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THE ACTIONS OF HETEROCYCLIC BISQUATERNARY COMPOUNDS, ESPECIALLY OF A PYRROLIDINIUM SERIES

BY

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In a previous paper (Wien and Mason, 1953b) the actions of a series of aromatic bisquaternary compounds were described, and we are now presenting the results on bisquaternary salts containing heterocyclic nuclei, especially of a pyrrolidinium series of compounds. This series was of outstanding interest because of the ganglion-blocking activity of the pentane member, which was about five times as active as hexamethonium. The compounds examined (Libman, Pain, and Slack, 1952) have the general formula $A.(CH_2)_n.A$, where A is a heterocyclic nucleus (1-methylpiperidino, 4-methylmorpholino, and 1-methylpyrrolidino), and from the examination of these and other series it was found that compounds with saturated nuclei were generally more active as ganglion-blocking substances than those with unsaturated nuclei (Wien, 1954b). Some preliminary results on the pyrrolidinium series have already been described (Wien and Mason, 1953a ; Wien, 1954a).

METHODS

These were the same as described previously (Wien and Mason, 1951) with the following additional procedures:

Perfusion of the superior cervical ganglion was performed (Kibjakow, 1933; Feldberg and Gaddum, 1934) using double dextrose Locke solution containing 1:100,000 eserine for the perfusion fluid, and the acetylcholine in the perfusate was assayed on the blood pressure of the eviscerated cat.

For experiments on parasympathetic ganglia in the whole animal two preparations were used, both in the cat under chloralose anaesthesia. In the first, the salivary flow was recorded from Wharton's duct in response to stimulation of the combined chorda tympani and lingual nerves. Square wave stimuli of 0.4 msec. duration at a rate of 5/sec. were used. In the second preparation the effects on the ciliary ganglion were studied using an extension of the method described by Schofield (1952). The superior cervical ganglion was removed on one side. In the presence of light, and after intravenous injection of the test compound, the pupil on the same side dilated in proportion to the degree of block of the ciliary ganglion. On the opposite side, both the ciliary and superior cervical ganglia were removed. This denervated eye provided a control against the possibility of a direct action of the compound on the iris.

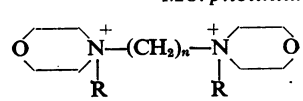
Experiments on neuromuscular transmission were made on the tibialis anterior and soleus muscles (Paton and Zaimis, 1951) as well as on the gastrocnemius of the cat. In addition, the isolated phrenic nerve-diaphragm preparation of the young rabbit was used in some experiments.

Eserine-like properties of the compounds were determined manometrically in the Warburg apparatus at 37° by Ammon's (1933) method, using horse erythrocyte enzyme and acetyl-β-methylcholine as substrate in a final concentration of 0.03 M. The preparation of the enzyme was essentially that of Adams and Thompson (1948).

Our thanks are due to our colleague, Dr. G. Fraser, who carried out the estimations of the anticholinesterase activity.

RESULTS

Morpholinium Series



n = 4 to 12
X = Br or I
R = Me or Et

(I)

In this series (I) we have examined compounds having four to twelve carbon atoms in the chain where R = Me and a compound having six carbon atoms in the chain where R = Et. Where R = Me maximum ganglion-blocking activity was found in the pentane and hexane members. Both were as active as hexamethonium bromide on the nictitating membrane preparation in the cat (on a molar basis they were 1.2 and 1.6 times as active where n = 5 and 6 respectively).

In the single instance where methyl groups on the nitrogen atoms were replaced by ethyl groups,

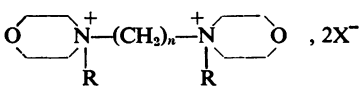
activity was markedly reduced, the resulting compound being only 6% as active as hexamethonium.

No marked neuromuscular-blocking properties were observed in this series; the compounds where n=9 to 12 only showed 5 to 6% of the activity of (+)-tubocurarine chloride on the sciatic-gastrocnemius preparation in the cat. The decane compound showed some anticholinesterase properties; on a relative molar basis it had an activity of 6.5×10^{-3}, where eserine=1.

These results are summarized in Table I.

