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EFFECTS OF SOME GUANIDINE DERIVATIVES ON NEUROMUSCULAR AND GANGLIONIC TRANSMISSION

BY

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The anticurare activity of some guanidine derivatives has been studied using the fowl sciatic nerve-gastrocnemius muscle preparation and the cat sciatic nerve-gastrocnemius and tibialis anterior muscle preparations. Among the compounds tested, and in decreasing order of potency, were *NN*-dimethylguanidine, *N*-methylguanidine, guanidine and *N*-aminoguanidine which antagonized or prevented tubocurarine or gallamine triethiodide-induced paralysis. None of the derivatives antagonized the effects of suxamethonium or decamethonium. *NN*-Dimethylguanidine, *N*-methylguanidine and guanidine antagonized or prevented the curare-like effects of magnesium without altering the activity of hemicholinium. At high doses *NN*-dimethylguanidine induced a decamethonium-like spastic paralysis in the fowl sciatic nerve-gastrocnemius muscle preparation. *NN*-Diethylguanidine, however, induced a tubocurarine-like flaccid paralysis. The derivatives possessing anticurare activity were also studied using the cat superior cervical ganglion-nictitating membrane preparation to check their possible effects against ganglionic blocking agents. Only guanidine antagonized or prevented the effects of hexamethonium, pentolinium and mecamylamine; it had no effect on the actions of pempidine and chlorisondamine. *NN*-Diethylguanidine was the only compound in the series to show a ganglionic blocking action.

The anticurare activity of guanidine was observed initially by Feng (1938). Later, Condouris & Ghazal (1957) studied the effects of the guanidinium ion on the paralysis produced by tubocurarine, gallamine triethiodide or decamethonium *in vitro*, using the phrenic nerve-diaphragm preparation from rats or young cats as the test system. These authors concluded that the effectiveness of guanidinium chloride varies with the blocking agent antagonized, being greatest against gallamine triethiodide and least against decamethonium. More recently Otsuka & Endo (1960) extended the initial studies carried out by Feng, and tested the effects of guanidine on neuromuscular transmission in the frog sciatic nerve-sartorius muscle preparation. By using intracellular electrodes, these authors showed that guanidine greatly increases the amplitude of the end-plate potential of the curarized muscle without altering the resting potential at the end-plate region. Cholinesterase activity and the sensitivity of the end-plate to acetylcholine are not altered. The possibility that the drug increases the amount of acetylcholine released from the nerve endings by a single nerve impulse was suggested.

The activity of guanidine against ganglionic blockade has been reported by Zamboni & Azzolini (1954). These authors observed that, in dogs, guanidine

antagonizes the hypotensive effects of hexamethonium and restores the excitability of the splanchnic nerve to normal. The excitability of the vagus was affected only slightly and inconsistently.

In the present investigation, guanidine and some related compounds were found to antagonize the paralysing effects of tubocurarine and gallamine triethiodide. Only guanidine, however, antagonized ganglionic blockade.

METHODS

Myoneural junction. The anticurare activity of the guanidine derivatives studied was tested initially on the fowl sciatic nerve-gastrocnemius muscle preparation. Only females were used in these experiments. This system reacts selectively to neuromuscular blocking agents in that it responds to depolarizing drugs with a spastic muscular paralysis and to tubocurarine-like drugs with a flaccid paralysis. Compounds shown to be active in this system were subsequently studied in cats with the sciatic nerve-anterior tibialis and gastrocnemius muscle preparations.

Gallamine triethiodide and decamethonium were used for the initial screening at intravenous doses of 400 μg/kg and 15 μg/kg respectively. These doses were sufficient to induce a complete paralysis of approximately constant duration. Prior to each experiment, nevertheless, the sensitivity of the preparation was tested with a standard dose of gallamine triethiodide or decamethonium, and any abnormally reacting animal was discarded.

The guanidine derivatives were injected 5 min after the administration of gallamine triethiodide. A second dose of gallamine triethiodide was given 15 min later if decurarization had occurred during this period. This procedure permitted the detection of both decurarizing and preventive effects. Each compound was given at doses of 12.5, 25 or 50 mg/kg, and each active dose was tested in a minimum of three different experiments. Since the effects of these agents were long-lasting, each preparation could be used for a single test only.

Cats. Pointed drill rods were driven through the proximal and distal ends of the femur and through the malleoli of a hind leg of cats under chloralose anaesthesia (80 mg/kg intravenously), so that the leg could be rigidly clamped to a Brown-Schuster myograph stand. Shielded silver electrodes were placed on the sciatic nerve, the proximal one being the anode. The nerve was ligated centrally to the electrodes. The tendons of the tibialis anterior and soleus muscles were connected to flat steel springs of the myograph and adjusted for isometric recording. Muscle twitches were elicited by rectangular pulses of 0.2 msec duration and of about twice the voltage required to evoke a maximal response. The frequency of the stimulation was 12 impulses/min. Tetani lasting 10 sec were elicited by increasing the frequency of stimulation. When necessary, artificial respiration was provided by means of a Palmer pump.

The drugs were injected intravenously through a cannula inserted in the femoral vein. The volume of the injected solutions and the speed of injection were kept constant. Carotid pressure was measured by means of a mercury manometer. Body temperature was kept constant by a thermocouple inserted in the rectum and connected, through a relay, to the heating table.

