Brit. J. Pharmacol. (1953), **8**, 115.

5 : 6-DITHIADECAMETHYLENE 1 : 10-BIS(TRIMETHYLAMMONIUM IODIDE): A NEW MYONEURAL BLOCKING AGENT OF THE DEPOLARIZING TYPE

BY

A. R. HUNTER

From the Department of Pharmacology, the University of Manchester

(RECEIVED MAY 27, 1952)

The compound 5:6-dithiadecamethylene 1:10-bis(trimethylammonium iodide) was synthesized by Andrews, Bergel, and Morrison (1953), and given the serial number Ro 3-0386. On preliminary investigation it proved to be a myoneural blocking agent with an action rather like that of decamethonium (Randall, personal communication). Since this latter drug has been found in clinical anaesthesia to have certain disadvantages (Gray, 1950 ; Hunter, 1950), it seemed worth while to study the new compound in some detail in the hope that it might be more satisfactory.

METHODS

In cats anaesthesia was induced by 40 mg./kg. of pentobarbitone given intraperitoneally. Maintenance doses of 5 to 10 mg./kg. were injected intravenously when the reflex contraction of the eyelids elicited by touching the inner canthus returned. In the curarized animal either an abnormally high blood pressure, over 185 mm. of mercury, not due to asphyxia, or irregular respiration was used as an indication for the administration of more pentobarbitone. In dogs, apart from the studies of ganglion blocking activity, only short experiments were carried out, and ether anaesthesia was used.

No direct records of muscular contraction were made. Instead the contraction of a foreleg muscle group in the cat was observed. Complete myoneural block was assumed to be present when stimulation of the appropriate fasciculus of the sciatic nerve, with what had previously been supramaximal shocks from an induction coil, no longer produced any visible movement of the unloaded foot or toes. Respiratory movements were recorded with the aid of tambours on the thorax and epigastrium. The mean blood pressure was measured with a mercury manometer with citrate as an anticoagulant.

Rabbit Head Drop Tests.—These were carried out on untrained animals. Small incremental doses of the drug were injected into the ear vein every 30 seconds until paralysis appeared. The dosage was so arranged that three to five increments were required to produce head drop.

Antagonists.—As antagonists are used in man to restore normal breathing, their action on the respiratory movements of experimental animals was used as the criterion of their effectiveness. In cats the relaxant was given until respiratory failure was indicated by depression of breathing movements and asphyxial hypertension. If the antagonist would then produce prompt restoration of respiration and reversal of the asphyxia, it was regarded as effective. These agents were also tested in less severe states of respiratory depression. They were given to animals in whom the relaxant drug had produced abolition of thoracic breathing and some reduction in the force of abdominal breathing. If both components of respiration were immediately restored the antidote was considered to be effective.

Antidotes were also studied in mice. A dose of the myoneural blocking agent was chosen which would kill a large percentage of the animals. The antidote was given, and after a suitable interval the selected dose of the myoneural blocking agent was given. Protection was assumed to have occurred if there was a significant reduction in the mortality in the second group.

Ganglion Blocking Activity.—This was tested directly for the parasympathetic only. Dogs and cats under pentobarbitone anaesthesia were submitted to bilateral vagotomy. The peripheral cut end of one vagus nerve was stimulated with an induction coil. The strength of the stimulus was the minimum which would produce a maximal response when applied to the adjacent sternomastoid muscle. Stimulation was continued until the blood pressure ceased to fall further, usually for some 10 to 15 seconds. After an interval of at least 5 minutes the drug to be tested was given,

and if it impaired the breathing artificial respiration was begun. If the same stimulus produced a fall in blood pressure of the same severity and duration it was considered that conduction in the parasympathetic ganglia was unimpaired. This method had previously been shown to be reliable when it was used to demonstrate the blocking effect of gallamine triethiodide on the terminal synapses of the cardiac vagus.

