[Chem. Pharm. Bull. 18(1) 14—21 (1970)]

UDC 615.31.015.11 : 547.495.9.015

Effects of Hexylguanidine on Cardiovascular System and Blood Glucose[1]

Hikaru Ozawa and Kazunobu Sugawara

Pharmaceutical Institute, Tohoku University[2]

(Received March 24, 1969)

The actions of hexylguanidine sulfate (HG) on the cardiovascular system and blood glucose were investigated using blood pressure in spinal cat, guinea-pig heart tissue, rabbit heart and perfused ear. And the following results were obtained.

In spinal cats, HG (5 mg/kg, *i. v.*) produces a rise in blood pressure of about 60 mmHg. The pressor actions are not affected by hexamethonium, by adrenalectomy and by pretreatment with reserpine, but are suppressed by phentolamine and by cocaine. Moreover, HG potentiates the pressor action of norepinephrine and inhibits those of tyramine, guanethidine and ephedrine.

On the perfused rabbit ear, also, HG (10^{-5} g/ml) potentiates the vasoconstriction induced by epinephrine and suppresses that induced by tyramine. However, high concentration of HG (10^{-4} g/ml) produces vasodilation.

The contractions of isolated guinea-pig atria and isolated rabbit papillary muscle are slightly decreased by HG.

From the results mentioned above, it is considered that the pressor action of HG is due to a direct sympathomimetic action on the α-adrenergic receptors, but part of its action is connected with catecholamine stores in the adrenergic nerves or with some other peripheral site except the adrenal medulla.

Furthermore, HG raises the blood glucose concentration of rabbit and potentiates the hyperglycaemic action by epinephrine.

The effects and the structure-activity relationship of monoalkylguanidines on the smooth muscle mobility, on the cardiac activity and on the blood pressure have been already reported.[3] And also, authors have observed that the mechanism of the action of hexylguanidine (HG) which has the most marked action among these compounds, was similar to that of cocaine, since HG potentiated the pressor actions of norepinephrine and epinephrine and inhibited that of tyramine on the blood pressure in cats.[4] In the experiment on the isolated smooth muscle preparation, furthermore, these facts were confirmed.[5]

Besides HG, there are several drugs which have the cocaine-like action; *e.g.* 3-phenoxypropylguanidine,[6] 1-(1-phenylcyclohexyl) piperidine,[7] 3,3-bis(*p*-aminophenyl)-propylamine[8] *etc.*, and these drugs potentiate the pressor actions of norepinephrine and epinephrine and

1) This work was reported at Meeting of Tohoku Branch, Pharmaceutical Society of Japan, Sendai, November 1968.
2) Location: *Aobayama, Sendai.*
3) H. Ozawa and K. Sugawara, *Chem. Pharm. Bull.*, **16**, 2376 (1968).
4) H. Ozawa and K. Sugawara, *Jap. J. Pharmacol.*, **19**, 343 (1969).
5) H. Ozawa and K. Sugawara, *Chem. Pharm. Bull.*, **18**, 8 (1970)
6) *a*) A.L. Bartlet, *Brit. J. Pharmacol.*, **18**, 475(1962); *b*) G. Chen, C.R. Ensor, D.A. McCarthy, J. R. McLean and A. Campbell, *J. Pharmacol. Exptl. Therap.*, **143**, 374 (1964).
7) *a*) K. F. Ilett, B. Jarrott, S.R. O'Donnell and J.C. Wanstall, *Brit. J. Pharmacol.*, **28**, 73 (1966); *b*) G. Chen, C.R. Ensor and B. Bohner, *J. Pharmacol. Exptl. Therap.*, **149**, 71 (1965); *c*) G. Chen, C.R. Ensor, D. Russell and B. Bohner, *ibid.*, **127**, 241 (1959); *d*) E.F. Domino, *Int. Rev. Neurobiol.*, **6**, 303 (1964).
8) *a*) G.P. Leszkovszky, L. Tardos and K. Takács, *Acta Physiol. Hung.*, **30**, 283 (1966); *b*) G.P. Leszkovszky, L. Tardos, J. Lendvai and K. Takács, *Experientia*, **23**, 336 (1967); *c*) *Idem.*, *Arzneimittel Forsch.*, **17**, 1590 (1967).

inhibit that of tyramine. Furthermore, these drugs have a sympathomimetic action. Ozawa, *et al.*[4] recognized, also, that HG showed an interesting pressor action in spinal cats.

