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Structure-activity Relationship of Alkylguanidines on Smooth Muscle Organs[1)]

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The actions and the structure–activity relationship of alkylguanidines on the smooth muscle organs were investigated and the following results were obtained.

On guinea–pig hypogastric nerve–vas deferens preparation and trachea preparation, the responses induced by the electrical stimulation of the nerve were potentiated with guanidine (G), methylguanidine (MG), N,N-dimethylguanidine (NN-DMG) and N,N′-dimethylguanidine (NN′-DMG), were not affected with ethylguanidine (EG) and were inhibited with propylguanidine (PG), butylguanidine (BG) and hexylguanidine (HG), in doses of 10^{-5}—5×10^{-3} M respectively. Also, the contractions of the vas deferens preparation induced by norepinephrine were potentiated with EG, PG, BG and HG, but the contractions induced by acetylcholine were not potentiated with these compounds.

The contractions of the cat nictitating membrane induced by the electrical stimulation of the cervical sympathetic nerve were not affected by all compounds.

In anaesthetized cats and rats, G, MG, NN–DMG and NN′–DMG, in dose of 5 mg/kg, produced a rise in the blood pressure. The rising actions induced by these compounds were not abolished with an adrenergic α-blockade. EG, PG, BG and HG, in dose of 5 mg/kg, produced a temporary fall followed by a rise. These compounds potentiated the pressor action of norepinephrine and suppressed that of tyramine with the increase of the carbon number. In spinal cats, a temporary fall of the blood pressure induced by these compounds were abolished and only a rise was observed. Also, the fall action in the blood pressure and the increase of the heart rate induced by isoproterenol were suppressed with HG.

HG (1%) had local anaesthetic action as potent as procaine, and its action was more lasting than that of procaine.

The pharmacological studies of guanidine derivatives have been reported by many investigators for years. In 1962, the review concerning to guanidine was published by Fastier.[3)] Particularly the effects of guanidine on the skeletal muscle preparation, neuromuscular preparation, were interested. Otsuka, *et al.*[4)] have demonstrated that guanidine increases the amplitude of the end–plate potential by increasing the quantity of acetylcholine released from the nerve ending by a nerve impulse, but that it does not change the sensitivity of the end–plate to acetylcholine.

According to the review of Fastier,[3)] it is described that guanidine has not a powerful action on smooth muscle preparations, and that guanidine in large doses raises the blood pressure of anaesthetized dogs and cats on the cardiovascular system.[5)]

Barzaghi, *et al.*[6)] have reported the effects of guanidine and N,N-disubstituted guanidines on the skeletal muscle and the ganglion, while Ozawa, *et al.*[7)] have reported the effects and the

1) This work was reported at the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968.
2) Location: *No.* 85, *Kita-4-bancho, Sendai.*
3) F.N. Fastier, *Pharmacol. Rev.* **14**, 37 (1962).
4) M. Otsuka and M. Endo, *J. Pharmacol. Exptl. Therap.*, **128**, 273 (1960).
5) R.H. Major and W. Stephenson, *Johns Hopk. Hosp. Bull.*, **35**, 186 (1924); F. Nakazawa and S. Abe, *Tohoku J. Exp. Med.*, **11**, 308 (1928).
6) F. Barzaghi, P. Mantegazza and M. Riva, *Brit. J. Pharmacol.*, **19**, 414 (1962); *ibid.*, **24**, 282 (1965).
7) *a*) H. Ozawa and M. Takeda, *Yakugaku Zasshi*, **85**, 991 (1965); *b*) H. Ozawa and K. Ikezawa, *Yakugaku Zasshi*, **87**, 461 (1967).

structure–activity relationship of monoalkylguanidines including guanidine on the skeletal muscle preparations.

In the present paper, authors investigated the effects and the structure–activity relationship of monoalkylguanidines on the smooth muscle preparations innervated by the sympathetic nerve, on the heart and on the blood pressure.