Fowls. General anaesthesia was achieved by the slow intravenous injection of 5 ml./kg of a solution of urethane (7.5%) and chloralose (0.75%). A leg was fixed to a Brown-Schuster myograph stand; twitches and tetani of the gastrocnemius muscle were elicited and recorded as previously described for cats. Femoral arterial pressure was measured by a mercury manometer. The drugs were injected through a cannula in the wing vein.

Superior cervical ganglion-nictitating membrane. For recording purposes, the tip of the membrane was connected to an isotonic frontal writing lever by means of a thin silk thread passing through a pulley. The weight on the nictitating membrane was 7 g and the contractions were magnified 12 times.

The pre- and post-ganglionic sympathetic nerves were isolated and prepared for electrical stimulation in the usual manner and covered with warm liquid paraffin.

Rectangular pulses, 0.3 msec in duration, from an electronic stimulator were applied through platinum electrodes for 30 sec every 2 min at the rate of 10 to 15 shocks/sec. In each experiment the voltage of the pulses was slightly greater than that required for maximal retraction of the membrane. Submaximal stimuli of graded intensity were obtained by varying the frequency of the pulses.

The drugs were injected intravenously into the femoral vein or intra-arterially into the lingual artery. In the latter, the external carotid artery was occluded during the injection so that the injected substance was diverted towards the superior cervical ganglion (Trendelenburg, 1954).

Drugs. The following drugs were used in the experiments; numbers in parenthesis following the name refer to Table 1, which gives the structure. Guanidine hydrochloride (1), *N*-methylguanidine hydrochloride (2), creatinine hydrochloride (11), creatine (10) and agmatine sulphate (13) were obtained from Hoffmann La Roche. *NN*-Dimethylguanidine sulphate (3), *NN*-diethylguanidine nitrate (4), *N*-aminoguanidine sulphate (5), *N*-nitroguanidine (6), dicyandiamide (7) and dicyandiamidine sulphate (8) were obtained from British Drug Houses. Eastman Organic Chemicals supplied *N*-amino-*N'*-nitroguanidine (9) and Aldrich supplied biguanide sulphate (12).

RESULTS

Nerve-muscle preparations

Fowls. The compounds shown in Table 1 were tested for possible antagonistic effects to gallamine-induced paralysis. Only *NN*-dimethylguanidine (3), *N*-methylguanidine (2), guanidine (1) and, to a lesser extent, *N*-aminoguanidine (5) were

TABLE 1

STRUCTURAL FORMULAE OF GUANIDINE DERIVATIVES USED IN THIS INVESTIGATION

(1)	$NH_2.C(:NH).NH_2,HCl$	Guanidine hydrochloride
(2)	$NH_2.C(:NH).NHCH_3,HCl$	*N*-Methylguanidine hydrochloride
(3)	$NH_2.C(:NH).N(CH_3)_2,\frac{1}{2}H_2SO_4$	*NN*-Dimethylguanidine sulphate
(4)	$NH_2.C(:NH).N(C_2H_5)_2,HNO_3$	*NN*-Diethylguanidine nitrate
(5)	$NH_2.C(:NH).NH.NH_2,\frac{1}{2}H_2SO_4,\frac{1}{2}H_2O$	*N*-Aminoguanidine sulphate
(6)	$NH_2.C(:NH).NH.NO_2$	*N*-Nitroguanidine
(7)	$NH_2.C(:NH).NH.CN$	Dicyandiamide
(8)	$NH_2.C(:NH).NH.CO.NH_2,\frac{1}{2}H_2SO_4,H_2O$	Dicyandiamidine sulphate
(9)	$NO_2.NH.C(:NH).NH.NH_2$	*N*-Amino-*N'*-nitroguanidine
(10)	$NH_2.C(:NH).N(CH_3).CH_2.CO_2H$	Creatine
(11)	$NH.C(:NH).N(CH_3).CH_2.CO,HCl$	Creatinine hydrochloride
(12)	$NH_2.C(:NH).NH.NH.C(:NH).NH_2,H_2SO_4$	Biguanide sulphate
(13)	$NH_2.C(:NH).NH.[CH_2]_4.NH_2,H_2SO_4$	Agmatine sulphate

capable of antagonizing and preventing the effects of gallamine triethiodide. These derivatives were also active against tubocurarine. None of the compounds, however, was capable of altering the spastic paralysis produced by decamethonium, suxamethonium or acetylcholine.

Figs. 1, 2, and 3 provide examples of the effects observed. The activity has a rapid onset and is long-lasting. For this reason, as mentioned previously, each animal was used for one experiment only. *NN*-Dimethylguanidine *N*-methylguanidine and guanidine, at doses comparable to those active against gallamine triethiodide, antagonized the curare-like effects of magnesium (Fig. 4) but not that of hemicholinium. At doses effective against gallamine triethiodide, *NN*-dimethylguanidine, *N*-methylguanidine and guanidine did not alter the amplitude of the

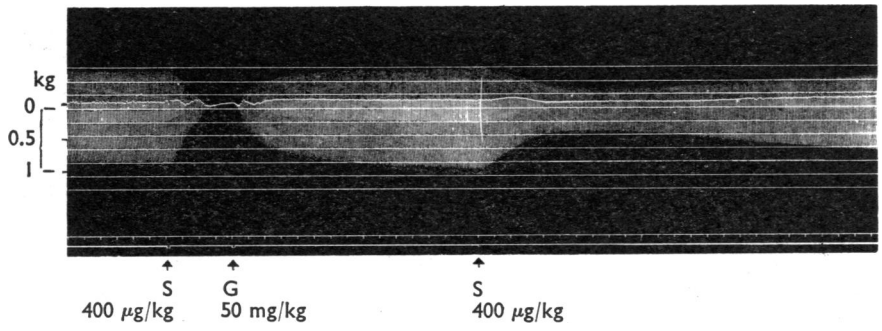

Fig. 1. Fowl, 1.8 kg. Maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every 5 sec. Time in min. S=intravenous injection of gallamine triethiodide, 400 μg/kg. G=intravenous injection of guanidine hydrochloride, 50 mg/kg.

