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EFFECTS OF SOME *NN*-DISUBSTITUTED GUANIDINES ON NEUROMUSCULAR AND GANGLIONIC TRANSMISSION

BY

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The activity of guanidine at the level of neuromuscular synapses was initially examined by Feng (1938), by Condouris & Ghazal (1957) and by Otsuka & Endo (1960). The last authors have demonstrated that guanidine antagonizes the activity of nondepolarizing muscle-paralysing agents, probably by increasing the amount of acetylcholine released from the nerve endings.

Zamboni & Azzolini (1954) reported also an antagonistic activity of guanidine against ganglionic blocking agents.

Recently Barzaghi, Mantegazza & Riva (1962) reported that *NN*-dimethylguanidine and *N*-methylguanidine are more active than guanidine as antagonists of (+)-tubocurarine and gallamine triethiodide, while *NN*-diethylguanidine shows a curare-like activity. As far as the antagonistic activity against ganglionic blocking agents is concerned, they found that only guanidine is effective, while *NN*-diethylguanidine blocks ganglionic transmission.

Having observed that *NN*-diethylguanidine has both curare-like and ganglionic blocking properties, it was considered interesting to study some new *NN*-disubstituted guanidines.

METHODS

Neuromuscular blocking activity in mice. All compounds were administered by rapid intravenous injection into the tail vein of albino mice (Swiss strain). The criterion of paralysis was the fall of the mouse from a screen inclined 60° from the horizontal (Hoppe, 1950). The median paralysing dose (EP50) and its standard error were calculated by the method of Litchfield & Wilcoxon (1949) using at least ten mice at each of three dose levels.

" *Head drop* " *test in rabbits.* The dose producing head drop in 50% of the rabbits (HD50) was calculated according to Litchfield & Wilcoxon (1949). At least six rabbits at each dose level were used.

Sciatic nerve-gastrocnemius muscle preparation in 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 of the gastrocnemius muscle were elicited and recorded as described for cats. Drugs were injected through a cannula in the wing vein.

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.

Sciatic nerve-gastrocnemius muscle preparation in cats. Pointed drill rods were driven through the proximal and distal ends of the femur and through the malleoli of a hind-leg of a cat anaesthetized with chloralose (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 central 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 stimulus frequency was 12 shocks/min. When necessary, artificial ventilation 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 arterial 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.

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

The pre- and postganglionic sympathetic nerves were isolated and prepared for electrical stimulation in the usual manner and were 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.

Drugs were injected intravenously into the femoral vein.

Drugs. The following compounds were used in the experiments, numbers in parentheses following the names refer to Table 1, which gives the structures. *NN*-Dimethylguanidine sulphate (1) and *NN*-diethylguanidine nitrate (2) were obtained from B.D.H. *NN*-Dipropylguanidine nitrate (3), *NN*-dibutylguanidine nitrate (4), *NN*-di-isopropylguanidine nitrate (5), *NN*-di-isobutylguanidine nitrate (6), *N*-ethyl-*N*-methylguanidine tartrate (7), *NN*-diphenylguanidine nitrate (8), *NN*-dibenzylguanidine hydrochloride (9), *N*-benzyl-*N*-phenylguanidine hydrochloride (10), *N*-methyl-*N*-phenylguanidine hydrochloride (11), *N*-benzyl-*N*-methylguanidine hydrochloride (12), *N*-ethyl-*N*-phenylguanidine hydrochloride (13) and *N*-benzyl-*N*-ethylguanidine tartrate (14) were synthetized at Vister Laboratory. Methods for the synthesis and chemical data will be published elsewhere (Bianchi & Barzaghi, 1964).

RESULTS

Curarizing action in mice. The results are summarized in Table 1, from which it appears that some of the guanidines examined have a strong curare-like activity. *N*-Benzyl-*N*-phenylguanidine is the most active compound, and the others, in decreasing order of potency, are *NN*-dibutylguanidine, *NN*-di-isobutylguanidine and *N*-ethyl-*N*-phenylguanidine. These compounds show an activity similar to that of gallamine triethiodide and a similar range between the EP50 and LD50.

In general, the paralysis is rapidly induced and of short duration.

Curarizing action in rabbits. The results obtained are shown in Table 1. From these it appears that some of the compounds are also quite active in this species. Furthermore, the compounds more effective in mice are also more effective in rabbits. None of the compounds appears to be more active than gallamine triethiodide.