In the present paper, authors investigated in more detail the mechanism of the hypertensive action of HG and the interaction of HG with other drugs on the cardiovascular system. In addition to, authors investigated the effect of HG on blood glucose.

Experimental

Methods

1. Blood Pressure and Nictitating Membrane of Spinal Cats——The cats of either sex, weighing 2—4 kg, were made spinal under ether anaesthesia by the method of Kumagai, *et al.*[9] Blood pressure was recorded from the left carotid artery with a mercury manometer. Drugs were injected into the left femoral vein. Contraction of the right nictitating membrane was recorded on a smoked paper with an isotonic writing level.

For acute bilateral adrenalectomy, the adrenolumbar vein and artery were tied off and the gland carefully freed.

Reserpinized cat was received 2 mg/kg of reserpine subcutaneously on each two successive days before use.

2. Isolated Heart Preparations——Atria from freshly killed guinea-pigs (400—600 g) were suspended in a 50 ml bath containing Tyrode solution at 30° and aerated with 95% O_2+5% CO_2. Contractions of atria were recorded on polygraph through a transducer.

Papillary muscles, from the right ventricles of rabbits stunned, were suspended in a 50 ml bath containing Tyrode solution at 37° and aerated with 95% O_2+5% CO_2. A tension of 1 to 2 g was applied to the muscle and they were stimulated at a frequency of 0.3 cps with 1 msec duration and at 10—20 V. Contractions of papillary muscle were recorded on polygraph using as isometric transducer.

3. Isolated Perfused Rabbit Ear——Rabbits with large ears were anaesthetized with ether and the ear was cut off. A polyethylene cannula was inserted into the central artery of the ear, and the auricular nerve freed along about 2 cm of its length. The ear was fixed on a plate and perfused with Tyrode solution at 38° from a Marriotte bottle. The venous outflow was recorded on a kymograph by a phototransistor drop counter. The drugs were usually injected into the arterial cannula in a volume of 0.1 ml. The stimulation to the auricular nerve was applied at a frequency 20 cps with 1 msec duration and at supramaximal voltage for 20 sec.

4. Blood Glucose after Epinephrine——The blood glucose concentration in rabbits was estimated by the method of Fujita, *et al.*[10] on 0.1 ml samples of blood withdrawn from the marginal ear vein. Six rabbits were used in a cross-over experiment, the two halves of the experiment being separated by an interval of two weeks. Before each half of the experiment the rabbits were kept without food for 24 hr. All injections were made subcutaneously on the flank. In the first half of the experiment three rabbits were injected with HG (20 mg/kg), and three were given a control injection, 18 hr before withdrawing blood. In the second half of the experiments the previously treated rabbits served as control and the previous control were injected with HG. Estimations of blood glucose were made on two samples of blood before injecting 50 μg/kg epinephrine and on samples taken 1, 2, 3, 4 and 5 hr after the injection.

Materials

The drugs used in these experiments were following: hexylguanidine sulfate (HG), *dl*-norepinephrine hydrochloride, *l*-epinephrine hydrochloride, tyramine hydrochloride, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), hexamethonium bromide, phentolamine methanesulfonate, cocaine hydrochloride, 2-(octahydro-1-azocinyl)-ethylguanidine sulfate (guanethidine), *l*-ephedrine hydrochloride.