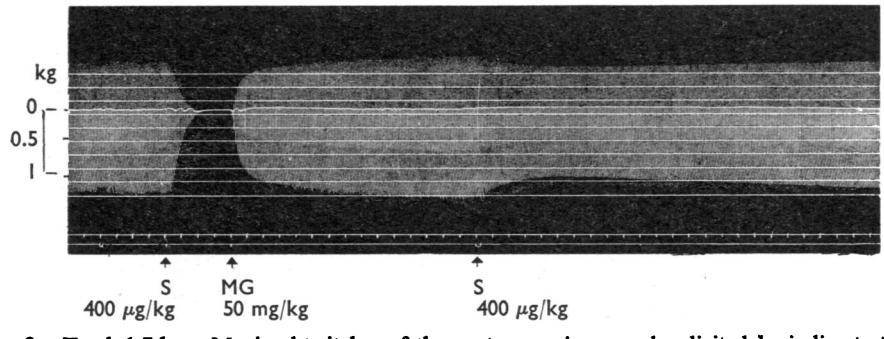

Fig. 2. Fowl, 1.7 kg. Maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every 5 sec. Time in min. S=intravenous injection of gallamine triethiodide, 400 μg/kg. MG=intravenous injection of methylguanidine hydrochloride, 50 mg/kg.

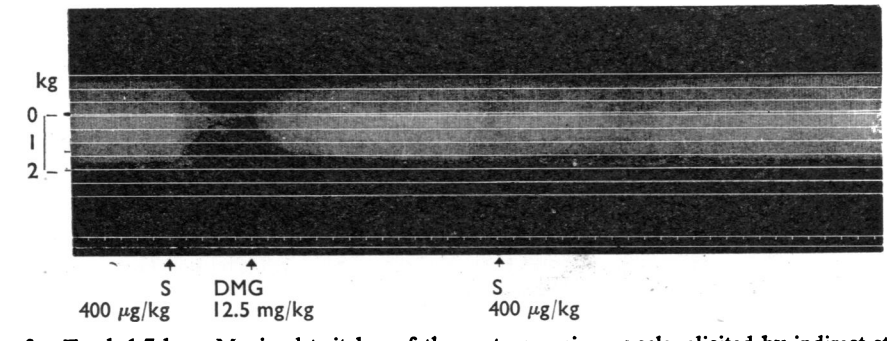

Fig. 3. Fowl, 1.7 kg. Maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every 5 sec. Time in min. S=intravenous injection of gallamine triethiodide, 400 μg/kg. DMG=intravenous injection of *NN*-dimethylguanidine sulphate, 12.5 mg/kg.

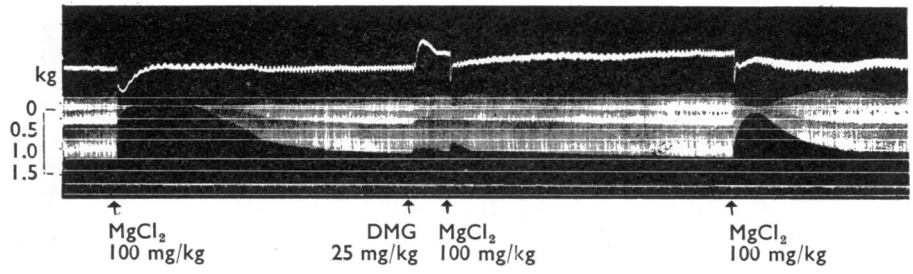

Fig. 4. Fowl, 1.9 kg. Upper record : blood pressure. Lower record : maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every 5 sec. Time in min. $MgCl_2$= intravenous injection of magnesium chloride, 100 mg/kg. DMG=intravenous injection of *NN*-dimethylguanidine sulphate, 25 mg/kg.

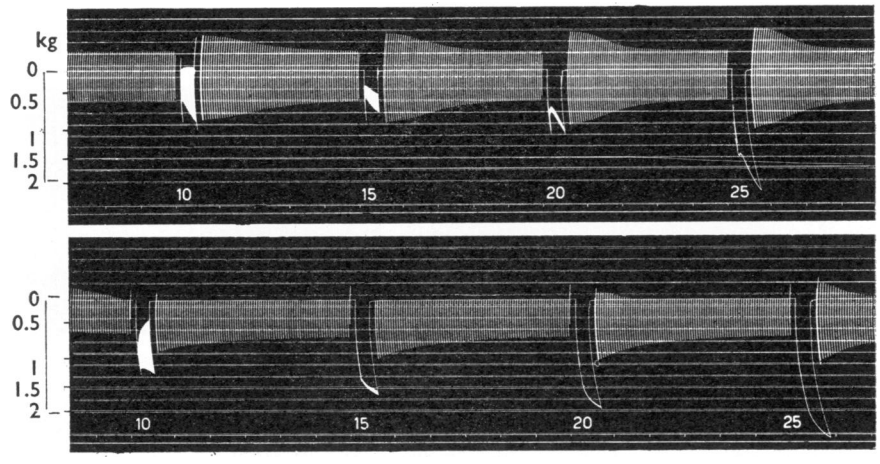

Fig. 5. Fowl, 1.9 kg. Maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every 5 sec. These were alternated with 10 sec tetani obtained by stimuli frequencies of 10–15–20–25 sec. *NN*-Dimethylguanidine sulphate (12.5 mg/kg) was given intravenously between the upper and lower recordings.