TABLE I

MORPHOLINIUM SERIES: RELATIVE POTENCIES FOR GANGLION AND NEUROMUSCULAR BLOCK



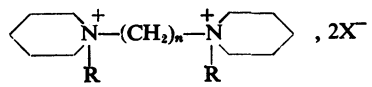
n	R	X	LD50 mg./g. i.v. (Mice)	Supr. Cerv. Ganglion (Cat)	Gastrocn. (Cat)
4	Me	Br	0·020	0·2	<0·01
5	Me	Br	0·168	1·0	<0·01
6	Me	I	0·108	1·0	<0·01
6	Et	I	0·060	0·06	<0·01
7	Me	I	0·016	0·8	<0·01
9	Me	I	0·008	—	0·05
10	Me	I	0·011	0·6	0·06
12	Me	I	0·001	—	0·06
Hexamethonium bromide			0·050	1·0	—
(+)-Tubocurarine chloride			0·0002	—	1·0

The hexane compound (R=Me) had a type of action similar to that of hexamethonium; for instance, when the effects of preganglionic excitation on the nictitating membrane were completely blocked, post-ganglionic excitation remained unaffected, and the stimulant action of nicotine on the guinea-pig ileum was abolished by a concentration that did not modify the effects of acetylcholine, histamine or pilocarpine.

After administration to rabbits the hexane compound (R=Me) was excreted in the urine to a similar extent to hexamethonium. Thus, both intravenously and subcutaneously, up to 96% was excreted within two days, but orally only 4.4% could be estimated in the urine; these estimations were by the reineckate method (Zaimis, 1950).

The hexane compound (R=Me) did not release histamine, since in the fully atropinized cat after sufficient nicotine had been injected to block the ganglia intravenous injections of the compound had no effect on the blood pressure. The compound had no anticholinesterase activity and no effect was observed on the salivary secretion stimulated by carbachol. In the conscious cat the intravenous injection of 20 mg./kg. caused marked dilatation of the pupils due to paralysis of the ciliary ganglia, and larger doses, up to 120 mg./kg., depressed respiration but were not fatal.

Piperidinium Series



n = 1 to 10
X = I or Br
R = Me or Et

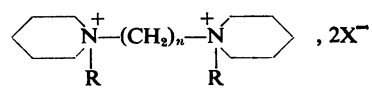
(II)

This series (II) is closely related to the morpholinium series, the oxygen atom in each heterocyclic nucleus being replaced by $-CH_2-$.

Where R=Me, compounds having 3 to 7 and 10 carbon atoms in the chain, and where R=Et, compounds having 6 and 10 carbon atoms, were examined. The results obtained in this series are shown in Table II.

TABLE II

PIPERIDINIUM SERIES: RELATIVE POTENCIES FOR GANGLION AND NEUROMUSCULAR BLOCK



n	R	X	LD50 mg./g. i.v. (Mice)	Supr. Cerv. Ganglion (Cat)	Gastrocn. (Cat)
3	Me	I	0·076	0·07	—
4	Me	I	0·006	0·8	<0·01
5	Me	Br	0·022	0·9	0·03
6	Me	I	0·014	0·8	0·10
6	Et	I	0·011	<0·1	0·10
7	Me	I	0·005	0·3	0·20
10	Me	I	0·020	0·05	0·15
10	Et	Br	0·001	<0·01	0·20
Hexamethonium bromide			0·050	1·0	—
(+)-Tubocurarine chloride			0·0002	—	1·0

Where R=Me, three members showed ganglion-blocking properties, where n=4, 5, and 6, and the potency of all three approached that of hexamethonium. On a molar basis the hexane compound was the most active, being 1.25 times as active as hexamethonium. Appreciable neuromuscular-blocking activity was found in the hexane and heptane members as well, but increasing the chain length to decane did not increase this activity. The substitution for methyl by ethyl (where R=Et), in both the hexane and decane members, decreased the ganglion-blocking activity without markedly affecting the neuromuscular-blocking actions.

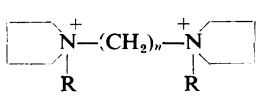
Other actions were displayed by certain members. The ganglion-blocking action of the hexane compound (R=Me) was complicated by slight atropine-like properties. On the salivary flow stimulated by carbachol it was effective at a dose of 1 mg./kg., which is near the dose which produces ganglion block. The decane compound (R=Me) was about one-tenth as active as eserine

against horse red cell enzyme, and in low doses increased the twitch tension of the gastrocnemius.

Neuromuscular-blocking activity of about the same order, it will be observed, occurred in the hexane, heptane and decane members, irrespective of whether the quaternating groups on the nitrogen atoms were methyl or ethyl, and the absence of a marked increase in activity at the decane member characterized this series, as it did also for the morpholinium series. In the morpholinium series, however, none of the compounds showed appreciable neuromuscular-blocking activity.

Several ring substituted compounds (with a chain of 6 carbon atoms) were also examined, in which a methyl group was substituted for a hydrogen atom in both nuclei in either the 2, 3, or 4 positions; all were less active than the parent compound in blocking ganglia, though they retained the same neuromuscular-blocking activity.