Sciatic nerve-gastrocnemius muscle preparation in fowls. All the guanidines, with the exception of *N*-methyl-*N*-phenylguanidine, *N*-benzyl-*N*-methylguanidine and *NN*-dimethylguanidine, provoke a flaccid tubocurarine-like paralysis.

N-Benzyl-*N*-ethylguanidine and *NN*-dibenzylguanidine are the most effective compounds, followed by *NN*-dipropylguanidine, *NN*-dibutylguanidine and *NN*-di-isobutylguanidine.

TABLE 1

TOXICITY AND NEUROMUSCULAR BLOCKING PROPERTIES OF *NN*-DISUBSTITUTED GUANIDINES

All quantities are given in terms of the free base. Values in parenthesis are 95% fiducial limits. Injections were intravenous

Compound No.	Structure (R=—C(:NH)NH$_2$)	LD50 (in mice)		EP50 (in mice)		HD50 (in rabbits)	
		mg/kg	Slope	mg/kg	Slope	mg/kg	Slope
1	R—N(CH_3)$_2$	50 (45–54)	1·1 (1·0–1·3)	43·6 (38·5–48·7)	1·24 (1·0–1·4)	>50·0	—
2	R—N(C_2H_5)$_2$	16·7 (7·1–36·8)	1·6 (0·8–3·3)	7·6 (7·1–8·1)	1·0 (0·8–1·4)	16·4 (14·8–17·4)	1·1 (0·7–1·5)
3	R—N(C_3H_7)$_2$	3·4 (3·1–3·6)	1·1 (0·8–1·2)	2·2 (2·0–2·3)	1·1 (0·8–1·4)	2·2 (2·0–2·4)	1·0 (0·7–1·4)
4	R—N(C_4H_9)$_2$	3·1 (2·5–3·7)	1·3 (1·0–1·7)	0·9 (0·5–1·6)	1·6 (1·0–2·5)	2·1 (1·7–2·7)	1·3 (1·0–1·5)
5	R—N(i—C_3H_7)$_0$	5·0 (4·6–5·4)	1·2 (1·1–1·3)	3·5 (3·1–3·8)	1·1 (0·9–1·3)	5·5 (4·7–6·3)	1·1 (0·7–1·6)
6	R—N(i—(C_4H_9)$_2$	1·7 (1·5–1·9)	1·1 (0·9–1·4)	1·0 (0·9–1·1)	1·1 (0·9–1·3)	1·6 (1·3–1·9)	1·2 (1·0–1·5)
7	R—N(CH_3)(C_2H_5)	52 (43–62)	1·3 (1·1–1·5)	35·3 (32·1–38·5)	1·2 (1·0–1·3)	31·3 (22·9–42·2)	1·2 (0·9–1·7)
8	R—N(C_6H_5)$_2$	7·1 (6·1–7·7)	1·3 (1·0–1·6)	2·8 (2·4–3·2)	1·2 (0·9–1·4)	3·0 (2·6–3·4)	1·1 (0·9–1·4)
9	R—N($CH_2.C_6H_5$)$_2$	1·1 (0·8–1·5)	2·3 (1·4–3·8)	1·1 (0·8–1·4)	1·4 (1·0–1·8)	2·8 (2·5–2·9)	1·1 (0·9–1·3)
10	R—N($CH_2.C_6H_5$)(C_6H_5)	3·7 (3·2–4·3)	1·2 (1·0–1·4)	0·02 (0·01–0·04)	15·6 (3·9–62·4)	2·2 (1·9–2·4)	1·1 (0·8–1·4)
11	R—N(CH_3)(C_6H_5)	14·4 (12·8–15·2)	1·1 (1·0–1·3)	1·8 (0·8–4·1)	2·6 (0·8–7·8)	7·3 (5·6–8·0)	1·2 (1·0–1·5)
12	R—N(CH_3)($CH_2.C_6H_5$)	10·4 (8·6–11·5)	1·2 (1·0–1·5)	5·4 (4·3–5·7)	1·2 (1·0–1·4)	6·4 (5·4–7·6)	1·1 (0·8–1·5)
13	R—N(C_2H_5)(C_6H_5)	5·7 (4·0–6·5)	1·3 (1·0–1·5)	1·1 (0·6–1·9)	2·9 (1·3–6·3)	3·4 (3·0–3·7)	1·1 (0·9–1·4)
14	R—N(C_2H_5)($CH_2.C_6H_5$)	4·4 (3·8–5·1)	1·2 (0·9–1·5)	2·3 (2·0–2·7)	1·1 (0·9–1·4)	2·7 (2·3–3·1)	1·2 (1·0–1·4)
Gallamine triethiodide		3·5 (3·2–3·7)	1·1 (0·9–1·6)	1·6 (1·4–1·7)	1·3 (1·1–1·4)	0·3 (0·2–0·35)	1·1 (0·8–1·3)