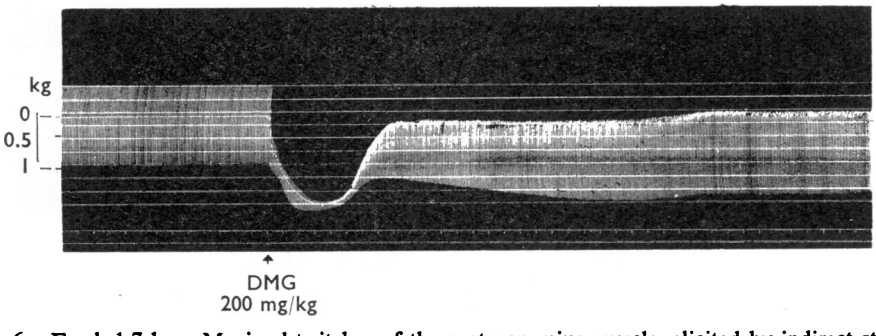

Fig. 6. Fowl, 1.7 kg. Maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every 5 sec. Time in min. DMG=intravenous injection of *NN*-dimethylguanidine sulphate, 200 mg/kg.

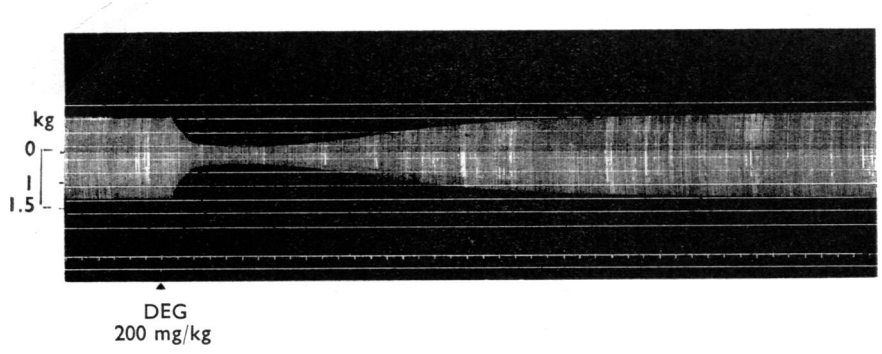

Fig. 7. Fowl, 1.8 kg. Maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every 5 sec. Time in min. DEG = intravenous injection of *NN*-diethylguanidine nitrate, 200 mg/kg.

twitches. As shown in Fig. 5, *NN*-dimethylguanidine favoured the tetanic fusion of stimuli repeated at increasing frequencies. The same compound, at doses higher than those causing the anticurare effects (200 mg/kg), caused a short-lasting spastic paralysis similar to that induced by suxamethonium or decamethonium (Fig. 6). This effect was not altered by neostigmine.

NN-Diethylguanidine (4), at a dose of 100 to 200 mg/kg, caused a tubocurarine-like flaccid paralysis (Fig. 7). This effect was not modified by cholinesterase inhibitors, and was additive with that of gallamine triethiodide or tubocurarine. The drug did not alter the effects of suxamethonium or decamethonium.

Cats. The effects observed with the neuromuscular preparations in cats were similar to those found in fowls and reported above. *NN*-Dimethylguanidine antagonized the effects of gallamine triethiodide and tubocurarine (Fig. 8), but not those of decamethonium and suxamethonium (Fig. 9). Also it antagonized the

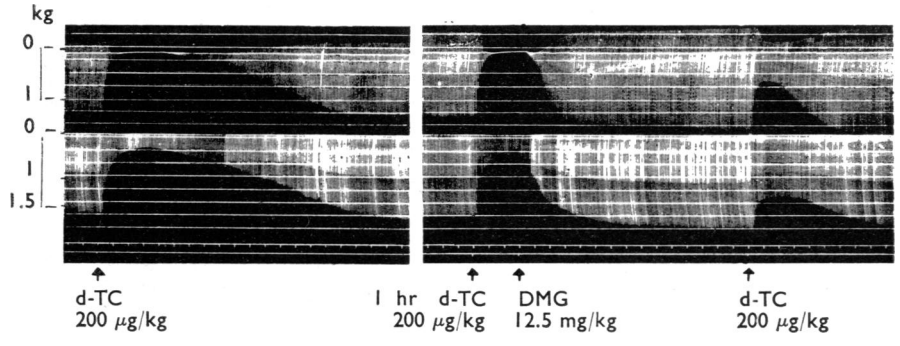

Fig. 8. Cat, 3.5 kg. Upper record: maximal twitches of the tibialis ant. muscle, elicited by indirect stimulation once every 5 sec. Lower record: maximal twitches of the gastrocnemius muscle, elicited by the same stimulation. Time in min. d-TC = intravenous injection of tubocurarine chloride pentahydrate, 200 μg/kg. DMG = intravenous injection of *NN*-dimethylguanidine sulphate, 12.5 mg/kg.