Pyrrolidinium Series



n = 3 to 12
X = I or Br
R = Me or Et

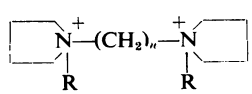
(III)

This series (III) was studied in more detail, particularly the pentane member, which is the most active ganglion-blocking drug we have encountered (Wien and Mason, 1953a; Smirk, 1953; Wien, 1954a).

Pyrrolidine, like piperidine and morpholine, is a saturated ring structure, but is a 5- instead of a 6-membered ring and it is of interest that the pyrrolidine ring occurs in several therapeutic substances. When the experimental work on this series was being carried out, Taylor (1951) independently described the tenth member.

TABLE III

PYRROLIDINIUM SERIES: RELATIVE POTENCIES FOR GANGLION AND NEUROMUSCULAR BLOCK



n	R	X	LD50 mg./g. i.v. (Mice)	Supr. Cerv. Ganglion (Cat)	Peristalsis (Guinea-pig)	Gastrocn. (Cat)	Diaphragm (Rabbit)
3	Me	I	0·127	0·05	0·1	<0·1	<0·1
4	Me	I	0·033	0·5	0·3	<0·06	<0·1
5	Me	I	0·030	5·0	1·5	<0·1	<0·1
6	Me	Br	0·017	3·0	1·0	<0·01	<0·1
6	Et	Br	0·012	0·6	—	—	0·02
10	Me	I	0·002	0·75	<0·1s	1·0	0·22
12	Me	I	0·003	0·10	0·5s	0·25	0·01
Hexamethonium bromide			0·050	1·0	1·0	—	—
(+)-Tubocurarine chloride			0·0002			1·0	1·0

s=Stimulant effect on intestine.

Where R=Me we have examined the compounds n=3 to 6, 10 and 12, and where R=Et we only examined the hexane compound. The results are summarized in Table III.

Our interest centred mainly on the pentane and hexane compounds (R=Me), which were very active ganglion-blocking substances, but the decane compound proved also to be a very interesting substance in its neuromuscular-blocking actions. This series was a fruitful source of investigation, for on one hand the pentane compound was several times as active as hexamethonium with a similar type of action, and on the other hand the decane compound was as active as tubocurarine, possessing a rather complex action, mainly resembling that of decamethonium.

Ganglion Paralysis with Penta- and Hexapyrrolidinium

"Pentapyrrolidinium," a name which can be applied for convenience to the cation only of the pentane compound, is pentamethylene-1:5-bis(1-methylpyrrolidinium), also known as M&B 2050 (iodide salt) or M&B 2050A and "Ansolysen" (tartrate salt); the approved common name is pentolinium tartrate. This is a white crystalline powder, readily soluble in water; the salt contains 48.6% of base. "Hexapyrrolidinium" (M&B 2024) was examined as the bromide salt.

The relative activities on different ganglia are summarized diagrammatically in Fig. 1, and are considered below in more detail.

Superior Cervical Ganglion.—Using the nictitating membrane preparation in the cat and preganglionic excitation, pentapyrrolidinium (iodide), given intravenously, was about five times as active as hexamethonium bromide. This was the mean of several experiments. These results were obtained by matching the observed effect at about a 50% reduction of the maximal contraction of the membrane (Fig. 2); an attempt was made to obtain a more precise figure by using a smaller ratio between the doses and plotting the percentage inhibition of the contraction against the log. dose. The result of such an assay is shown in Fig. 3.

The slopes of the dose-response lines differ, and consequently it is difficult to evaluate precisely one in terms of the other. The duration of the effect of pentapyrrolidinium was slightly longer than that of hexamethonium, a difference which was usually but not always observed. Hexapyrrolidinium was three times as active as hexamethonium on this preparation. The peak of

activity, it will be noted, occurred at the pentane, not the hexane, member as in the methonium series.

Ciliary Ganglion.—On this preparation pentapyrrolidinium was slightly more active than it was on the nictitating membrane preparation, but hexapyrrolidinium was equally active on both preparations.

Salivary Flow.—In the cat under chloralose, the flow of saliva to chorda-lingual excitation was recorded from Wharton's duct, and the inhibitory effects of compounds injected intravenously were determined. Stimulation was applied continuously during the recording of the effect of a dose, but intervals of 20 min. rest were necessary between doses to allow full recovery (Fig. 4).