The approximate doses which are able to produce a 50% inhibition of the muscle twitches were 5.1 mg/kg for *N*-benzyl-*N*-ethylguanidine, 7.7 mg/kg for *NN*-dibenzylguanidine and 8.3 mg/kg for *NN*-dibutylguanidine. They were calculated graphically from the regression line obtained by plotting the intensity of paralysing effect against the logarithm of the doses. The paralysing effect is, in general, rapidly induced and lasts for a short time, as demonstrated in Fig. 1.

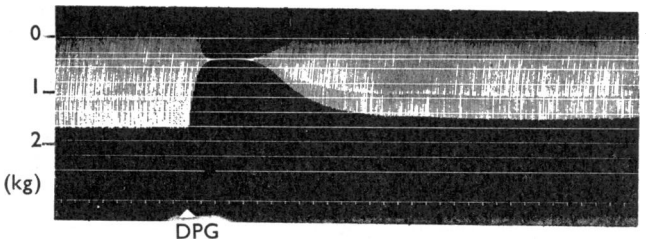


Fig. 1. Fowl, 2.4 kg. Maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every 5 sec. Time in minutes. At DPG, intravenous injection of *NN*-dipropylguanidine nitrate (12.5 mg/kg).

As mentioned before, *N*-methyl-*N*-phenylguanidine and *NN*-dimethylguanidine provoke a contracture of gastrocnemius muscle similar to that induced by depolarizing muscle-paralysing agents. But, while *NN*-dimethylguanidine is active at doses of 200 mg/kg, *N*-methyl-*N*-phenylguanidine is active at doses fifteen-times lower, as demonstrated in Fig. 2.

N-Benzyl-*N*-methylguanidine provokes a dual type of action, namely contracture and then paralysis, as shown in Fig. 3.

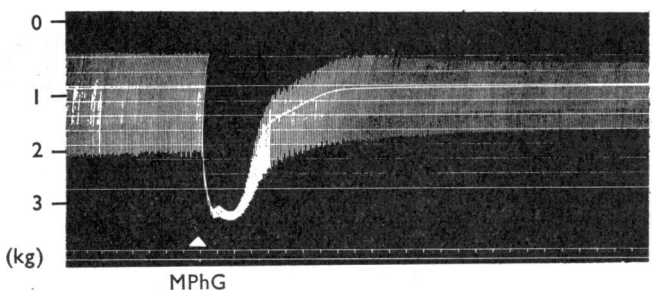


Fig. 2. Fowl, 2.0 kg. Maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every 5 sec. Time in minutes. At MPhG, intravenous injection of *N*-methyl-*N*-phenylguanidine hydrochloride (12.5 mg/kg).

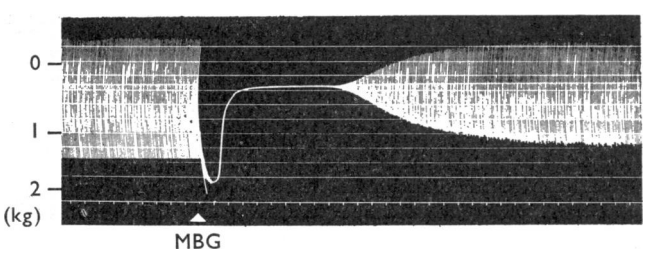


Fig. 3. Fowl, 2.8 kg. Maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation once every second. Time in minutes. At MBG, intravenous injection of *N*-benzyl-*N*-methylguanidine hydrochloride (25 mg/kg).

As *NN*-dimethylguanidine was reported to antagonize tubocurarine-like agents (Barzaghi *et al.*, 1962), *N*-methyl-*N*-phenylguanidine was also studied from this point of view but we did not observe such an antagonistic activity.

Sciatic nerve-gastrocnemius muscle preparation in cats. On this preparation the guanidines are on the whole more active than in fowls. *NN*-Dibenzylguanidine, *NN*-di-isobutylguanidine and *N*-benzyl-*N*-ethylguanidine are the most effective compounds. The approximate doses able to produce 50% inhibition of the muscle twitches are respectively: 1.7, 1.8 and 2.4 mg/kg. In this instance, as well as in those illustrated in Figs. 4, 5, 9 and 10, the activity is rapidly induced, is short lasting, and there is no evidence of any antagonistic activity by the cholinesterase inhibitor.