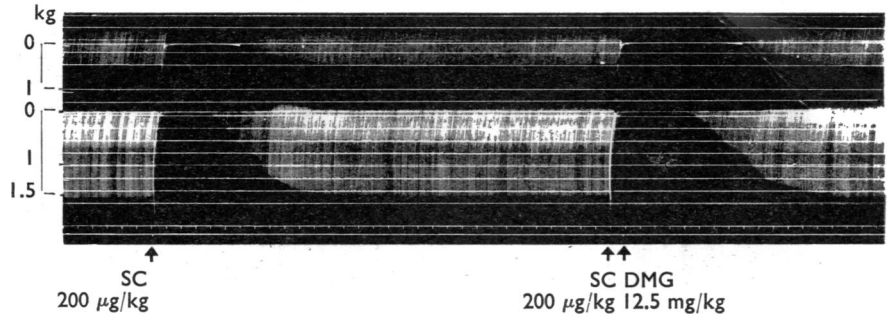

Fig. 9. Cat, 2.5 kg. Upper record: maximal twitches of the tibialis ant. muscle, elicited by indirect stimulation once every 5 sec. Lower record: maximal twitches of the gastrocnemius muscle, elicited by the same stimulation. Time in min. SC=intravenous injection of suxamethonium chloride, 200 μg/kg. DMG=intravenous injection of *NN*-dimethylguanidine sulphate, 12.5 mg/kg.

curare-like activity of magnesium (Fig. 10), but not that of hemicholinium. *N*-Methylguanidine and guanidine had the same effects as had *NN*-dimethylguanidine.

In contrast to the observations in fowls, in cats *NN*-dimethylguanidine, at doses capable of antagonizing tubocurarine and gallamine triethiodide, did not facilitate the tetanic fusion of stimuli repeated at various frequencies.

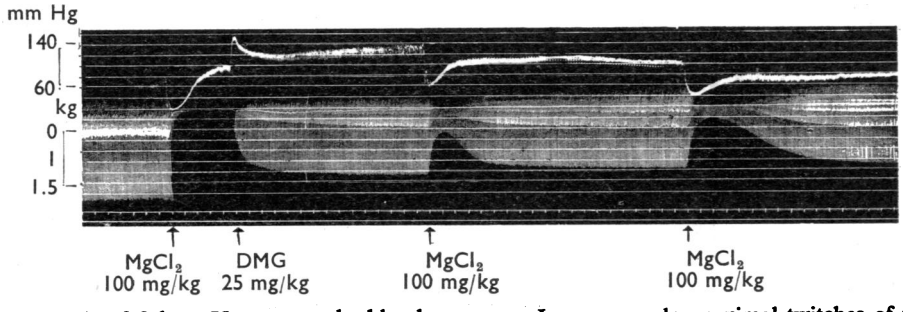

Fig. 10. Cat, 3.2 kg. Upper record: blood pressure. Lower record: maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every 5 sec. Time in min. $MgCl_2$ =intravenous injection of magnesium chloride, 100 mg/kg. DMG=intravenous injection of *NN*-dimethylguanidine sulphate, 25 mg/kg.

NN-Diethylguanidine caused curarization in cats at doses lower than those effective in fowls (Fig. 11), but, as found with fowls, this curare-like activity was additive with that of gallamine triethiodide and was not altered by cholinesterase inhibitors. In addition, the drug did not modify the effects of decamethonium and suxamethonium.

Superior cervical ganglion-nictitating membrane

Cats. Only guanidine antagonized the effects of ganglion blocking agents in the nictitating membrane system. This activity was evident at doses which were also effective against curare-like agents (25, 50 and 100 mg/kg).

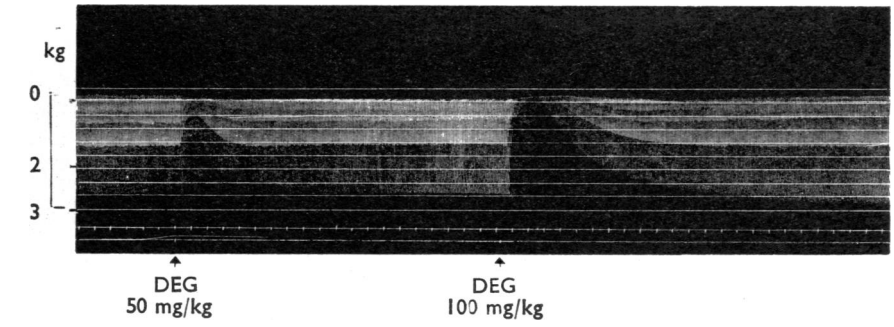

Fig. 11. Cat, 3.4 kg. Maximal twitches of the gastrocnemius muscle elicited by indirect stimulation once every 5 sec. Time in min. DEG=intravenous injection of *NN*-diethylguanidine nitrate, 50 and 100 mg/kg.

The antagonism exerted by guanidine was demonstrated against various types of ganglion blocking agents. In view of the difficulty of inducing a ganglion blockade of comparable intensity and duration by various drugs it is only possible to state with some approximation that guanidine antagonized, in decreasing order of potency, the effects of hexamethonium, pentolinium and mecamylamine (Figs. 12, 13 and 14). No clear activity was seen against chlorisondamine and pempidine.