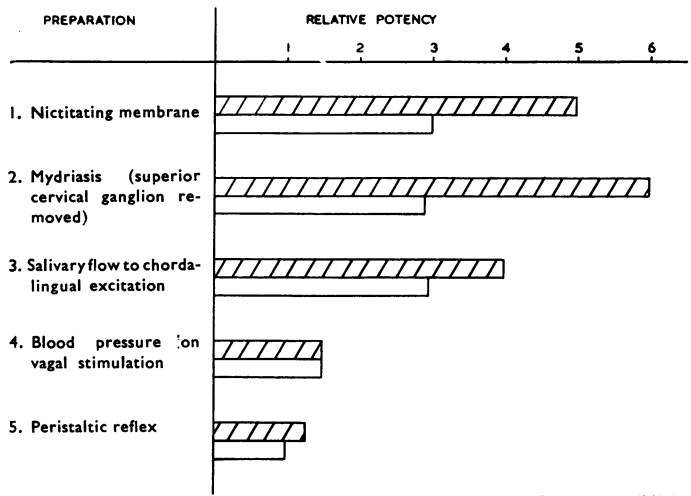


FIG. 1.—Diagram showing the relative ganglion-blocking activities of pentapyrrolidinium (cross-hatched) and hexapyrrolidinium (open) on five different preparations (see text). On each preparation hexamethonium bromide = 1.

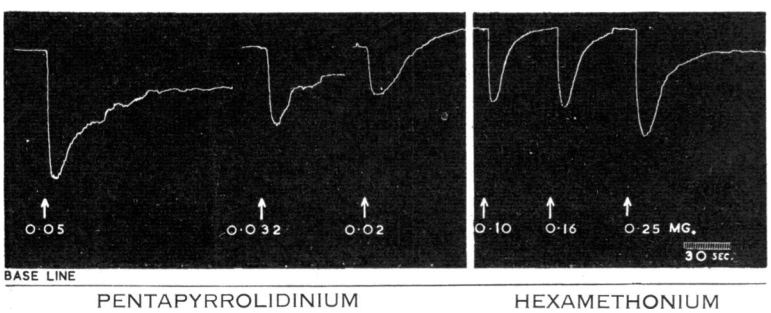


FIG. 2.—Cat, chloralose. Nictitating membrane contractions to sustained preganglionic excitation of the cervical sympathetic nerve. As indicated, intravenous injections in mg. of pentapyrrolidinium (iodide) and hexamethonium (bromide). The data in Fig. 3 were compiled from a similar experiment.

On this preparation, penta- and hexa-pyrrolidinium had similar potencies, relative to hexamethonium, as they had on other preparations.

Peristaltic Reflex and Cardiac Vagus.—The two compounds were also examined for ganglion-blocking properties using the peristaltic reflex in isolated guinea-pig ileum, and the bradycardia and fall in blood pressure following stimulation of the peripheral end of the cut vagus in the cat. These methods involve intermittent stimulation, and it was interesting to observe (Fig. 1) that in these two preparations penta- and hexa-pyrrolidinium

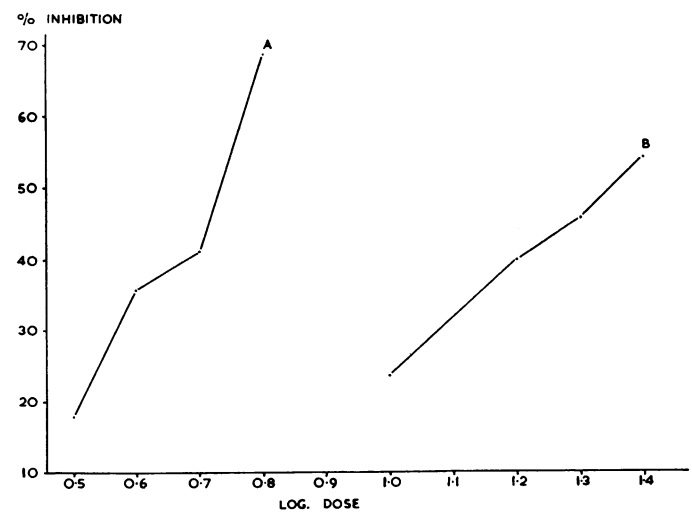


FIG. 3.—A comparison of the effects of (A) pentapyrrolidinium (iodide) and (B) hexamethonium (bromide) on the nictitating membrane preparation in the cat from a single experiment (using sustained preganglionic stimulation), showing the relation of the relaxation of the membrane to intravenous doses of ganglion-blocking agents.

were less active, relative to hexamethonium, than in the three preparations using continuous stimulation.