Results

1. Blood Pressure and Nictitating Membrane of Spinal Cats

a) **Effect of HG on Blood Pressure and Nictitating Membrane**——In spinal cats an intravenous injection of HG (5 mg/kg) produced a rise in blood pressure of about 60 mmHg together with the contraction of the nictitating membrane (Fig. 1). A second dose administered immediately after the effects of the first dose had worn off showed tachyphylaxis. When

9) H. Kumagai, T. Yui, K. Ogawa and H. Ohga, *Seitai no Kagaku*, **5**, 132 (1953).
10) A. Fujita and D. Iwatake, *Biochem. Zeitsch.*, **242**, 43 (1931).

the interval of the administration was more than 30 min tachyphylaxis did not occur. In all experiments, therefore, HG was administered at the interval of more than 30 min.

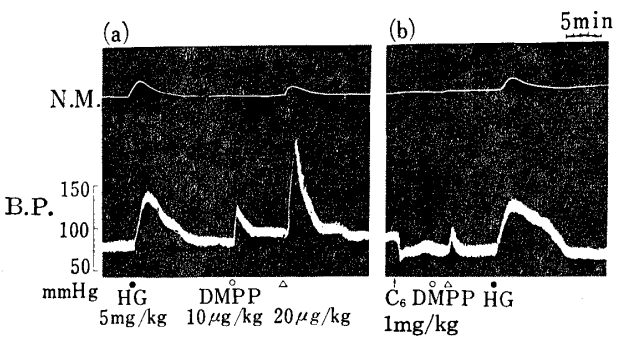

Fig. 1. Blood Pressure and Nictitating Membrane in Spinal Cat

HG (5 mg/kg, *i.v.*) and DMPP (10 and 20 μg/kg) were administered at dots.
(a) control
(b) after administration of hexamethonium (1 mg/kg, *i.v.*) at C_6

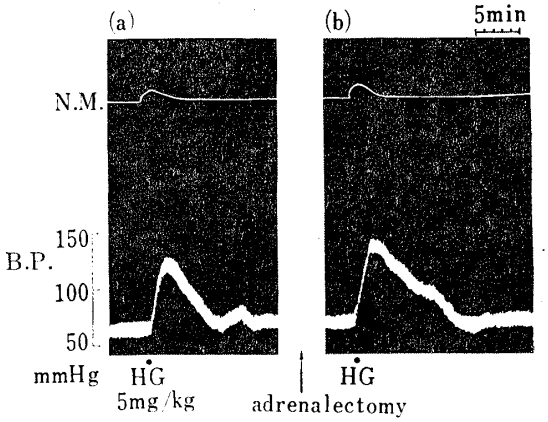

Fig. 2. Blood Pressure and Nictitating Membrane in Spinal Cat

HG (5 mg/kg, *i.v.*) were administered at dots. Adrenalectomy was made between (a) and (b).

b) Effect of Ganglion Blocking Agent——The rises in blood pressure induced by DMPP (10 and 20 μg/kg) were abolished by hexamethonium (2 mg/kg), but those by HG (5 mg/kg) were not abolished (Fig. 1).

c) Effect of Adrenalectomy——The rise in blood pressure induced by HG (5 mg/kg) was not affected by acute, bilateral adrenalectomy (Fig. 2). The rises in blood pressure by HG before and after adrenalectomy were 59.3±1.4 mmHg and 56.8±1.6 mmHg, and the difference between two results was not statistically significant.

d) Effect of Reserpine——In spinal cats pretreated with reserpine, HG (5 mg/kg) produced a rise in blood pressure of 64.2±2.1 mmHg. And this response was not significantly different from that in untreated cats. In all experiments, tyramine (1 mg/kg) was administered to confirm that norepinephrine stores were depleted.

e) Effect of *α*-Adrenergic Blocking Agent——The rise in blood pressure induced by HG (5 mg/kg) was blocked by phentolamine (5 mg/kg) (Fig. 3).