Methods and Materials

1. Guinea–pig Hypogastric Nerve–vas Deferens Preparation——After the vas deferens was dissect together with the hypogastric nerve from guinea–pig, the preparation was suspended in a 10 ml bath containing a Tyrode solution at 32° and aerated with 95% O_2+5% CO_2. The contraction of the vas deferens was recorded on a smoked paper with an isotonic writing lever. The electrical stimulation to the hypogastric nerve was applied at 3 min intervals at a frequency of 50 cps with 1 msec duration and at supramaximal voltage for 3 sec. The postganglionic stimulation of the hypogastric nerve was carried out according to the method of Birmingham.[8]

2. Guinea–pig Trachea Preparation——The experiment was carried out according to the method of Foster.[9] A cannula was tied into each end of the trachea and a long platinum wire electrode was passed up through the lower cannula and tracheal lumen until its end lay in the upper carotid artery. The preparation was fitted into a 20 ml bath containing a Tyrode solution at 37° and aerated with 95% O_2+5% CO_2. The other platinum electrode lay in the bath opposite the tracheal muscle. The electrical stimulation was applied at 10 min intervals at a frequency of 10 cps with 0.4 msec duration and at 60 V for 30 sec. Since the relaxant response was object of study, atropine (4×10^{-7} g/ml) was included in all Tyrode solution which came into contact with trachea.

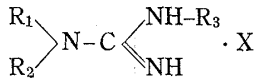
3. Cat Blood Pressure and Nictitating Membrane——The cats were anaesthetized with 1.4 g/kg urethane subcutaneously or were made spinal. Blood pressure was recorded from the right carotid artery. The contractions of the left nictitating membrane were recorded on a smoked paper with an isotonic writing lever. The injections of drugs were made into a femoral vein. The left preganglionic cervical sympathetic nerve was stimulated supramaximally by a square–wave stimulator for 10 sec at a frequency of 20 cps with 1 msec duration. The heart rate was recorded on a polygraph through a tachometer.

4. Rat Blood Pressure——The rats were anaesthetized with 1.4 g/kg urethane subcutaneously. Blood pressure was recorded from a carotid artery and the drugs were injected into a jugular vein.

5. Isolated Guinea–pig Heart Preparation——The heart of guinea–pig was isolated by Langendorff's method.[10] The movement and rate of heart were recorded on a polygraph.

6. Intradermal Test for Local Anaesthesia——A modification of the method of Bülbring, *et al.*[11] was used. Solutions of local anaesthetics made isotonic with sodium chloride were injected intradermally, six injections of 0.1 ml being made on the depilated back of each guinea–pig. The reactions of each weal to five pin–pricks were tested at 3, 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90 min after the injection.

Table I. Chemical Structures of Test Compounds



Compd.	R_1	R_2	R_3	X
Guanidine (G)	H	H	H	H_2SO_4
Methylguanidine (MG)	CH_3	H	H	$\frac{1}{2}H_2SO_4$
N,N–Dimethylguanidine (NN–DMG)	CH_3	CH_3	H	$\frac{1}{2}H_2SO_4$
N,N'-Dimethylguanidine (NN'–DMG)	CH_3	H	CH_3	HCl
Ethylguanidine (EG)	C_2H_5	H	H	$\frac{1}{2}H_2SO_4$
Propylguanidine (PG)	C_3H_7	H	H	$\frac{1}{2}H_2SO_4$
Butylguanidine (BG)	C_4H_9	H	H	$\frac{1}{2}H_2SO_4$
Hexylguanidine (HG)	C_6H_{13}	H	H	$\frac{1}{2}H_2SO_4$

8) A.T. Birmingham, *Brit. J. Pharmacol.*, **27**, 145 (1966).
9) R.W. Foster, *J. Pharm. Pharmacol.*, **16**, 125 (1964).
10) O. Langendorff, *Arch. Ges. Physiol.*, **61**, 291 (1895).
11) E. Bülbring and I. Wajda, *J. Pharmacol. Exptl. Therap.*, **85**, 78 (1945).