Mode of Action

The mode of action of pentapyrrolidinium was assessed by criteria similar to those used by Paton (1951). Firstly, the ability of the nictitating membrane to contract, and, secondly, the ability of the postganglionic nerves to conduct, were unimpaired during a complete paralysis to preganglionic excitation as shown by the unmodified responses to adrenaline and postganglionic stimulation. Thirdly, during perfusion of the superior cervical ganglion acetylcholine was liberated normally to preganglionic stimulation although the contraction of the nictitating membrane was completely inhibited by injection of the compound into the perfusion fluid. This was observed with doses up to 2 mg., which is several times the dose necessary to block the ganglion. Fourthly, the stimulant effect of nicotine on the guinea-pig ileum was abolished by concentrations of the drug which did not modify the effects of acetylcholine, histamine, and pilocarpine. Fifthly, the effect of stimulation of the peripheral vagus on the blood pressure in the anaesthetized cat was abolished, but the effect of acetylcholine was unmodified.

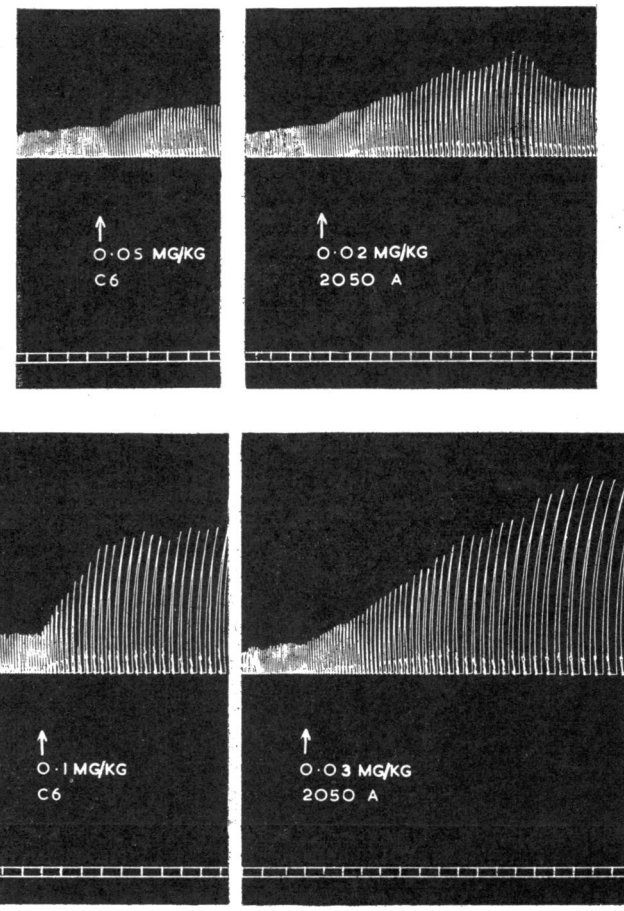


FIG. 4.—Cat, chloralose. Record of the salivary flow from Wharton's duct to electrical stimulation of the chorda tympani at a rate of 5/sec. C6=hexamethonium (bromide) and 2050A=pentapyrrolidinium (bitartrate). Between each dose the preparation was rested for 20 min. Time=30 sec.

Penta- and hexa-pyrrolidinium had no marked atropine-like action; for example, hexapyrrolidinium slightly antagonized salivary flow to infusion of carbachol in an intravenous dose of 10 mg., but this effect was less than that of 4 μg. atropine. There was no release of histamine, as shown by the absence of a depressor action in the atropinized cat under chloralose after ganglionic paralysis with nicotine.

Toxicity of Pentapyrrolidinium

Mice.—The acute LD50 figures by the intravenous, subcutaneous and oral routes were 0.030 mg./g. (limits of 93–107% for P=0.05, b=20.0), 0.090 mg./g. (limits of 88–114%, b=8.1), and 0.33 mg./g. (limits of 85–119%, b=7.8) respectively. The wide difference between the parenteral and oral toxicities indicated poor gastro-intestinal absorption.

Rats.—Daily subcutaneous injections of 0.02 mg./g. for two weeks had no effect on the growth rate of young rats (10 rats in a group). Uninjected controls grew from a mean weight of 52 to 72 g. and the animals in the treated group increased from 52 to 73 g.

Rabbits.—Daily intravenous injections of 3 mg./kg. to 6 rabbits over a period of three weeks did not visibly affect the health of any of them. The injections did not cause any local reaction or damage to the veins. Single doses up to 10 mg./kg. produced no obvious marked symptoms, other than slight mydriasis and vasodilatation of the

ear vessels; doses of 20 mg./kg. and more were fatal, and the symptoms seen were paralysis of the neck muscles, dyspnoea and respiratory depression.