When ganglionic blockade was followed by administration of guanidine the responses of the nictitating membrane to post-ganglionic stimuli were slightly potentiated and at the same time the responses to pre-ganglionic stimuli rapidly approached normal levels (Fig. 15). In addition, guanidine restored blood pressure to normal levels, and sometimes hypertension developed. However, the compound has pressor effects of its own at doses as low as 50 mg/kg.

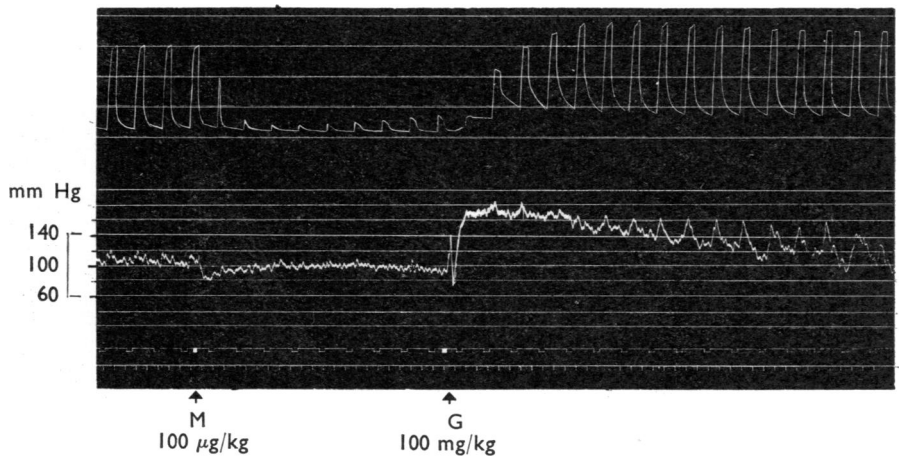

Fig. 12. Cat, 2.3 kg. From the top to bottom: nictitating membrane response to pre-ganglionic stimulation; blood pressure; injection and stimulation signal; time in min. M=intra-arterial injection of mecamylamine, 100 μg/kg. G=intravenous injection of guanidine hydrochloride, 100 mg/kg.

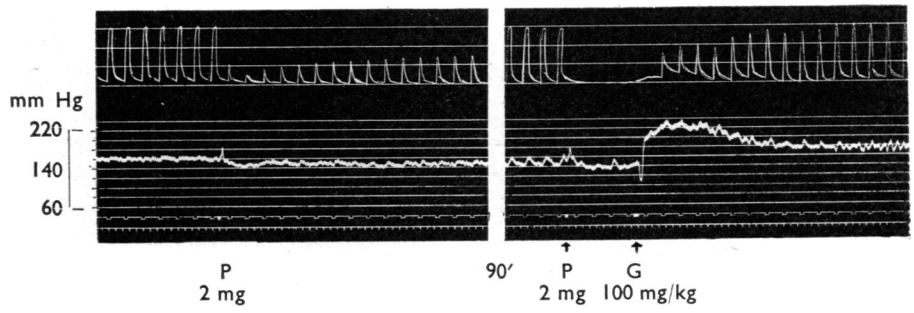

Fig. 13. Cat, 2.9 kg. From the top to bottom: nictitating membrane response to pre-ganglionic stimulation; blood pressure; injection and stimulation signal; time in min. P=intra-arterial injection of pentolinium tartrate, 2 mg. G=intravenous injection of guanidine hydrochloride, 100 mg/kg.

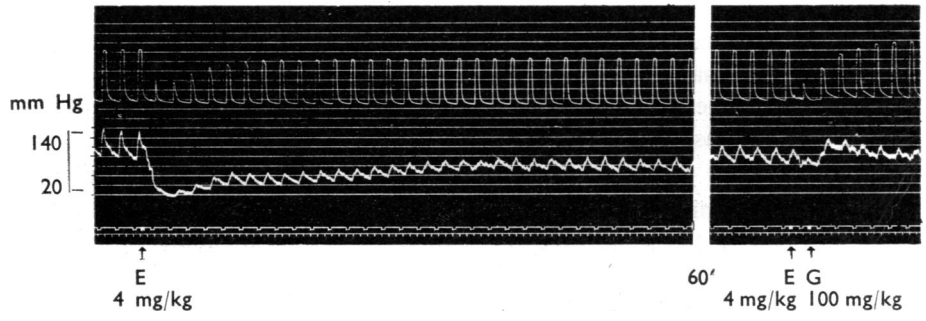

Fig. 14. Cat, 2.6 kg. From the top to bottom: nictitating membrane response to pre-ganglionic stimulation; blood pressure; injection and stimulation signal; time in min. E=intravenous injection of hexamethonium iodide, 4 mg/kg. G=intravenous injection of guanidine hydrochloride, 100 mg/kg.