Materials

The chemical structures of the test compounds were shown in Table I. These compounds were synthetized by the method of Angyal, *et al.*[12]

The other drugs used in these experiments were following: norepinephrine hydrochloride, acetylcholine chloride (ACh), tyramine hydrochloride, isoproterenol hydrochloride, epinephrine hydrochloride, phentolamine methanesulfonate, procaine hydrochloride, dibucaine hydrochloride.

Results

1. Guinea-pig Hypogastric Nerve-vas Deferens Preparation

a) The Effect on the Contraction induced by the Electrical Stimulation of the Hypogastric Nerve

The effects of alkylguanidines on the contraction of the vas deferens induced by the electrical stimulation of the preganglionic fiber were shown in Table II and Fig. 1. The contraction was potentiated by guanidine (10^{-5}—5×10^{-3} M). The degree of the potentiation

Table II. Effects of Test Compounds on Hypogastric Nerve–Vas Deferens Preparation of Guinea-Pig[a]

Compd.	Concn. (M)				
	10^{-6}	10^{-5}	10^{-4}	10^{-3}	5×10^{-3}
G	(−)	↑	↑	↑↑	↑↑↑
MG	(−)	↑	↑	↑↑	↑↑
NN-DMG	(−)	(−)	↑	↑	↑
NN′-DMG	(−)	(−)	↑	↑	↑
EG	(−)	(−)	(−)	(−)	(−)
PG	(−)	(−)	↓	↓	↓
BG	(−)	(−)	↓	↓↓	↓↓
HG	(−)	↓	↓	↓↓	↓↓

potentiation: ↑↑↑ (>200%), ↑↑ (150—200%), ↑ (110—150%)
inhibition: ↓↓ (50—100%), ↓ (10—50%), (−) no effect
a) Electrical stimulation to preganglionic fiber was applied at 50 cps, 1 msec, supramaximal voltage.

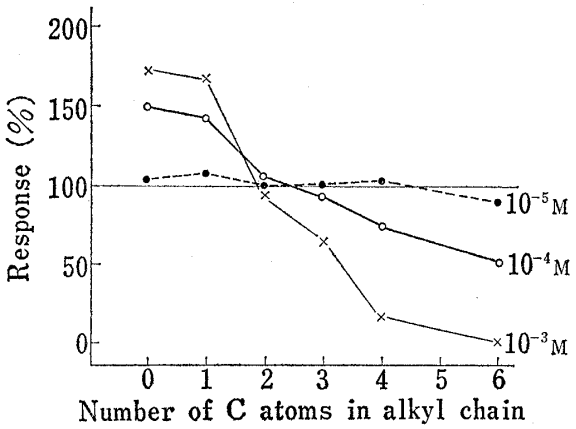


Fig. 1. Effects of Test Compounds on Hypogastric Nerve–Vas Deferens Preparation of Guinea-pig

Electrical stimulation to preganglionic fiber was applied at 50 cps, 1 msec, supramaximal voltage.

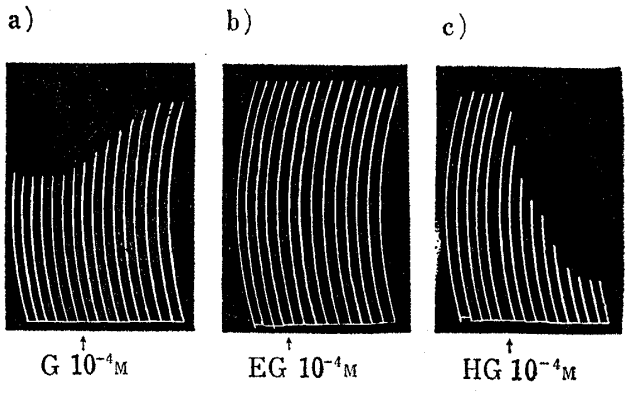


Fig. 2. Effects of G, EG and HG on Hypogastric Nerve–Vas Deferens Preparation of Guinea-pig

