 5% solutions of mecamylamine hydrochloride caused erythema and necrosis at the injection site.

The LD50 in mice of pempidine and of mecamylamine are shown in Table III. Our estimate

TABLE III
ACUTE TOXICITY IN MICE

Compound	Route of Injection	LD50 (mg. cation/kg.)	Oral LD50 / Intravenous LD50
Pempidine ..	Oral	275	
	Subcutaneous	134	6·9
	Intravenous	39·5	
Mecamylamine	Oral	97·5	
	Intravenous	12·9	7·6

of the intravenous toxicity of mecamylamine is higher than that found by Stone *et al.* (1956) and consequently we have obtained a higher oral LD50/intravenous LD50 ratio than these authors. In our experiments the intravenous injections were given at a rate of 1.0 ml./15 to 20 sec. and, as the acute toxicity is related to the speed of injection, this may account for the difference between results. By both oral and intravenous routes pempidine was about one-third as toxic as mecamylamine.

Toxic effects after oral administration of either compound appeared within minutes. In addition to dyspnoea and clonic convulsions, large doses caused generalized tremors and slight ataxia. In rats the acute oral LD50 of pempidine was 470 mg./kg.

When large doses of either compound were administered orally to young rats, inhibition of growth occurred. Table IV shows % inhibition of growth after 10 daily doses. The last column of Table IV shows the calculated dose to produce 50% inhibition of growth.

TABLE IV
EFFECT ON GROWTH RATE OF YOUNG RATS

Compound	Daily Dose (mg. cation/kg.)	% Inhibition of Growth	Deaths	Calculated Dose to Produce 50% Inhibition of Growth (mg. cation/kg.)
Pempidine ..	40	45·3	0/5	48·0
	80	64·0	1/5	
	160	95·6	3/5	
Mecamylamine	15	47·9	0/5	
	30	63·6	1/5	16·5
	60	89·6	4/5	

No abnormalities occurred in the blood pictures of a group of guinea-pigs injected subcutaneously for 4 weeks (5 times/week) with 4 mg./kg. of pempidine. The tissues from groups of rabbits, guinea-pigs, and rats which had received up to 4 weeks' treatment with the compound were kindly examined by Dr. R. Williamson and Professor C. V. Harrison. Guinea-pigs and rabbits which had received 6.4 mg./kg. subcutaneously and intravenously respectively and rats which had received 12.8 mg./kg. orally were examined by Dr. Williamson, who reported: " In all the animals (rabbits, guinea-pigs, and rats) sections of the liver, kidneys, heart, lungs, spleen, pancreas, suprarenals, and bone marrow were examined. In addition sections of the spinal cord were examined in the rabbits and guinea-pigs. At post-mortem examination no gross changes were observed in the organs of any of the animals. Histological examination did not reveal any significant pathological lesion (one rabbit and one rat had mild inflammatory changes in the bronchi). . . . In my opinion (the compound) has not produced any significant histological lesions in any of the organs of the animals examined."

Professor Harrison reported on guinea-pigs and rabbits which had received 12.8 mg./kg. subcutaneously and intravenously respectively and rats which had received 51 mg./kg. orally for 1½ weeks. The tissues examined were: salivary gland, oesophagus, stomach, jejunum, pancreas, liver, gall bladder, lung, spleen, bone marrow, heart muscle, voluntary muscle and included nerves, cervical lymph node, kidney (including pelvis), fallopian tube, ovary, testis, suprarenal,

thyroid, parathyroid, and brain. Professor Harrison concluded that "all tissues examined appeared healthy except for an occasional focus of foreign body reaction in the lungs."

Discussion

Although non-quaternary ammonium ganglion-blocking compounds have been known for some time, the first such compound shown to possess a high specificity of action combined with a high potency was mecamylamine (Stone *et al.*, 1956). The present compound, pempidine, like those described by Koppanyi and Vivino (1946), Norton and Phillips (1953) and Phillips (1955, 1957), is a piperidine derivative and a tertiary amine whereas mecamylamine is a secondary amine and an isocamphane derivative. However, the secondary amine corresponding to pempidine possesses similar activity and the quaternated compound is also a potent (3 times hexamethonium) but short acting ganglion-blocking compound (Corne and Edge, unpublished observation ; Spinks and Young, 1958).

Pempidine, although not devoid of other actions, is a selective ganglion-blocking compound and combines a prolonged duration of action with good absorption from the gastro-intestinal tract as can be judged from the low ratio of oral to intravenous toxicity, the rapidity with which mydriasis develops after oral administration of small doses to mice and the effectiveness of the hypotensive properties of the compound when introduced directly into the duodenum of the anaesthetized cat. Furthermore, there is a rapid rise in the blood concentration after oral administration to rats (Reading, personal communication). The weak muscarinic properties which can sometimes be seen on the isolated guinea-pig ileum would tend to counteract the parasympathetic ganglion-blocking activity of the compound and suggests that a reduced incidence of parasympathetic side-effects may be encountered clinically. This stimulant action on the isolated ileum was potentiated by physostigmine and this suggests that the effect may be due not to an acetylcholine-like action but to the liberation of acetylcholine (Renshaw, Green and Ziff, 1938).

The prolonged duration of action of these amines and their chemical dissimilarity to the quaternary ammonium compounds suggests that the two classes of compound may have fundamentally different modes of action. Using mecamylamine, Bennett *et al.* (1957) have described a difference in the type of response obtained from the preganglionically stimulated nictitating membrane, but we have not been able to confirm this observation and can offer no explanation for the difference observed.