Results

The degree of myoneural block produced by various doses of Ro 3-0386 is given in Table I. For comparison the amounts of decamethonium with the same action are also given where the figures are available. The duration of the effect of comparable doses seemed to be about the same. The dose of Ro 3-0386 which would produce complete myoneural block in a nerve muscle preparation was just slightly less than that which would cause respiratory failure. At lighter levels of the drug's action, weakening of the response to single stimuli was apparent. Tetani were, however, fairly well sustained, a contrast to the effect of *d*-tubocurarine.

Table I

Comparative Doses of Ro 3-0386 and Decamethonium

Species	Criterion of Myoneural Block	Myoneural Blocking Dose	
		Ro 3-0386 mg./kg.	Decamethonium mg./kg.
Mouse ..	LD50	1·95*	0·84†
Rabbit ..	Head drop	About 0·1	0·08–0·22
Cat ..	Paralysis thoracic breathing	0·01	—
	Apnoea	0·02	0·08
Dog ..	,,	About 0·1	—

* Parkes (personal communication). † Paton and Zaimis (1949).

Ro 3-0386 proved to have a muscle-stimulating action. Violent contractions of the limbs, enough to make the animal jump several inches into the air, quite often followed its intravenous administration in mice. Fasciculation and sometimes writhing movements could be observed when the drug was given to cats under pentobarbitone. These changes did not, however, appear in dogs anaesthetized with ether or pentobarbitone. When Ro 3-0386 was given by close intra-arterial injection into the tibialis anticus of the cat a sharp contraction of the muscle occurred, while when it was given into the femoral artery a less forceful response of all the muscles of the limb was seen. All these phenomena have been observed after the administration of decamethonium.

Action on Respiratory Movements.—In cats under pentobarbitone Ro 3-0386, like decamethonium, produced first intercostal paralysis with compensatory increase in the diaphragmatic movement. Then as the action of the drug became more profound diaphragmatic respiration became weaker and was lost in the fasciculation of the abdominal muscles which was a prelude to their complete paralysis. In three dogs under ether anaesthesia to which the drug was given in large doses the respiration was first depressed, and then, as the dose approximated to that which was going to kill the animal, there appeared jerky, gasping slow breathing which caused only very inadequate ventilation; but this was sufficient to maintain the animal in a state of chronic suboxygenation until the paralysing effect of the drug had passed off. Similar breathing has been observed in human subjects who have been kept deeply under the influence of decamethonium for long periods (Gray, 1950; Hunter, 1950).

Summation with Decamethonium.—The results of the investigation so far indicated that Ro 3-0386 was a depolarizing blocking agent like decamethonium. It was therefore thought worth while to see whether a small dose of one drug would sum with a comparatively ineffective dose of the other. It was found that, if to a cat which received half the dose of Ro 3-0386 which would cause apnoea a similar half dose of decamethonium was given, breathing was stopped for a few minutes and a period of intercostal paralysis followed. A similar summation of action could be seen in mice, in which harmless doses of drugs given in quick succession caused death in a proportion of animals (Table II).

Table II

The Additive Effects of Decamethonium and Ro 3-0386

Drugs	Dose mg./kg. (about ½ LD50)	Number of Mice Died
Ro 3-0386	0·75	0/6
Decamethonium	0·4	0/2
Decamethonium and Ro 3-0386	0·4 + 0·75	5/6

Antagonists.—Hexamethonium given by intraperitoneal injection in doses of 40 mg./kg. failed to afford appreciable protection to mice against the muscle-paralysing action of Ro 3-0386 in doses of 1.0 mg./kg. In the cat hexamethonium (2 mg./kg. intravenously), a dose which in a previous study (Hunter, 1950) had produced reversal of the action of decamethonium, failed to cause any appreciable restoration of the breathing of animals partly

paralysed by Ro 3-0386. 3-Thiapentamethylene 1:5-bis(trimethylammonium iodide) (Ro 3-0438), however, in a dose of 2.0 mg./kg. produced a restoration of abdominal but not of thoracic respiration. On the other hand, neither Ro 3-0438 in a dose of 100 mg./kg. nor the related Ro 3-0468 (3-thiahexamethylene 1:6-bis(trimethylammonium iodide)) in doses of 50 mg./kg. by intraperitoneal injection reduced the mortality in mice subsequently given Ro 3-0386 in lethal doses.