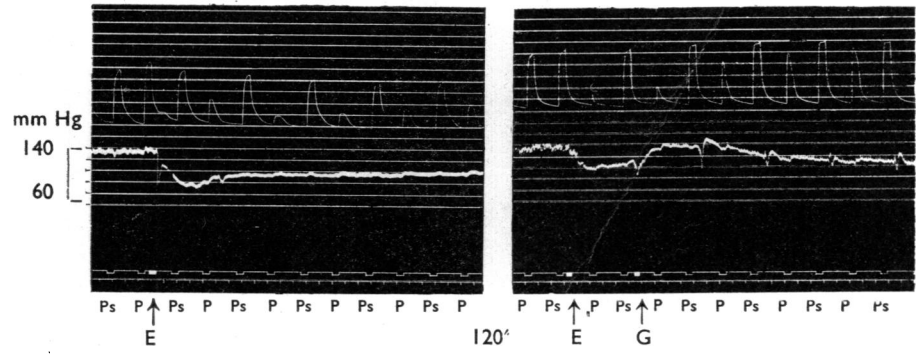

Fig. 15. Cat, 3.2 kg. From the top to bottom: nictitating membrane response to pre-ganglionic (P) and post-ganglionic (Ps) alternate stimulation; blood pressure; injection and stimulation signal; time in min. E=intravenous injection of hexamethonium iodide, 4 mg/kg. G=intravenous injection of guanidine hydrochloride, 50 mg/kg.

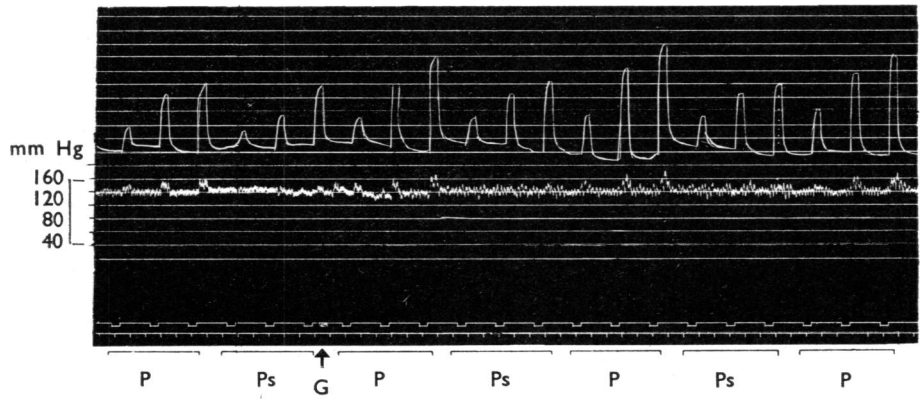

Fig. 16. Cat, 2.0 kg. From the top to bottom: nictitating membrane response to graduated pre- (P) and post-ganglionic (Ps) stimulation (rectangular pulses of 0.3 msec ; rates 1–5–10 shocks/sec); blood pressure; injection and stimulation signal; time in min. G=intravenous injection of guanidine hydrochloride, 25 mg/kg.

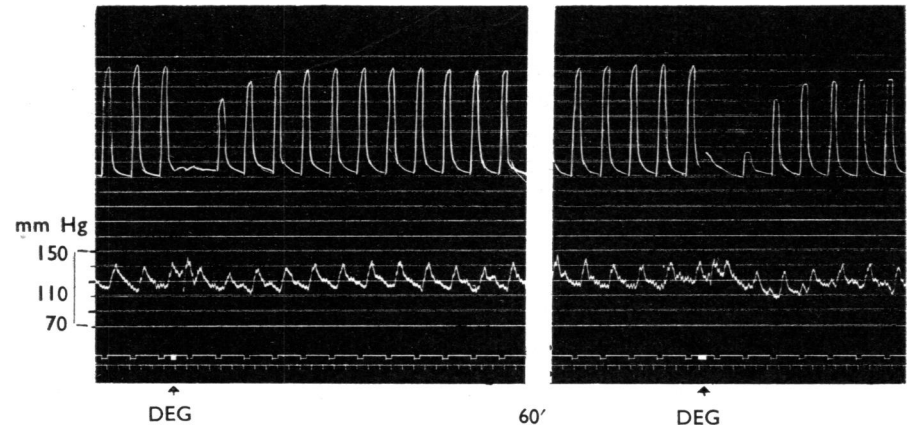

Fig. 17. Cat, 4.0 kg. From the top to bottom: nictitating membrane response to pre-ganglionic stimulation; blood pressure; injection and stimulation signal; time in min. DEG=intravenous injection of *NN*-diethylguanidine nitrate, 6.25 mg/kg, and after 60 min intravenous injection of *NN*-diethylguanidine nitrate, 12.5 mg/kg.

In the absence of any ganglionic blockade guanidine potentiated the responses of the nictitating membrane to pre- and post-ganglionic stimulation (Fig. 16), but the pre-ganglionic responses were always more clearly potentiated. The potentiation of post-ganglionic stimuli may be related to the fact that the drug increased the sensitivity of the membrane to the sympathetic mediators. Responses to adrenaline and noradrenaline were potentiated only by guanidine of all the compounds studied.

Guanidine did not alter the reactivity of the parasympathetic system. Neither hypotension nor bradycardia induced by vagus stimulation were altered by guanidine. Also, in agreement with previous observations by Zamboni & Azzolini (1954),

guanidine did not antagonize the vagus blockade induced by ganglion blocking agents.

Finally, it is of interest that *NN*-diethylguanidine possessed ganglion blocking effects in addition to its curare-like activity. The contractions of the nictitating membrane induced by pre-ganglionic stimuli were inhibited by this compound at doses of 6.25 and 12.5 mg/kg (Fig. 17). These doses are lower than those necessary to cause a curare-like effect at the level of the myoneural junction.