Guinea-pigs.—A complete blood-picture examination was made by our colleague, Mr. W. A. Freeman, for each of six guinea-pigs at twice-weekly intervals over a period of four weeks during which each animal received daily a subcutaneous injection of 6 mg./kg. No abnormalities were observed. At the end of this period the animals were killed and were kindly examined by Dr. Williamson of the Department of Pathology, Cambridge, who found no pathological changes in any of the tissues except a small localized area of catarrhal inflammation in the lung of one animal. The tissues examined were kidney, lung, bone marrow, heart, liver, suprarenal, spleen, and spinal cord.

Cats.—In unanaesthetized cats the intravenous injection (saphena vein) of 5 mg./kg. had no effect; 10 mg./kg. produced only a slight mydriasis, and 20 mg./kg. diminished muscular power; 30 mg./kg. caused pronounced mydriasis, ataxia and depression of respiration, but recovery took place within an hour. The mydriasis lasted for at least an hour after the symptoms of respiratory depression and motor incoordination had passed off.

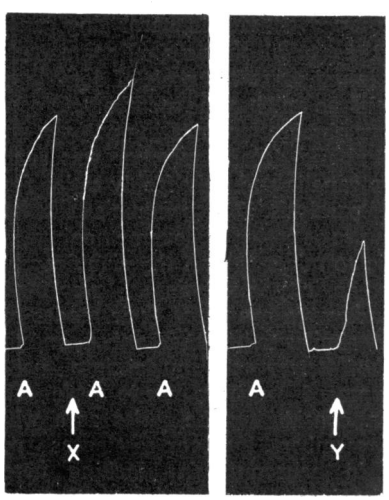


FIG. 5.—Contractions of the isolated frog rectus muscle showing the increased response to acetylcholine (A) in the presence of low concentrations of decapyrrolidinium (X) and the contractions of the muscle to high concentrations of decapyrrolidinium (Y). A=5.0 μg. acetylcholine: X=20 μg. and Y=200 μg. of decapyrrolidinium in a 5 ml. bath.

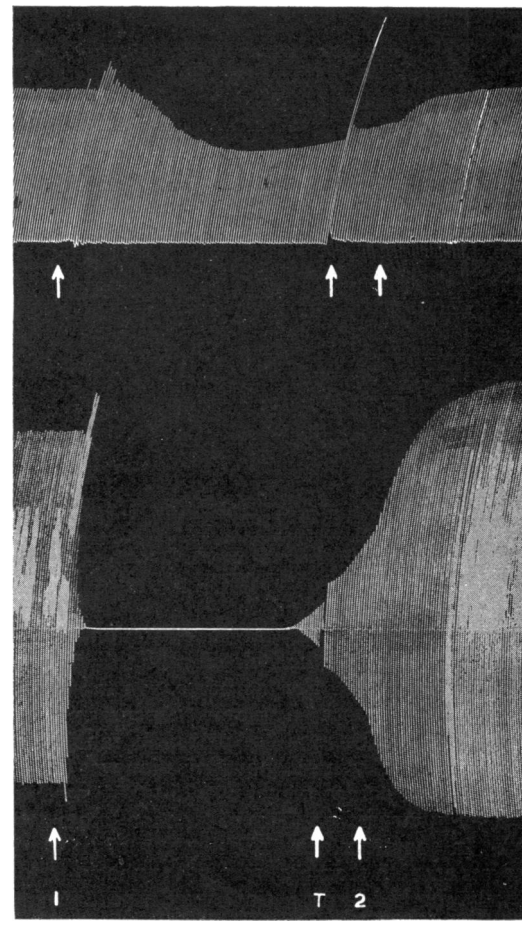


FIG. 6.—Cat, chloralose. Contractions of the soleus (upper record) and tibialis (lower record) to maximal stimulation of the sciatic nerve at a rate of 6/min., 0.5 msec. duration. All injections were intravenous. At (1) 0.5 mg. of decapyrrolidinium; T = tetanus of 50/sec. for 10 sec.; (2) 20 mg. of pentapyrrolidinium.

Urinary Excretion of Pentapyrrolidinium in Rabbits

By the reineckate method the following results were obtained, using 3 rabbits for each route of administration: (*a*) after 15 mg./kg. intravenously, 77.2, 91.3, and 59.3% was eliminated within two days; (*b*) after 30 mg./kg. subcutaneously, 79.0, 74.9, and 82.8% was excreted; and (*c*) after 50 mg./kg. orally 20.1, 23.2, and 19.4% was excreted in the urine.