Electrical stimulation to preganglionic fiber was applied at 50 cps, 1 msec, supramaximal voltage.
Drugs were administered at arrow.
a) guanidine 10^{-4} M b) ethylguanidine 10^{-4} M
c) hexylguanidine 10^{-4} M

12) S.J. Angyal and W.K. Warburton, *J. Chem. Soc.*, **1951**, 2492.

was less with the increase of carbon atoms number of alkyl substituted group, and the contraction was not almost affected by EG. Moreover, when carbon number was increased the response shifted for the inhibition, and a marked inhibitory action was observed with HG (10^{-5}—5×10^{-3} M). The experimental examples were shown in Fig. 2. The effects on the contraction induced by the transmural electrical stimulation of the postganglionic fiber, also, were the same as that on the preganglionic fiber.

b) The Effect on the Contraction induced by Norepinephrine and ACh

The contractions of the vas deferens induced by norepinephrine (5×10^{-6}M) were potentiated by EG, PG, BG and HG (Fig. 3a), but those by ACh (5×10^{-6} M) were little affected (Fig. 3b).

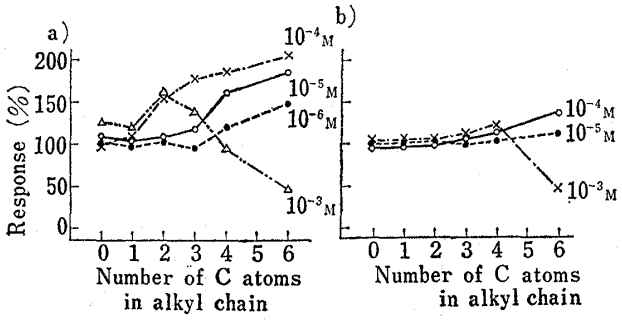


Fig. 3. Effects of Test Compounds on Contractions of Vas Deferens of Guinea-pig induced by Norepinephrine and Ach.

Contraction was induced by a) norepinephrine 5×10^{-6} M and b) Ach 5×10^{-6} M.

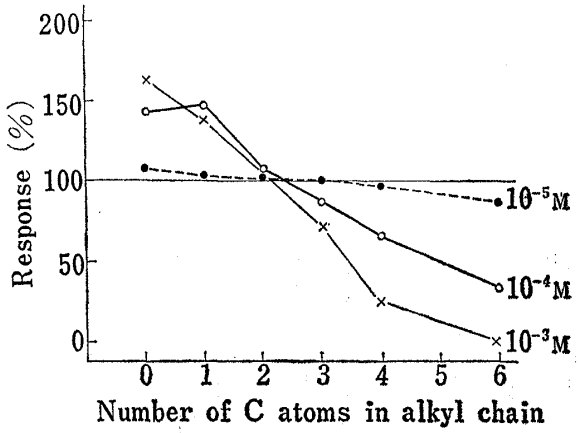


Fig. 4. Effects of Test Compounds on Trachea Preparation of Guinea-pig

Electrical stimulation was applied transmurally at 10 cps, 0.4 msec, 60 V.

Table III. Effects of Test Compounds on Trachea Preparation of Guinea-pig[a)]

Compd.	Concn. (M)			
	10^{-6}	10^{-5}	10^{-4}	10^{-3}
G	(−)	↑	↑	↑↑
MG	(−)	(−)	↑	↑
NN-DMG	(−)	(−)	↑	↑
NN'-DMG	(−)	(−)	(−)	(−)
EG	(−)	(−)	(−)	(−)
PG	(−)	(−)	↓	↓
BG	(−)	(−)	↓	↓↓
HG	↓	↓	↓↓	↓↓

potentiation: ↑↑ (>150%), ↑ (110—150%)
inhibition: ↓↓ (50—100%), ↓ (10—50%), (−) no effect
a) Electrical stimulation was applied transmurally at 10 cps, 0.4 msec, 60 V.