DISCUSSION

The results reported here are consistent with previous observations made *in vitro* by Condouris & Ghazal (1957) on the rat phrenic nerve-diaphragm preparation, and confirm that guanidine antagonizes the curare-like effects of tubocurarine and gallamine triethiodide without altering those of suxamethonium and decamethonium. In addition, *NN*-dimethylguanidine and *N*-methylguanidine proved to be more potent than guanidine, whereas other analogues such as *N*-nitroguanidine, dicyandiamide or dicyandiamidine were completely inactive.

The results reported are also consistent with the hypothesis suggested by Otsuka & Endo (1960). These authors believe, on the basis of electrical phenomena evaluated at the myoneural junction, that guanidine favours the liberation of acetylcholine from the terminations of the motor nerves, and that this is the mechanism of its antagonistic activity against tubocurarine. The fact that *NN*-dimethylguanidine, *N*-methylguanidine, and guanidine antagonize the curare-like agents that prevent depolarization (tubocurarine and gallamine triethiodide) but not those that cause depolarization (suxamethonium and decamethonium) is in agreement with the hypothesis mentioned above. Also consistent with this hypothesis is the observation that the guanidines antagonize or prevent the curare-like effects of magnesium. It is known that magnesium reduces the liberation of acetylcholine from the terminations of the motor nerves (Del Castillo & Engbaek, 1953). Furthermore, the lack of antagonism between guanidine and hemicholinium fits the hypothesis discussed. The curare-like effects of hemicholinium are dependent upon the inhibition of acetylcholine biosynthesis (MacIntosh, Birks & Sastry, 1956; Gardiner, 1957).

The observation that *NN*-dimethylguanidine favours the tetanic fusion of stimuli repeated at increasing frequencies is also in agreement with the ideas of Otsuka & Endo (1960). This phenomenon, however, was not observed in cats. Potentiation of the twitches was never observed. In this regard it is worth mentioning that Fastier (1949) noticed potentiation of the twitches by guanidine in the rat phrenic nerve-diaphragm preparation, but did not do so in the cat sciatic nerve-gastrocnemius system.

The fact that in fowls the guanidines did not alter the spastic paralysis induced by acetylcholine, decamethonium or suxamethonium also suggests that their anti-curare activity does not take place directly at the level of the neuromuscular plate.

A mechanism of action at the neuromuscular plate level, however, could explain the decamethonium-like spastic paralysis induced in fowls by *NN*-dimethylguanidine at doses much higher than those capable of antagonizing tubocurarine or gallamine triethiodide. Indeed, cholinesterase inhibitors did not alter this paralysis.

One of the most striking features of the guanidines is the duration of their activity. This characteristic had been clearly noted by Fastier (1949) on the rat phrenic nerve-diaphragm preparation, and led him to state that "even ten or fifteen washings failed to restore the normal reaction of preparation, consequently little could be learned from the effect of a second dose."

As far as the antagonism against ganglionic blocking agents is concerned, several considerations are possible. It must be stressed that only guanidine was active among the compounds studied. Since guanidine is the only compound, of those tested on the nictitating membrane system, which was capable of potentiating the effects of adrenaline, noradrenaline and post-ganglionic stimulation, it is reasonable to assume that this antagonistic activity is the result of a potentiation of the peripheral effects of the sympathetic chemical mediators.

Some data suggest, however, that guanidine also acts at the ganglionic level. In the first place the drug is active even after complete ganglion blockade, that is to say, when pre-ganglionic stimulation should not liberate the chemical mediator at the peripheral termination. Secondly, by alternating pre- and post-ganglionic stimulation of the nictitating membrane one can show that, after ganglion blockade, guanidine produces a rapid reappearance of the contractions due to pre-ganglionic stimuli without a significant potentiation of those due to post-ganglionic impulses. On the other hand, in absence of any ganglion blockade, guanidine clearly potentiates the pre-ganglionic responses and has much less effect on the post-ganglionic ones. Finally, guanidine is active against some ganglion blocking agents, such as hexamethonium, pentolinium or mecamylamine, but it is not active against others such as chlorisondamine or pempidine. Should a peripheral mechanism of action be the only one involved, comparable antagonism by guanidine would be expected against all of these blocking agents.

These observations suggest, therefore, that a ganglionic site of action may condition the activity of guanidine against ganglion blocking agents ; only the study of action potentials in the ganglia will clarify this problem completely.

It is difficult to say whether or not the ganglionic action of guanidine is similar to the peripheral action at the neuromuscular junctions. An increased liberation of acetylcholine from pre-ganglionic fibres could explain the antagonism exerted against hexamethonium and pentolinium, both of which are considered to be non-depolarizing ganglionic blocking agents of the competitive, surmountable type (Trendelenburg, 1961). The lack of effect against the non-competitive, unsurmountable pempidine and chlorisondamine would be consistent with this possibility. The antagonism of guanidine against mecamylamine is not easily explained since the mechanism of this blocking agent is considered to be analogous to that of both pempidine and chlorisondamine (Trendelenburg, 1961). However, mecamylamine has also been reported to have an intermediate type of action, being both a competitive and a non-competitive antagonist (Van Rossum, 1961).

In conclusion, the present report of the activity of some guanidine derivatives at the myoneural junction and ganglionic levels emphasizes the need for further studies of the intimate mechanism involved in the effects observed.

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