Superior cervical ganglion-nictitating membrane preparation in cats. In general, it may be said that the *NN*-bisaliphatic derivatives of guanidine show a typical ganglionic blocking

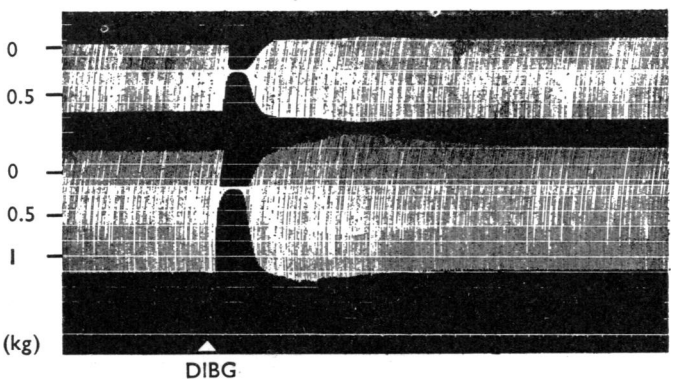


Fig. 4. Cat, 2.2 kg. From the top to bottom: maximal twitches of the tibialis anterior muscle, elicited by indirect stimulation once every 5 sec; maximal twitches of the gastrocnemius muscle, elicited by the same stimulation; time in minutes. At DIBG, intravenous injection of *NN*-di-isobutylguanidine nitrate (6.2 mg/kg).

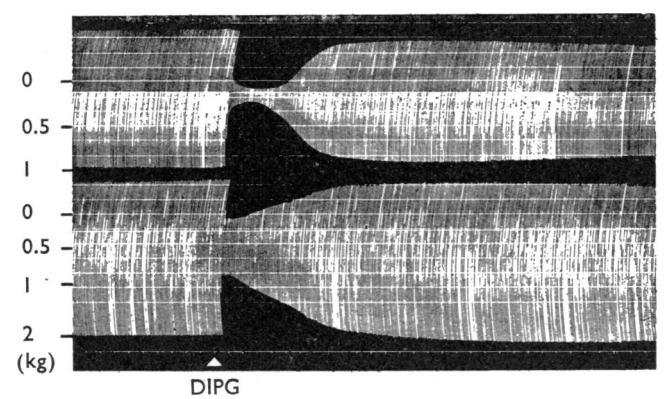


Fig. 5. Cat, 2.2 kg. From the top to bottom: maximal twitches of the tibialis anterior muscle, elicited by indirect stimulation once every 5 sec; maximal twitches of the gastrocnemius muscle, elicited by the same stimulation; time in minutes. At DIPG, intravenous injection of *NN*-di-isopropylguanidine nitrate (6.2 mg/kg).

activity, while all the compounds having one aromatic substituent demonstrate a different activity which we shall briefly describe later.

NN-Di-isopropylguanidine is the most active compound. At doses of 0.5 mg/kg it provokes a reduction of the nictitating membrane contraction and hypotension lasting for about 15 to 30 min. At the same doses it also abolishes for a longer time both hypotension and bradycardia caused by vagal stimulation, as shown in Fig. 6. At doses of 2 to 3 mg/kg

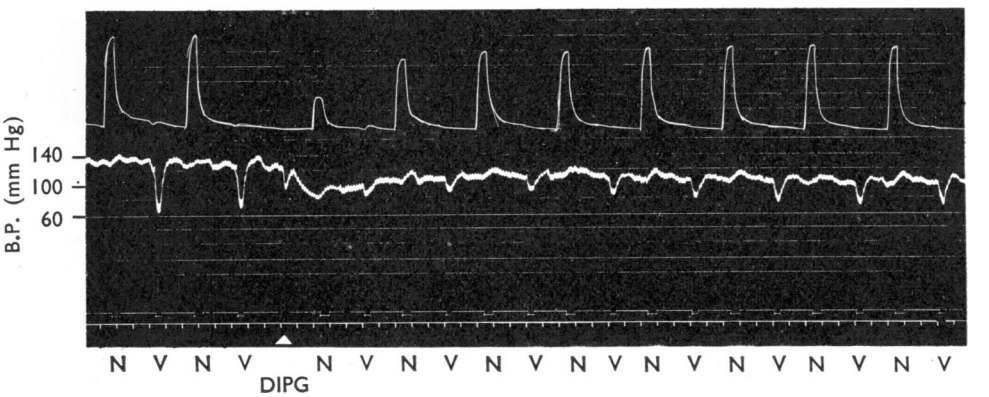


Fig. 6. Cat, 1.9 kg. From the top to bottom: nictitating membrane response to preganglionic stimulation; blood pressure (B.P.); injection and stimulation signal; time in minutes. At N, preganglionic stimulation of the nictitating membrane; at V, stimulation of vagus; at DIPG, intravenous injection of *NN*-di-isopropylguanidine nitrate (0.75 mg/kg).