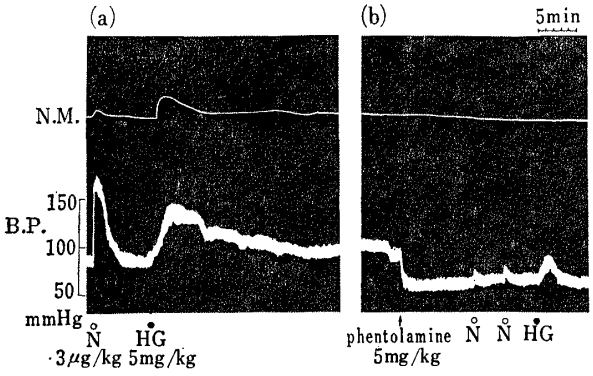

Fig. 3. Blood Pressure and Nictitating Membrane in Spinal Cat

N: norepinephrine 3 μg/kg *i.v.*
(a) HG 5 mg/kg *i.v.*
(b) Phentolamine (5 mg/kg, *i.v.*) was applied at arrow.

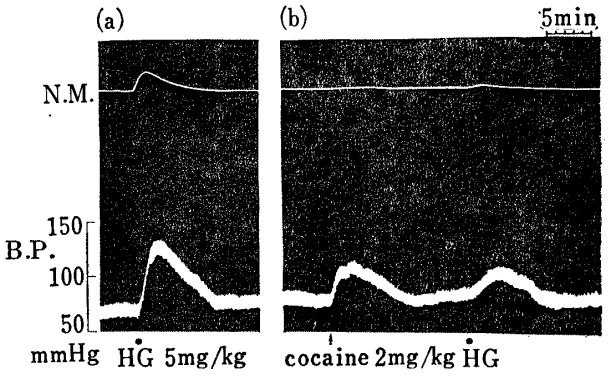

Fig. 4. Blood Pressure and Nictitating Membrane in Spinal Cat

(a) HG 5 mg/kg *i.v.*
(b) Cocaine (2 mg/kg, *i.v.*) was applied at arrow.

f) Effect of Cocaine——The rise in blood pressure induced by HG (5 mg/kg) was blocked by cocaine (2 mg/kg) (Fig. 4).

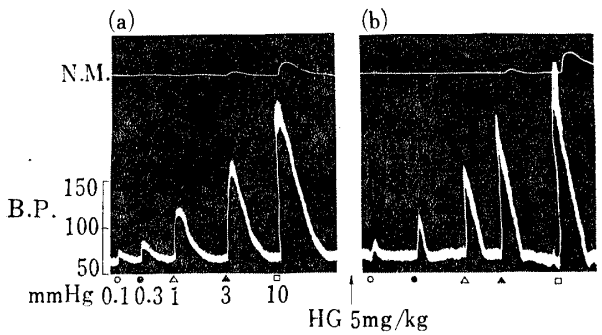

Fig. 5. Blood Pressure and Nictitating Membrane in Spinal Cat

Norepinephrine 0.1, 0.3, 1, 3 and 10 μg/kg were administered intravenously at dots, respectively. HG (5 mg/kg, *i.v.*) was administered between (a) and (b).

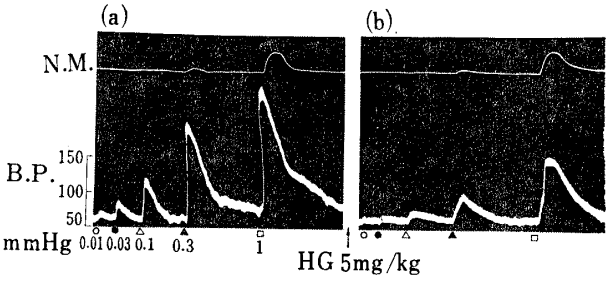

Fig. 6. Blood Pressure and Nictitating Membrane in Spinal Cat

Tyramine 0.01, 0.03, 0.1, 0.3 and 1 mg/kg were administered intravenously at dots, respectively. HG (5 mg/kg, *i.v.*) was administered between (a) and (b).