2. Guinea-pig Trachea Preparation

The effects of alkylguanidines on the dilation of the trachea induced by transmural electrical stimulation were shown in Table III and Fig. 4. The action on this preparation, also, was similar to that on the vas deferens, that is, the dilation was potentiated with G, not almost affected with EG and inhibited markedly with HG.

3. Rat and Cat Blood Pressure, Cat Nictitating Membrane and Heart Rate

The results were shown in Table IV. In anaesthetized cats, G, MG (Fig. 5a), NN-DMG and NN'-DMG, in dose of 5 mg/kg, produced a rise in the blood pressure. The pressor actions

Table IV. Effects of Test Compounds on Blood Pressure in Rat and Cat[a)]

Compd. (5 mg/kg) *i.v.*	Action	Norepinephrine (5 μg/kg *i.v.*)	Tyramine (1 mg/kg *i.v.*)	Isoproterenol (2 μg/kg *i.v.*)
G	slight rise	no change	no change	no change
MG	rise	no change	no change	no change
NN–DMG	rise	pot. +	no change	no change
NN′–DMG	slight rise	no change	no change	no change
EG	rise following fall	no change	no change	no change
PG	rise following fall	pot. +	inhib. +	inhib. (slight)
BG	rise following fall	pot. +	inhib. +	inhib. (slight)
HG	rise following fall	pot. ++	inhib. ++	inhib. +

pot.: potentiation inhib.: inhibition
a) Rat and cat were anaesthetized with urethane 1.4 g/kg *s.c.*

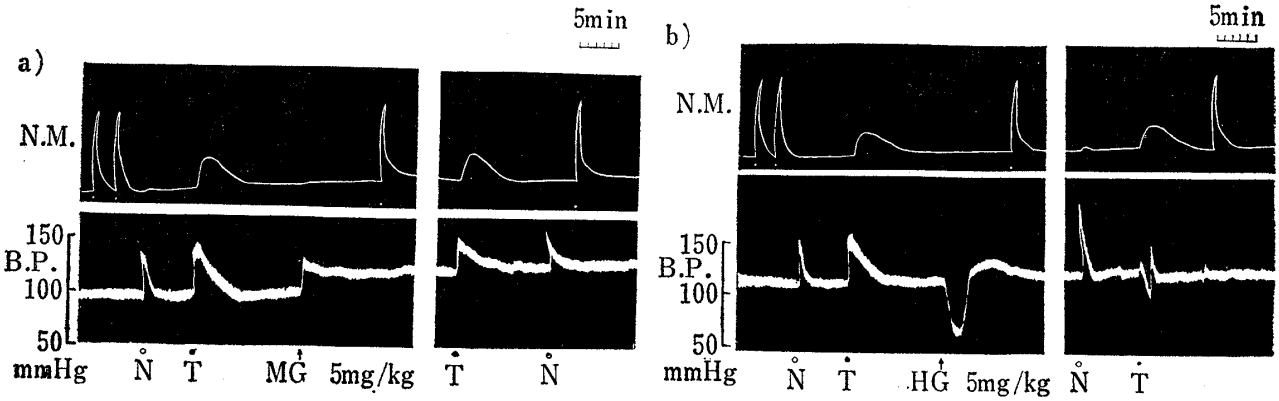


Fig. 5. Effects of MG and HG on Blood Pressure and on Contraction of Nictitating Membrane (Cat, Urethane Anaesthesia)

Stimulation was applied at dots on preganglionic fiber of cervical sympathetic nerve (frequency 20 cps, duration 1 msec, at supramaximal voltage).
N: norepinephrine 5 μg/kg *i.v.* T: tyramine 1 mg/kg *i.v.*
a) MG 5 mg/kg, b) HG 5 mg/kg were applied at arrow, respectively.