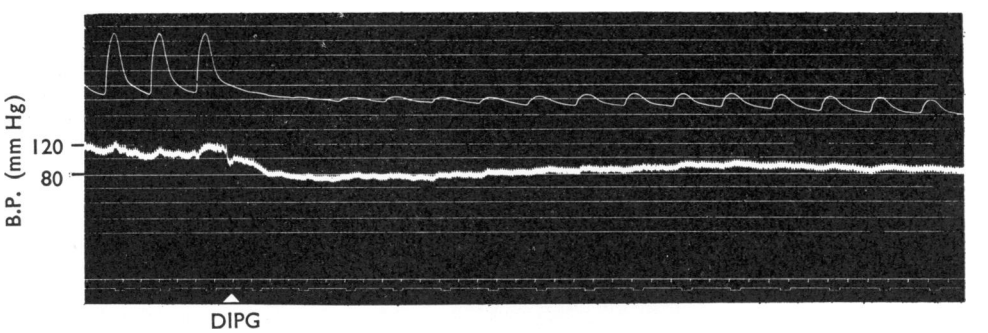


Fig. 7. Cat, 3.0 kg. From the top to bottom: nictitating membrane response to preganglionic stimulation; blood pressure (B.P.); injection and stimulation signal; time in minutes. At DIPG, intravenous injection of *NN*-di-isopropylguanidine nitrate (3.1 mg/kg).

it completely abolishes the nictitating membrane contraction and provokes a hypotension, as seen in Fig. 7. At these doses the effect lasts for more than 1 hr and the hypotension is still present even when the nictitating membrane begins to react again to the preganglionic stimulations. The site of action of *NN*-di-isopropylguanidine is at the ganglionic level and not at the periphery, because the effects of the postganglionic stimulation are unchanged, as is seen in Fig. 8. *NN*-Di-isopropylguanidine also antagonizes the hypertension due to nicotine or to carotid occlusion.

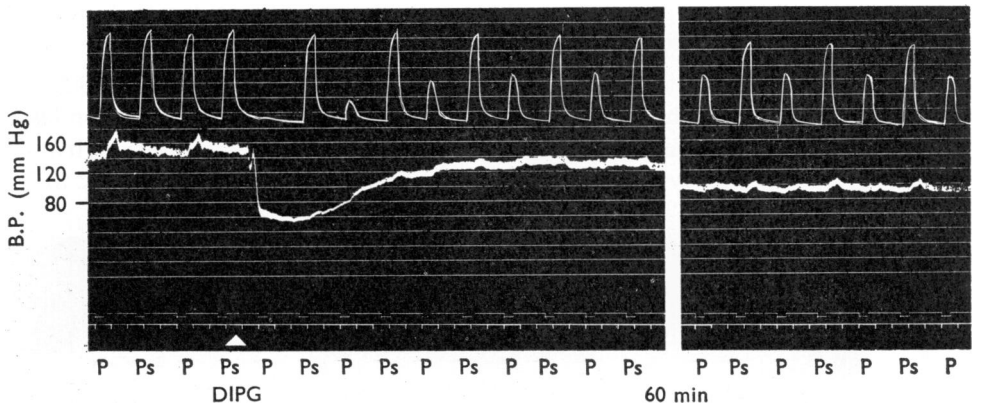


Fig. 8. Cat, 2.5 kg. From the top to bottom: nictitating membrane response to preganglionic (P) and postganglionic (Ps) alternate stimulation; blood pressure (B.P.); injection and stimulation signal; time in minutes. At DIPG, intravenous injection of *NN*-di-isopropylguanidine nitrate (1.5 mg/kg).