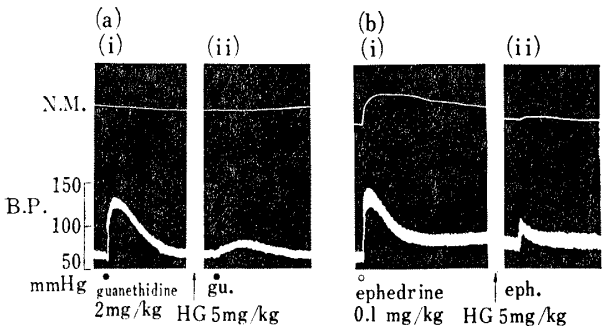

Fig. 7. Blood Pressure and Nictitating Membrane in Spinal Cat

HG (5 mg/kg, *i.v.*) was administered between (i) and (ii).
(a) Effect of HG on the pressor action of guanethidine (2 mg/kg, *i.v.*).
(b) Effect of HG on the pressor action of ephedrine (0.1 mg/kg, *i.v.*).

g) Effect of HG on Pressor Actions of Norepinephrine and Tyramine——As shown in Fig. 5, the pressor actions induced by norepinephrine were potentiated by HG (5 mg/kg). And the potentiation were still remained after 1 hr from administration. Also, the pressor actions induced by tyramine were inhibited by HG (5 mg/kg) (Fig. 6). But the stimulant action of tyramine on the nictitating membrane was never abolished by HG.

h) Effect of HG on Pressor Actions of Guanethidine and Ephedrine——As shown in Fig. 7, the pressor actions induced by guanethidine (2 mg/kg) and ephedrine (0.1 mg/kg) were suppressed by HG (5 mg/kg).

2. Isolated Heart Preparations

a) Effect of HG on Atria of Guinea-pig——As shown in Fig. 8a, the contraction of isolated guinea-pig atria was slightly suppressed by 2×10^{-5} g/ml of HG.

b) Effect of HG on Papillary Muscle of Rabbit——The contraction of isolated rabbit papillary muscle induced by the electrical stimulation, also, was only slightly suppressed by 2×10^{-5} g/ml of HG as well as the contraction of guinea-pig atria (Fig. 8b).

3. Effect of HG on Isolated Perfused Rabbit Ear

The vasoconstrictions induced by the electrical stimulation, by epinephrine and by tyramine were suppressed at a concentration of HG 10^{-5} g/ml in the perfusion fluid. When the ear was perfused with normal Tyrode solution, the vasoconstrictor action of epinephrine was potentiated compaired with the response to the initial injections of this substance, but that of tyramine was remained antagonized (Fig. 9a). Fig. 9b showed the vasodilation of HG 10^{-4} g/ml.

4. Effect of HG on Blood Glucose Concentration and on Hyperglycaemic Action of Epinephrine

HG (20 mg/kg, *s.c.*) raised the blood glucose concentration of rabbit by about 27% (Table I).

Also, the hyperglycaemic action of epinephrine (50 μg/kg, *s.c.*) was potentiated by HG (Table II).