Compound 49-204 of de Beer *et al.* (1951) (1-methyl - 2-(dimethylaminophenethyl) - piperidine-1:4'-bis-methiodide) in doses of 1.0 mg./kg. reversed the action of Ro 3-0386 in the cat, restoring both the diaphragmatic and thoracic components of breathing. After such a reversal the animals had an increased resistance to the myoneural blocking agent. In mice significant protection against lethal doses of Ro 3-0386 was given by 3.0 mg./kg. of compound 49-204 given intravenously five minutes before (Table III). This

TABLE III

THE PROTECTIVE EFFECT OF COMPOUND 49-204 AGAINST LETHAL DOSES OF Ro 3-0386

Drug	Dose mg./kg.	Mice Survived
Ro 3-0386	1·8	1/12
49-204	3·0	6/6
49-204 and Ro 3-0386 ..	{3·0 / 1·8}	9/12

$\chi^2=8{\cdot}4$. $P<0{\cdot}01$.

amount of the latter compound seemed unlikely to be responsible for any of the deaths, as it caused no deaths when given alone and is less than half the LD50 (7.2 mg./kg.; de Beer, personal communication).

Ro 3-0386 is also antagonized by gallamine triethiodide. Both in the cat and the dog the dose of the former agent needed to produce apnoea is increased in animals which have recovered from the muscle-paralysing action of the latter (Table IV). Mice which have received a sublethal dose of gallamine triethiodide (2 mg./kg.) are also protected to a significant extent against poisonous

TABLE IV

THE PROTECTIVE EFFECT OF GALLAMINE TRIETHIODIDE AGAINST THE ACTION OF Ro 3-0386 IN CATS AND DOGS

Animal	Myoneural Blocking Dose of Ro 3-0386 (mg./kg.)	
	Alone	After Gallamine Triethiodide
Cat	0·02 (3)	0·16 (2)
Dog	0·09 (4)	0·13 (2)

The figures indicate the means of a number of experiments (in brackets).

TABLE V

THE PROTECTIVE EFFECT OF GALLAMINE TRIETHIODIDE AGAINST THE ACTION OF Ro 3-0386

	Group	Dose of Ro 3-0386 mg./kg.	Mice Survived
Without gallamine triethiodide	I	1·2	0/6
	II	1·8	3/9
After gallamine triethiodide (2 mg./kg.)	I	1·2	4/6
	II	1·8	8/9

$\chi^2=7{\cdot}115$. $P>0{\cdot}02$, $<0{\cdot}05$.

doses of Ro 3-0386 (Table V). A more positive antagonism between the two drugs was seen when a temporary deepening in the respiration of a cat partially curarized by gallamine triethiodide was produced by the administration of 0.02 mg./kg. of Ro 3-0386.

Ganglion Blocking Activity.—Ro 3-0386 had no immediate effect on the blood pressure of any of the animals to which it was given. An asphyxial rise in blood pressure was observed when respiration failed, and it therefore seemed as if it did not block the sympathetic ganglia. This was confirmed directly when Pellmont (personal communication) found that Ro 3-0386 in doses of 0.03 mg./kg. had no effect on the conduction of impulses through the superior cervical ganglion of the cat. It did reduce the force of the contraction of the nictitating membrane, but the site of the block was distal to the superior cervical ganglion, since the response to postganglionic stimulation was equally reduced.

In the present study it was found that Ro 3-0386 had no effect on conduction through one type of parasympathetic ganglion. Doses up to twice that which would cause apnoea had no influence on the fall in blood pressure produced by stimulating the peripheral cut end of the vagus nerve.