A similar action is shown by *NN*-diethylguanidine, *NN*-dipropylguanidine, *NN*-dibutylguanidine, *NN*-di-isobutylguanidine, *N*-ethyl-*N*-methylguanidine, *NN*-diphenylguanidine, *NN*-dibenzylguanidine and *N*-benzyl-*N*-phenylguanidine. However, these compounds are less effective than *NN*-di-isopropylguanidine. Their ganglionic blocking and hypotensive activities are present at higher doses, 6 to 12 mg/kg, and last for a shorter time (Figs. 9 and 10).

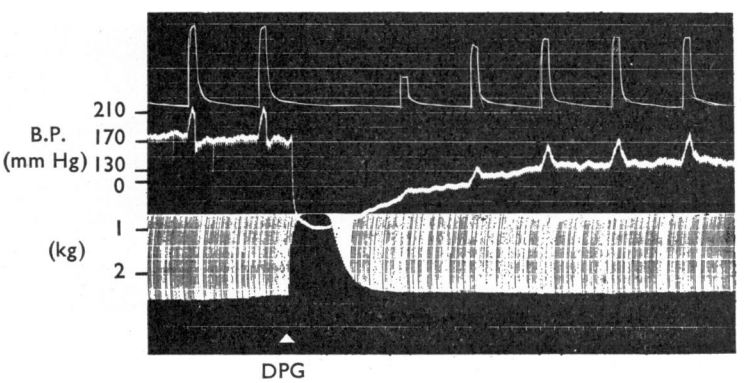


Fig. 9. Cat, 3.0 kg. From the top to bottom: nictitating membrane response to preganglionic stimulation; blood pressure (B.P.); maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation; time in minutes. At DPG, intravenous injection of *NN*-dipropylguanidine nitrate (12.5 mg/kg).

Unlike most of the compounds, *N*-methyl-*N*-phenylguanidine causes a transient hypotension followed by hypotension and a contracture of the nictitating membrane, as is shown in Fig. 11.

N-Benzyl-*N*-methylguanidine, *N*-ethyl-*N*-phenylguanidine and *N*-benzyl-*N*-ethylguanidine provoke a contraction of the nictitating membrane, but at the same time abolish the

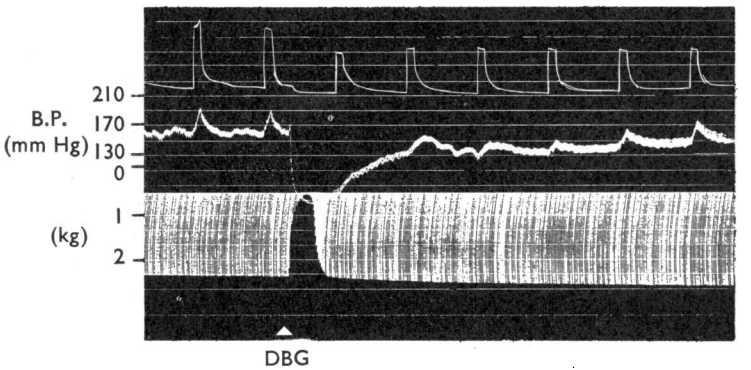


Fig. 10. Cat, 2.4 kg. From the top to bottom: nictitating membrane response to preganglionic stimulation; blood pressure (B.P.); maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation; time in minutes. At DBG, intravenous injection of *NN*-dibenzylguanidine hydrochloride (12.5 mg/kg).

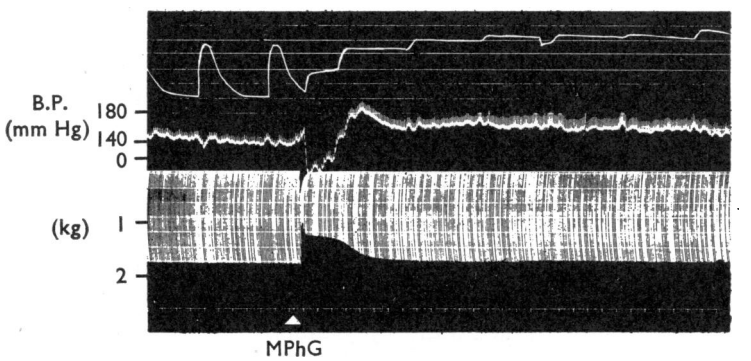


Fig. 11. Cat, 2.8 kg. From the top to bottom: nictitating membrane response to preganglionic stimulation; blood pressure (B.P.); maximal twitches of the gastrocnemius muscle, elicited by indirect stimulation; time in minutes. At MPhG, intravenous injection of *N*-methyl-*N*-phenylguanidine hydrochloride (6.2 mg/kg).

response to preganglionic stimulation. The contraction of the nictitating membrane induced by these compounds is present even when the superior cervical ganglion had been removed, and is almost completely abolished by adrenalectomy.