Neuromuscular Paralysis with the Decapyrrolidinium Compound

The decapyrrolidinium compound had powerful neuromuscular-blocking properties; it reduced the twitch tensions of the gastrocnemius, soleus, and

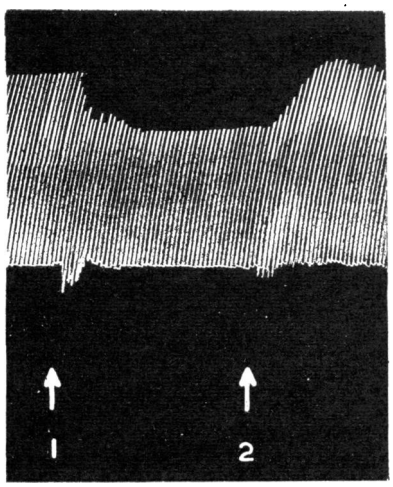


FIG. 7.—Cat, chloralose. Contractions of the gastrocnemius muscle to maximal stimulation of the sciatic nerve at a rate of 6/min., 0.5 msec. duration. All injections were intravenous. At (1) 0.5 mg. decapyrrolidinium and at (2) 0.25 mg. neostigmine injected.

tibialis anterior of the cat to excitation of the sciatic nerve, and of the isolated rabbit diaphragm to excitation of the phrenic nerve. In the anaesthetized cat it was as active as tubocurarine, but its mode of action was complex. In rabbits the head-drop dose by intravenous injection was 0.53 ± 0.21 mg./kg. compared with 0.25 ± 0.04 mg./kg. for (+)-tubocurarine (8 rabbits on a cross-over test). In conscious cats the induction of paralysis was less smooth than that seen with tubocurarine; initially, marked fasciculations were noted, respiration was quickly depressed and there was salivation. On intravenous injection of 0.1 to 0.5 mg./kg. the duration of the paralysis lasted from 5 to 30 min., and respiration was depressed from 2 to 10 min.; when respiration was arrested a Magill tube was passed under direct vision and artificial respiration maintained.

Most of its effects were like those of decamethonium. In the chick on intravenous injection of 0.1 μg./g. there was a spastic paralysis and addition of the compound to the isolated frog's rectus abdominis muscle produced a contracture. Moreover, it had an appreciable anti-esterase effect; against the red cell enzyme its molar activity relative to eserine = 1 was 2.3×10^{-2}, and it potentiated the contractions of acetylcholine on the frog's rectus (Fig. 5).

After an almost complete paralysis of the tibialis anterior muscle in the cat a tetanus was well maintained (Fig. 6), and the muscle twitch was easily restored by an intravenous injection of the pentapyrrolidinium compound, an effect which is analogous to that of the reversal of the action of decamethonium by pentamethonium.

The resemblance to decamethonium was increased by the differential action of decapyrrolidinium on the tibialis and soleus muscles; the tibialis was more readily blocked than the soleus by either decapyrrolidinium or decamethonium (Fig. 6; Paton and Zaimis, 1951).

On the other hand, neostigmine partially reversed a decapyrrolidinium block of the tibialis when in the same experiment it did not reverse a decamethonium block, and a partial paralysis of the gastrocnemius by decapyrrolidinium was also easily reversed by neostigmine. In this way paralysis by decapyrrolidinium resembled that by tubocurarine; the paralysis was accompanied, however, by marked fasciculations (Fig. 7).

DISCUSSION

Extension of our work now covers a pharmacological study of three series of heterocyclic bisquaternary salts, of which the most interesting was the pyrrolidinium series. Of other series examined it was found that compounds with saturated nuclei of the structure $A.(CH_2)_n.A$, where A was a heterocyclic nucleus, were more active as ganglion-blocking agents than those with unsaturated nuclei (Wien, 1954b). In all three series, morpholinium, piperidinium and pyrrolidinium, replacement of methyl by ethyl groups on the quaternary nitrogen atoms reduced activity in paralysing ganglia.

With the methyl quaternated compounds in the morpholinium series the peak of activity occurred at the pentane and hexane members, in conformity with other observations relating potency and chain length, but the decane member was surprisingly active on ganglia and only feebly active for neuromuscular paralysis. The pentane and hexane compounds were as active as hexamethonium and were less toxic to mice, though the significance of this decreased toxicity is not clear. In so far as these compounds were examined their modes of action were very similar to hexamethonium, and the introduction of heterocyclic nuclei into the cationic head appeared to have made little difference. However, considering the series as a whole, the decane member by contrast was markedly inferior in neuromuscular-blocking potency to decamethonium.