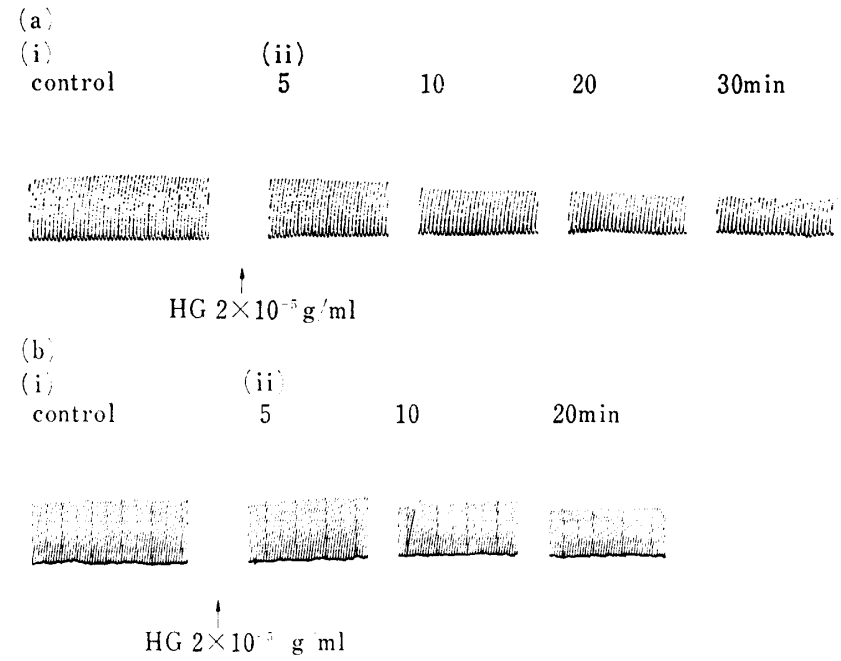

Fig. 8. Effect of HG on Isolated Heart Tissues

HG (2×10^{-5} g/ml) was applied at arrow.
(a) Isolated guinea-pig atria.
(i) control
(ii) after 5, 10, 20 and 30 min from the administration of HG, respectively.
(b) Isolated rabbit papillary muscle.
Electrical stimulation was applied at 0.3 cps, 1 msec and 10—20 V.
(i) control
(ii) after 5, 10 and 20 min from the administration of HG, respectively.

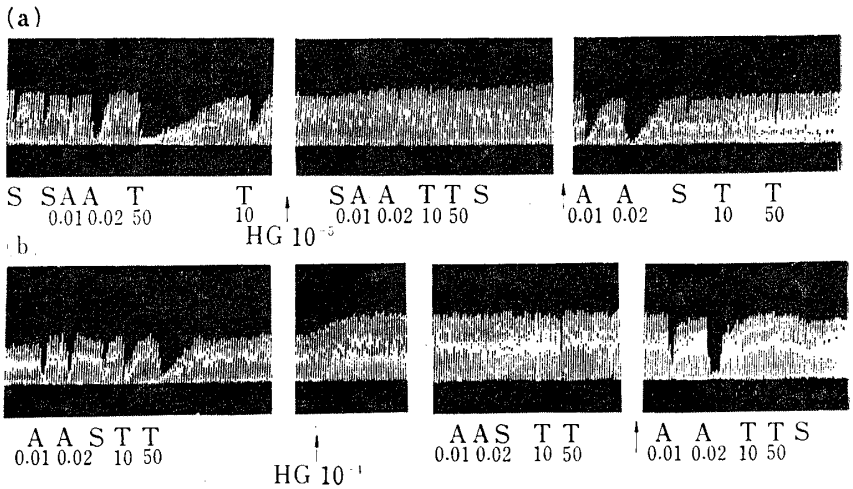

Fig. 9. Effect of HG on Perfused Rabbit Ear

Electrical stimulation was applied for 20 sec at S on the auricular nerve (20 cps, 1 msec, supramaximal voltage). Drugs were injected into the arterial cannula. HG 10^{-5} g/ml (a) and 10^{-4} g/ml (b) were present in the perfusion fluid between the arrows.
A: epinephrine 0.01and 0.02 μg T: tyramine 10 and 50 μg

Table I. Effect of HG on Blood Glucose Concentration

Rabbit	Blood glucose (mg/100 ml) 18 hr after *s.c.* injection of		Increase in blood glucose after HG (mg/100 ml)	Mean increase	*P* value of mean increase
	0.9% NaCl solution	HG (20 mg/kg)			
1	112.4	180.6	68.2	27.8	$p<0.05$
2	99.2	148.8	49.6		
3	116.6	120.1	3.5		
4	93.1	113.1	20.0		
5	100.1	100.9	0.8		
6	107.9	132.2	24.3		

Table II. Effect of HG on Hyperglycaemic Response to 50 μg/kg Epinephrine

Time (hr after epinephrine)	Mean rise in blood glucose (mg/100 ml ±S.E.) after epinephrine		Mean increase in epinephrine hyperglycaemia after HG	p value of mean increase
	Control	18 hr after 20 mg/kg HG		
1	182.4±3.6 (6)	223.9±4.0 (6)	41.5±4.6 (6)	$p<0.1$
2	199.2±3.0 (6)	269.3±6.0 (6)	70.1±6.2 (6)	$p<0.1$
3	184.2±3.6 (5)	273.5±6.3 (5)	89.3±6.4 (5)	$p<0.1$
4	159.8±4.3 (5)	271.8±5.5 (5)	112.0±5.1 (5)	$p<0.05$
5	117.5±5.7 (5)	187.4±6.1 (5)	69.9±6.1 (5)	$p<0.1$