The Use of Ro 3-0386 in Man

The methods used were similar to those employed in a previous study of decamethonium, i.e., the administration of a drug to patients already intubated and stabilized under plane I nitrous oxide and oxygen anaesthesia (Hunter, 1950). A barbiturate, usually "Kemithal" (sodium cyclohexenyl-allyl-thio-barbiturate), was used for induction, and analgesic drugs were given to supplement the nitrous oxide when necessary. Because previous experience with decamethonium indicated that the type of relaxation was likely to be more satisfactory for lower abdominal operations, the cases here studied were herniotomies and appendicectomies. There were 22 in all, of ages ranging from 11 to 60 (mean 33.9 years).

Relaxation.—The relaxation afforded by Ro 3-0386 was very like that produced by decamethonium. It began very shortly after the injection of the drug ; it disappeared, if anything, more abruptly than when decamethonium had been given. Some patients passed from the state requiring assistance with their breathing to apparently complete recovery in two to three minutes. Continuation of the expiratory pull of the flat muscles of the abdomen was a marked feature of this phase of the drug's action. Unlike succinylcholine, Ro 3-0386 caused no visible muscular contractions or fibrillary twitchings in man, though one or two of the early patients to whom inadequate doses were given had deviations of the eyeballs into odd positions.

Dosage.—The experimental work in the cat suggested that Ro 3-0386 was more potent than decamethonium. It soon, however, became apparent that the dose of the two drugs was about the same in man. Indeed Ro 3-0386 was slightly less potent. In adults 3–4 mg. usually depressed the respiration so much that artificial ventilation became necessary. Forceful breathing was resumed some 5–10 minutes later and more or less simultaneously the relaxation disappeared. As recommended by Paton and Zaimis (1950) for decamethonium, test doses were not used after it had become apparent that tachyphylaxis to Ro 3-0386 developed even more rapidly than with decamethonium.

Respiration.—Like decamethonium, Ro 3-0386 markedly spared the diaphragm. Intercostal paralysis developed early, and rocking-boat respiration with pronounced diaphragmatic breathing was not infrequently observed. The gasping, jerky respiration characteristic of prolonged administration of decamethonium was not observed, though, as has been noted above, this disturbance was seen in some of the dogs to which the drug was given. The absence of this complication is, however, probably due to limitation of the total dosage of the drug to an arbitrary maximum of 10 mg. and the total duration of its action to some 40 minutes. It does not imply any special merit on the part of Ro 3-0386.

Side-effects.—Ro 3-0386 had no action on the pulse rate, which remained unaltered at all rates. Usually the drug was equally without effect on the blood pressure, but in 4 of the 22 patients of this series there was some fall, the maximum decline being 25 mm. of mercury. There is little doubt that these falls in blood pressure were due to the relaxant, as they developed immediately after its administration and lasted only a few minutes. Hypotension recurred in only one of these four cases after a second dose of the drug. Neither of these features is characteristic of ganglion block, and it seems more likely that Ro 3-0386 may possess in a minority of individuals a direct depressor action comparable to that exerted by acetylcholine and many other substances with a trimethylammonium group. It is interesting that decamethonium also lowers the blood pressure occasionally in the human subject (Hunter, 1950).

Discussion

The main interest in Ro 3-0386 centres in its similarity to the relaxant decamethonium, the prototype of the depolarizing myoneural blocking agents. Thus, like decamethonium, Ro 3-0386 produces stimulation before paralysis. With both agents tetani are much better sustained than in animals paralysed to a comparable extent with *d*-tubocurarine. Both show a weak antagonism to gallamine triethiodide and are themselves rendered less toxic by the previous administration of this drug. Harmless doses of both drugs sum with one another to produce a fatal effect. The variation in species sensitivity to the drugs is somewhat similar, though the cat is more sensitive and the mouse less sensitive to Ro 3-0386 than it is to decamethonium. Both agents are successfully antagonized by compound 49-204. The action of the two drugs in the human subject is also very similar and they are approximately equipotent. The onset and disappearance of relaxation are abrupt. The same sparing of diaphragmatic respiration can be seen. There is also the same tendency to tachyphylaxis.