of norepinephrine (5 μg/kg) and tyramine (1 mg/kg) were not affected by these compounds except that the action of norepinephrine was potentiated with NN–DMG. Also, the pressor actions of G, MG, NN–DMG and NN′–DMG were not inhibited by adrenergic α-blockade. EG, PG, BG, HG (Fig. 5b), in dose of 5 mg/kg, produced a temporary fall followed by a rise in blood pressure. The pressor actions of norepinephrine (5 μg/kg) were markedly potentiated and those of tyramine (1 mg/kg) were inhibited with the increase of carbon number. The hypotensive action of isoproterenol (2 μg/kg) was slightly inhibited by PG, BG and HG, and the inhibition with HG was about 50 per cent. The contractions of the nictitating membrane induced by the electrical stimulation on preganglionic fiber of the cervical sympathetic nerve were not affected by all compounds.

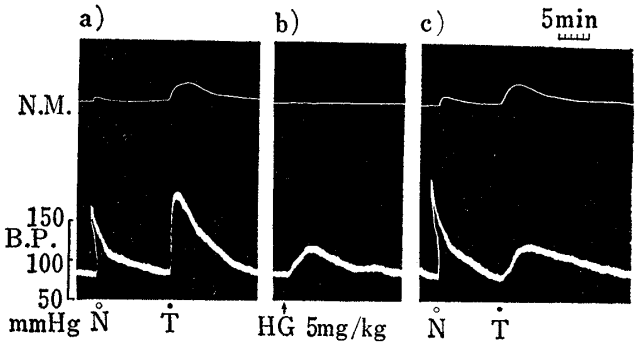


Fig. 6. Effect of HG on Blood Pressure and on Contraction of Nictitating Membrane in Spinal Cat

N: norepinephrine 3 μg/kg *i.v.* T: tyramine 0.3 mg/kg *i.v.*
HG 5 mg/kg was applied at arrow.
a) control of norepinephrine and tyramine
b) after HG 5 mg/kg *i.v.* c) after 1 hour

In spinal cats, a temporary fall of the blood pressure induced by HG was abolished and only a rise was observed (Fig. 6).

Table V. Effects of Test Compounds on Cat Heart Rate[a]

Compd. (5 mg/kg) *i.v.*	Action	Isoproterenol (2 μg/kg *i.v.*)
G	(−)	no change
MG	(−)	no change
NN–DMG	(−)	no change
NN′–DMG	(−)	no change
EG	(−)	no change
PG	(−)	no change
BG	slight decrease	inhib. (slight)
HG	decrease	inhib. +

inhib.: inhibition (−) no effect
a) Cat was anesthetized with urethane 1.4 g/kg *s.c.*

As shown in Table V, the heart rate of anaesthetized cat was unaffected by G, MG, NN–DMG, NN′–DMG, EG and PG. It was slightly decreased by BG and HG, and the increase of the heart rate induced by isoproterenol was slightly suppressed.

4. Isolated Guinea-pig Heart Preparation

On isolated guinea-pig heart preparation, each compound did not almost affect except that MG and NN–DMG increased slightly the cardiac force and HG decreased slightly.

5. Intradermal Test for Local Anaethesia

As shown in Fig. 7, the activity of HG (1%) in a local anaesthesia was the same degree with procaine (1%) and longer duration than that of procaine and dibucaine. BG, also, produced a weak local anaesthetic action.