Milne, Rowe, Somers, Meuhrcke, and Crawford (1957), and Payne and Rowe (1957), have shown that certain organs selectively store mecamylamine, whilst Payne and Rowe (1957) have further shown that carbon dioxide inhalation increases the plasma level and potentiates the hypotensive and neuromuscular blocking effects of mecamylamine. Furthermore, Zawoiski, Baer, Braunschweig, Paulson, Shermer, and Beyer (1958) have shown that mecamylamine is excreted into the lumen of the stomach and absorbed by the small intestine so that it undergoes a cycle of absorption and excretion which will also contribute to its prolonged action. However, these mechanisms cannot be solely responsible for the long duration of action of these compounds since small doses injected intra-arterially close to the superior cervical ganglion also exert a more prolonged action than is seen with hexamethonium. These compounds may act intracellularly, or, by virtue of their subsequent slow release from the ganglion cell body, they may block the acetylcholine membrane receptors in the same way as has been postulated for the quaternary ammonium compounds. Alternatively, their prolonged action may be due to stronger binding at the membrane receptors themselves.

There is some evidence (Crawford, cited by Payne, 1957) that mecamylamine inhibits the synthesis of acetylcholine. Although the acetylcholine output of the preganglionically stimulated superior cervical ganglion was not abolished by a large dose of pempidine, inhibition of choline-acetylase could contribute to the long duration of action of these compounds by preventing the replenishment of available acetylcholine. However, the perfusion experiments show that pempidine does not appreciably diminish the release of acetylcholine.

Pempidine has a similar pK_a value (10.4) to mecamylamine (11.3) and is also lipoid soluble so that it is likely to have similar distribution and excretion characteristics to mecamylamine. However, there is evidence (Harington, Kincaid-Smith and Milne, 1958) that pempidine exhibits less protein-binding than does mecamylamine and this will undoubtedly modify its distribution and excretion pattern, and hence its duration of action. Blood and urine levels in both rats (Reading, personal communication) and patients have shown that pempidine is excreted more rapidly than mecamylamine and in clinical use pempidine has been

found to have a less prolonged action than mecamylamine (Harington *et al.*, 1958).

The cause of the rise in blood pressure which can be seen with large doses of pempidine remains obscure since it occurs against a background of complete ganglion paralysis and to a very slight extent in preparations in which the central nervous system is completely destroyed. It cannot be attributed to the release of adrenaline from the adrenal medulla since it occurred after adrenalectomy, and Spinks and Young (1958) have shown that small doses inhibit the effects of stimulation of the splanchnic nerve. Lockett (1949) found that the pressor action of piperidine was not greatly affected by adrenalectomy but was considerably reduced by acute total sympathectomy and reversed by dibenamine. It could be that pempidine in large doses overcomes its own ganglion-blocking action and stimulates sympathetic ganglia, but phenoxybenzamine did not inhibit the effect and no evidence of ganglion stimulation was obtained during perfusion of the superior cervical ganglion. Unless there is a species difference the pressor response cannot be accounted for by a direct vasoconstrictor action since vasodilatation was the only response observed during perfusion of the hind limb of the dog. In this preparation, mecamylamine was considerably more potent than pempidine in causing vasodilatation, and this, together with its direct inhibitory action on the heart, could account for the sharp fall in blood pressure which occurs when this compound is injected into a preparation in which autonomic paralysis is already maximal.

The facility with which mecamylamine and pempidine enter the central nervous system is indicated by the occurrence of tremors when large doses are given orally or parenterally and it seems likely that ganglion-blocking compounds, which are readily absorbed from the gastro-intestinal tract, will, for similar reasons, exhibit effects on the central nervous system. However, the lower toxicity and more rapid excretion of pempidine suggest that these symptoms may occur less frequently in patients than with mecamylamine. It appears that mecamylamine and pempidine have intrinsically less effect on the brain than the quaternary compounds, hexamethonium and pentolinium, since when given by intracerebroventricular injection, the former compounds were less effective in causing tremors than were the latter compounds.

Laurence and Stacey (1953) showed that quaternary ammonium ganglion-blocking compounds antagonize nicotine convulsions by inhibiting the peripheral release of adrenaline by nicotine. In our experiments mecamylamine and pempidine were found to be considerably more effective than hexamethonium and pentolinium in this property, and it seemed that this enhanced effect may have been due to inhibition of the central effect of nicotine. However, pempidine was found to have no inhibitory effect on convulsions induced by leptazol or strychnine except in near-toxic doses.

Inhibitory effects of nicotine on histamine induced contractions of the guinea-pig ileum have been previously described by several authors (for references see Ambache and Rocha e Silva, 1951), but the effects we have observed appear to be different from those previously described. Ambache and Rocha e Silva (1951) distinguished two separate blocking actions. With large paralysing doses of nicotine, histamine contractions were reduced only whilst the nicotine was still present, whereas after removal of nicotine from the bath the histamine responses were unaffected despite the persistence of nicotine paralysis. With smaller stimulating doses there was a prolonged residual inhibition of histamine responses and this resembled the Cantoni and Eastman (1946) effect following large doses of other agonists. However, we have found that small stimulating and even non-stimulating doses of nicotine will reduce the effect of succeeding histamine responses whilst having no effect on acetylcholine responses. The effect we have observed is analogous to that recently described by Dale (1958), who found that small stimulating doses of acetylcholine inhibit the response of the guinea-pig ileum to histamine.

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