Similarly in the piperidinium series the pentane and hexane members (when the groups on the nitrogens were methyl) were about as active as hexamethonium. In this series, however, mixed

actions were more evident, since the hexane compound had appreciable neuromuscular-blocking properties as well, and the decane compound was a fairly potent inhibitor of cholinesterase. The hexane, heptane, and decane members were all from one-tenth to one-fifth as active as (+)-tubocurarine and in this respect differed from other series, since there was not the sharp differentiation between ganglion- and neuromuscular-blocking properties.

The pyrrolidinium series included several pharmacologically active compounds of potential value as therapeutic substances. The variation in properties on lengthening the chain was very well illustrated in this series.

In all the tests to which the pentane compound was submitted it resembled hexamethonium very closely. Though the fivefold increase in potency was not accompanied by a similar increase in duration of action, there was evidence of a slightly more prolonged effect, and Smirk (1953) has, indeed, found a more prolonged hypotensive action in patients. There was a correspondence between the relative ganglion-blocking values for certain compounds obtained experimentally (on the superior cervical ganglion preparation in the cat), and the ability of these compounds to lower the blood pressure in hypertensive patients. Smirk (1952a, 1952b, and 1953) found that the relative activities in patients for hexamethonium bromide: its bis(ethyldimethyl)analogue: pentapyrrolidinium: and hexapyrrolidinium, were as 1:2:5:1.7 and experimentally in cats these ratios were 1:1.5:5:3. Other clinical work on pentapyrrolidinium has been reported by Hetherington (1953), Freis, Partenope, Lilienfield, and Rose (1954), Rønnov-Jessen (1954), and others.

We have endeavoured to find any selective action that might exist for some of these compounds on different ganglia, and it was considered that the results should preferably be compared in the same species with continuous, rather than intermittent, excitation of the autonomic nervous system. There were three preparations in the cat which roughly fulfilled these conditions (Fig. 1, (1), (2), and (3)). Comparing these results it was found that, relative to hexamethonium, penta- and hexa-pyrrolidinium had about the same order of activity (5:3, 6:3, and 4:3) on each preparation; there was no evidence of a selective action on different ganglia. However, in all these assays there was evidence of some modification of the activity of hexamethonium by pentapyrrolidinium (and vice-versa), which was not completely eliminated even by extended rest periods between doses.

The neuromuscular-blocking properties of the decane compound were interesting; the characteristic features mainly resembled those of a decamethonium block. In the cat under chloralose the maximal twitch of the tibialis was potentiated before the ensuing block, the tibialis was more readily antagonized than the soleus, and the block was not easily, or completely, antagonized by a tetanus or neostigmine; decapyrrolidinium also had anticholinesterase properties. Just as decamethonium can exert a dual mode of action on the muscles, so was there evidence of a variable action with decapyrrolidinium; for example, on the gastrocnemius a partial block could be easily reversed by neostigmine (Fig. 7) resembling block by competition rather than by depolarization. This is not surprising when it has been demonstrated that not only are there species differences in response to neuromuscular-blocking substances, but that differences exist between muscles within any one species (Jewell and Zaimis, 1953). Decapyrrolidinium is another example, like decamethonium and succinylcholine (Zaimis, 1953; Hall and Parkes, 1953), of a compound possessing a dual mode of action.

Summary

1. The actions are described of three series of heterocyclic bisquaternary salts having the generic formula $A.(CH_2)_n.A$, where A is a heterocyclic nucleus (1-methylpiperidino, 4-methylmorpholino, and 1-methylpyrrolidino).

2. When the groups on the nitrogen atoms were methyl, the butane, pentane, and hexane members of the piperidinium series and the pentane and hexane members of the morpholinium series had ganglion-blocking activity of the same order as hexamethonium. There was only negligible neuromuscular-blocking activity in the morpholinium series; the decane members (either methyl or ethyl groups on the nitrogen atoms) of the piperidinium series were about one-fifth as active as (+)-tubocurarine. The types of action in these two series are described.

3. In the pyrrolidinium series the peak of ganglion-blocking activity occurred at the pentane member (with methyl groups on the nitrogens), which was about five times as active as hexamethonium on the nictitating membrane preparation in the cat.

4. By several tests the mode of action of "pentapyrrolidinium" (pentamethylene-1:5-bis(1-methylpyrrolidinium)) was similar to that of hexamethonium.

5. When examined by experiments in the cat on (i) the salivary flow to chorda-lingual excitation and (ii) the mydriatric response to light (superior cervical ganglion removed), pentapyrrolidinium did not show any marked selective action on either preparation.

6. Decapyrrolidinium was as active as (+)-tubocurarine in producing neuromuscular block in the anaesthetized cat; it had a mixed type of action, which was predominantly decamethonium-like.

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