There is, however, something specific about the action of the two drugs. The true polymethylene compounds are antagonized to some extent by the shorter-chain polymethylene compounds, while the thiapolymethylene agents are antagonized by thiapolymethylene substances. On the other hand, hexamethonium does not antagonize the paralysis produced by Ro 3-0386, nor do Ro 3-0438 and Ro 3-0468 (the thiapentamethylene and thiahexamethylene compounds) antagonize the action of decamethonium.

Succinylcholine is the other depolarizing blocking agent in common use to-day. The pattern of its action is similar to that of decamethonium in that the onset of paralysis is abrupt and recovery rapid. It, too, sums with decamethonium and antagonizes *d*-tubocurarine and like drugs. The preliminary administration of gallamine triethiodide protects animals against dangerous doses of succinylcholine. On the other hand, this drug is

not antagonized by compound 49-204 nor yet by hexamethonium. There is, too, a very marked difference in the effective dose of succinylcholine and the other drugs. In the author's hands this agent has proved to be more than three times as toxic to mice as Ro 3-0386 and about twice as toxic as decamethonium. In cats the effective doses of decamethonium and succinylcholine are about the same. By contrast in man it takes 5–10 times the amount of succinylcholine to produce a degree of paralysis comparable to that caused by decamethonium or Ro 3-0386. It is possible that these quantitative differences are in some way related to the fact that succinylcholine depends on cholinesterase for its elimination, and it seems possible that in the human subject enough of the drug to overwhelm this enzyme must be given before paralysis can be produced. Further experiment would, however, be required to test this hypothesis.

The main point which emerges, however, from these considerations is the fact that the linking chain between the two quaternary nitrogen atoms is of importance in determining some at least of the properties of myoneural blocking agents. The intervening groups are thus more than a prop to maintain a distance of 14Å between the active trialkylammonium groups. A partial explanation of this finding may be found in the stresses in the molecule of substances of this type which result in bending of the central chain, with the result that there must be slight differences in the orientation at the muscle end-plate. In consequence only those of the same series have the required molecular configuration to displace the corresponding active myoneural blocking agents from the end-plate receptors.

The small tentative trial in the human subject of Ro 3-0386 could not provide a true indication of the place of the drug in clinical anaesthesia. The near identity of its actions with those of decamethonium, however, suggests that the two drugs might have about the same applicability, and in fact the brevity of action of Ro 3-0386 has already been put to useful purpose in electric convulsion therapy. The problem of whether Ro 3-0386 would produce cumulative effects in man like those of decamethonium has not been solved by this investigation, though rather similar effects were noted in some dogs to which the drug was given.

Summary

The drug Ro 3-0386, 5:6-dithiadecamethylene 1 : 10-bis(trimethylammonium iodide), has been shown to be a myoneural blocking agent with an action very similar to that of decamethonium, both in experimental animals and in man. It was used in 22 patients to produce relaxation for surgical operations under general anaesthesia.

The author is indebted to Roche Products Ltd. for generous supplies of this substance and for much helpful advice.

References

Andrews, K. M., Bergel, F., and Morrison, A. L. (1953). *J. chem. Soc.* (in press).

de Beer, E. J., Castillo, J. C., Phillips, A. P., Fanelli, R. V., Wnuck, A. L., and Norton, S. (1951). *Ann. N.Y. Acad. Sci.*, **54**, 362.

Gray, A. J. (1950). *Lancet*, **258**, 253.

Hunter, A. R. (1950). *Brit. J. Anaes.*, **22**, 218.

Paton, W. D. M., and Zaimis, E. J. (1949). *Brit. J. Pharmacol.*, **4**, 381.

—— —— (1950). *Lancet*, **259**, 568.