DISCUSSION

Our results, in addition to those previously reported (Barzaghi *et al.*, 1962), confirm that asymmetric guanidines can interfere with the neuromuscular and ganglionic transmission.

As regards the neuromuscular transmission, the sciatic nerve-gastrocnemius muscle preparation in fowls permitted more interesting observations. On this preparation most of the guanidines examined provoke a tubocurarine-like flaccid paralysis, and a few of them a decamethonium-like spastic paralysis. As far as the tubocurarine-like activity is concerned, it is rapidly induced, of short duration and quite similar in potency on the various species examined. It is not antagonized by cholinesterase inhibitors. With reference to the decamethonium-like activity, only *NN*-dimethylguanidine and *N*-methyl-*N*-phenylguanidine,

among the compounds examined, are active, but the latter is 15- to 20-times more potent than the former.

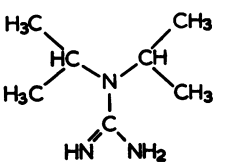
N-Benzyl-*N*-methylguanidine also induces a contracture of gastrocnemius muscle in fowls, but the contracture is followed by a flaccid paralysis; this compound has therefore a " dual action." An analogous behaviour was described for some quaternary ammonium salts by Thesleff & Unna (1954), Ginzel, Klupp & Werner (1951) and Mantegazza (1956).

The fact that among the compounds examined only *NN*-dimethylguanidine, *N*-methyl-*N*-phenylguanidine and *N*-benzyl-*N*-methylguanidine are able to contract the fowls'muscles, suggests that probably one methyl group at the disubstituted nitrogen atom of guanidine is necessary to induce this type of action.

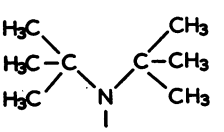
It is also interesting to observe that, while *NN*-dimethylguanidine antagonizes tubocurarine, *N*-methyl-*N*-phenylguanidine is completely devoid of this activity. Since *N*-methyl-*N*-phenylguanidine is much more active than *NN*-dimethylguanidine in inducing a spastic paralysis in fowls, it may be concluded that the antagonistic activity of guanidines against nondepolarizing agents is not related to the fact that they behave like depolarizing agent on the striated muscles of fowls.

As regards the ganglionic blocking activity, it can be observed that it is not so widely present among the various guanidines examined as is the curare-like activity. In fact, only the *NN*-bisaliphatic derivatives shows a ganglionic blocking activity. Among all the compounds having ganglionic blocking properties, *NN*-di-isopropylguanidine (I) is by far the most effective. The site of action of these compounds is at ganglionic level, because the effects of the postganglionic stimulation are left completely unchanged.

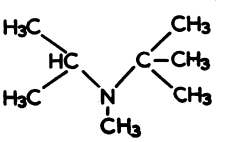
By far the most pronounced activity of *NN*-di-isopropylguanidine in respect to all the other compounds examined may be explained on the basis of structural analogy with other ganglionic blocking agents. In fact, a di-t-butylamino-structure (II) had been identified in the molecule of pempidine as responsible for its ganglionic blocking activity (Bretherick, Lee, Lunt, Wragg & Edge, 1959). Furthermore, among several aliphatic derivatives obtained by opening the pempidine ring (Hiltmann, Wollweber, Wirth & Gösswald, 1960), the t-butylisopropyl amine (III) had been found to be the most active and to exhibit a level of activity similar to that of pempidine.



(I)



(II)



(III)

From the above results it appears that a short branched aliphatic structure seems to be the structural requirement for the highest ganglionic blocking activity, just as for the guanidines we have examined.

Finally, little can be said on the mechanism of action of the asymmetric guanidines studied. Probably it is related to their guanidine moiety, which is strongly basic and highly ionized, just like a quaternary ammonium base.

However, this analogy does not seem to justify a similar mechanism of action at the neuromuscular or ganglionic synapses. This is shown, for instance, by the absence of an antagonism by cholinesterase inhibitors against curare-like activity.

For these reasons a wider analysis, both from a pharmacological and chemical point of view, seems necessary for a better understanding of the mechanism of action of this new class of ganglionic blocking and curare-like agents.