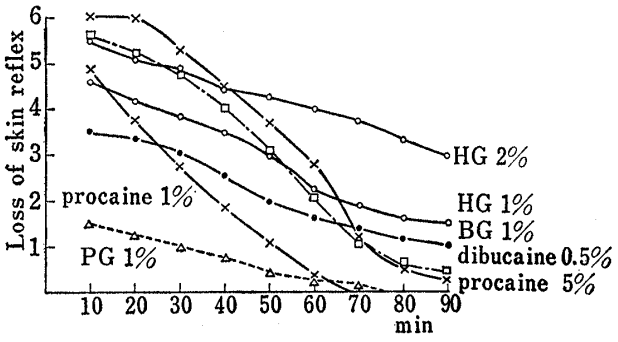


Fig. 7. Local Anaesthetic Actions of PG, BG and HG by Intradermal Weal Test in Guinea–pig

Discussion

As described in the introduction, Ozawa, *et al.*[7] have reported the actions and the structure–activity relationship of monoalkylguanidines on the skeletal muscle preparation using frog sciatic–sartorius preparation in 1965. They have concluded that monoguanidines were classified into three groups; curare antagonists, curare potentiators and muscle relaxants, and that G, MG and NN–DMG possessed the anti–curare activity and guanidino compounds with bulky substituents possessed a muscle relaxing action. Authors have investigated, in the present experiments, the actions and the structure–activity relationship of these compounds on the smooth muscle preparations innervated by the sympathetic nerve, on the heart and on the blood pressure.

In the experiments of the isolated guinea–pig hypogastric nerve–vas deferens preparation and the trachea preparation, the responses induced by the electrical stimulation of the corresponding nerve were potentiated with G, were not almost affected with EG, and were inhibited with HG possessed the bulky carbon numbers. These results of the authors shown in Fig. 1, 2 and 4, also, were similar to that of Ozawa, *et al.*[7]

Thus, since the consistent structure–activity relationship of monoalkylguanidines was obtained on the response of the skeletal and smooth muscles induced by the electrical stimulation, it is considered that the actions of these compounds are perhaps neurotropic. The act-

tion of HG which suppresses the contraction induced by the electrical stimulation was about 1/10 compared with that of guanethidine possessed an adrenergic neurone blocking action.

It has been elucidated by Muscholl[13] that the catecholamine potentiating action of cocaine is due to inhibit the uptake of the catecholamine into a tissue at an adrenergic nerve ending. Among the alkylguanidines used in the present experiment, PG, BG and HG which suppressed the contraction of the vas deferens induced by the electrical stimulation of the nerve potentiated the contraction by norepinephrine without affecting that by ACh. These compounds potentiated the pressor action of norepinephrine and suppressed that of tyramine also on the anaesthetized rats and cats. Therefore, it would be considered that these compounds might be inhibit the uptake of the amine as well as cocaine. And this action was remarkable as much bulky of the carbon number. The action of HG was about 1/3 compared with that of cocaine.

These compounds suppressed slightly the action of isoproterenol on the blood pressure and the heart, but from only these results it could not explain that they possessed β-blocking action. It would be perhaps due to suppress directly a myocardium.

On spinal cat, the temporary falls induced by EG, PG, BG and HG in blood pressure of the anaesthetized cat were abolished and only a rise was observed. Since guanidine derivatives does not pass through a blood brain barrier because of the strong base, it is considered that the temporary fall will be a reflex stimulation.

The blood pressure was rised by G, MG, NN–DMG and NN′–DMG, and the pressor actions were not abolished with an adrenergic α-blockade. Fastier, *et al.*[14] have described that the rises of blood pressure produced by the intravenous injection of guanidine (100 mg/kg) were due in large part to a direct constrictor action on blood vessels. Therefore, the rises of blood pressure produced by these compounds, also, would be perhaps due to a direct constrictor action on blood vessels.

On the local anaesthetic action, HG possessed a bulky carbon number showed the strongest action and BG showed a slight action.

Considering from these points, the actions of monoalkylguanidines were reverse between compounds with a small substituent and a bulky substituent, and the action tended to be strong with the increase of the carbon number.

13) E. Muscholl, *Pharmacol. Rev.*, **18**, 551 (1966).
14) F.N. Fastier and F.H. Smirk, *J. Pharmacol. Exptl. Therap.*, **89**, 256 (1947).