SUMMARY

1. The activities of some new *NN*-disubstituted guanidines have been examined on the sciatic nerve-gastrocnemius muscle preparation in fowls and on the sciatic nerve-gastrocnemius and tibialis anterior muscle preparation in cats. The curare-like activities of the compounds were also compared by their effects on the fall of mice from an inclined screen and by the " head drop " test in rabbits. Their actions on pre- and postganglionic stimulations to the superior ganglion nictitating membrane preparation in cats were also examined.

2. In fowls, most of the compounds induce a tubocurarine-like flaccid paralysis which is not antagonized by cholinesterase inhibitors. *NN*-Dimethylguanidine and *N*-methyl-*N*-phenylguanidine, on the other hand, provoke a decamethonium-like spastic paralysis and *N*-benzyl-*N*-methylguanidine causes a " dual type " of paralysis.

3. In cats, mice and rabbits all the compounds induce a flaccid paralysis which is also not antagonized by cholinesterase inhibitors. In mice some compounds (*N*-benzyl-*N*-phenyl-, *NN*-diphenyl-, *NN*-dibutyl- and *NN*-di-isobutyl-guanidine) appear to be more active than gallamine; in rabbits, *NN*-di-isobutylguanidine, the most active compound, is about five-times less active.

4. Among the guanidines only some *NN*-derivatives were found to be ganglionic blocking agents and *NN*-di-isopropylguanidine is by far the most active derivative.

5. While a short branched aliphatic structure appears to be required for the highest ganglionic blocking activity, a less specific structure seems to be required for the curare-like activity. However, in this case, some structural modifications were found to be capable of producing, in fowls, a tubocurarine- or a decamethonium-like type of activity.

REFERENCES

BARZAGHI, F., MANTEGAZZA, P. & RIVA, M. (1962). Effects of some guanidine derivatives on neuromuscular and ganglionic transmission. *Brit. J. Pharmacol.*, **19**, 414–426.

BIANCHI, M. & BARZAGHI, F. (1964). Sintesi ed attività farmacologiche di alcune guanidine NN-bisostituite. *Boll. chim. farm.*, **103**, 490–498.

BRETHERICK, L., LEE, G. E., LUNT, E., WRAGG, W. R. & EDGE, N. D. (1959). Congeners of pempidine with high ganglion-blocking activity. *Nature (Lond.)*, **184**, 1707–1709.

CONDOURIS, G. A. & GHAZAL, A. (1957). Evaluation of the interaction of the guanidinium ion with several neuromuscular blocking agents on a mammalian neuromuscular preparation. *Fed. Proc.*, **16**, 289.

FENG, T. P. (1938). Studies on the neuromuscular junction. X. The effects of guanidine. *Chin. J. Physiol.*, **13**, 119–140.

GINZEL, K. H., KLUPP, H. & WERNER, G. (1951). Die Wirkung einiger aliphatischer α-ω-Bis-quaternärer Ammonium-Verbindungen auf die Skelettmuskulatur. *Naunyn-Schmiedeberg's Arch. exp Path. Pharm.*, **213**, 453–466.

HILTMANN, R., WOLLWEBER, H., WIRTH, W. & GÖSSWALD, R. (1960). Einfache aliphatische Amine mit ganglioplegischer und blutdrucksenkender Wirksamkeit. *Angew. Chem.*, **72**, 1001.

HOPPE, J. O. (1950). A pharmacological investigation of 2,5-bis-(3-diethylaminopropylamino)benzoquinone-bis-benzylchloride (Win 2747) : a new curarimimetic drug. *J. Pharmacol. exp. Ther.*, **100** 333–345.

LITCHFIELD, J. T. & WILCOXON, F. (1949). A simplified method of evaluating dose-effect experiments. *J. Pharmacol. exp. Ther.*, **96**, 99–113.

MANTEGAZZA, P. (1956). Caratteristiche della attivita curarica in una nuova serie di sali di ammonio quaternari simmetrici. (Sali di 2-2′-polimetilen-bis-piperidinio e bis-piridinio.) *Farmaco, Ed. sci.* **11**, 357–377.

OTSUKA, M. & ENDO, M. (1960). The effect of guanidine on neuromuscular transmission. *J. Pharmacol. exp. Ther.*, **128**, 273–282.

THESLEFF, S. & UNNA, K. R. (1954). Differences in mode of neruomuscular blockade in a series of symmetric bis-quaternary ammonium salts. *J. Pharmacol. exp. Ther.*, **111**, 99–113.

ZAMBONI, P. & AZZOLINI, G. (1954). Farmaci antagonistici dei ganglioplegici. I. Nitrato di guanidina. *Ateneo parmense*, **25**, 5.