Discussion

It has been already reported that the intravenous injection of HG produced a temporary fall followed by a rise in the blood pressure of anaesthetized cats and that the temporary fall was abolished and only a rise was observed in spinal cats.[4] Therefore, it was considered that the temporary fall of the blood pressure induced by HG would be perhaps due to the effect to central nervous system. Furthermore, the actions of HG was similar to those of cocaine since HG potentiated the pressor actions of norepinephrine and epinephrine and inhibited that of tyramine on the blood pressure in cats.[4] Also, HG has an interesting pressor action.

In the present experiments, the pressor response to HG was not affected by ganglion blockade, hexamethonium. And the pressor response was not abolished by adrenalectomy. Thus HG does not stimulate sympathetic ganglia nor discharge catecholamines from the adrenal medulla.

The pressor response was markedly reduced by α-adrenergic blocking agent, phentolamine, but it was unaffected by the pretreatment of reserpine. Therefore, it is considered that HG perhaps acts directly on the α-adrenergic receptors.

However, the pressor response to HG was abolished by cocaine. Judging from that cocaine suppresses the pressor actions of the sympathomimetic amines which had the indirect action such as tyramine and had the mixed action such as ephedrine,[11] it is considered that HG has an indirect action. And this fact contradicts with the statement of the pretreatment

11) U. Trendelenburg, A. Muskus, W.W. Fleming and G.A. de la Sierra, *J. Pharmacol. Exptl. Therap.*, **138**, 181 (1962).

of reserpine. Tainter[12] reported that the pressor action of ephedrine was inhibited by cocaine, whereas Moore, *et al.*[13] reported that the pressor action of ephedrine was not inhibited by reserpine pretreatment. Generally, it has been observed that the supersensitivity of the vascular smooth muscle to directly acting amines could be produced by the pretreatment of reserpine and that the subsensitivity of that to indirectly acting amines could be produced by reserpine because the depletion of norepinephrine.[13] It would be considered, therefore, that since a direct action and an indirect action of ephedrine were offset the pressor action of ephedrine was unaffected by reserpine pretreatment. If it is postulated, the same idea might be applied to the action of HG. Thus, the action of HG seems be parallel to that of ephedrine.

If HG were successively administered to cats in less than 30 min tachyphylaxis was observed. And since even that time the response to norepinephrine was not reduced it would be considered that the tachyphylaxis induced by HG might be due to depletion of catecholamine in store acting indirectly.

Moreover, as a suggestion which HG acts indirectly to nerve terminal, it is necessary to consider the potentiating action of the exogenous catecholamines by HG, that is cocaine-like action.[4] As described in the introduction, there are several drugs which have the cocaine-like action, and these drugs potentiate the actions of direct-type catecholamines such as norepinephrine and epinephrine since they inhibit the uptake of catecholamine into the adrenergic nerve terminals and lead to a greater concentration of catecholamine in the receptor.[6–8] In the present experiment, also, HG potentiated the pressor action of norepinephrine in cats and the vasoconstrictor action of epinephrine of rabbit ears. Thus it is considered that the potentiating actions of catecholamine induced by HG are due to inhibiting the uptake of catecholamine into the store at adrenergic nerve terminals. Although no direct evidence for this view is available, there is appeared indirect evidence from the observation that the pressor action and the vasoconstrictor action of tyramine are inhibited by HG. As it was made by Iversen,[14] the application of labelled catecholamine seems to be rather direct evidence. Furthermore, HG suppressed the pressor actions induced by a low concentration of guanethidine[15] which produces the release of catecholamine and that induced by ephedrine which has mixed action. Considering from that cocaine and cocaine-like drugs suppress the action of tyramine by inhibiting its uptake into the adrenergic store and resulting in the inability of tyramine to release endogenous norepinephrine,[16] it seems to be quite all right that the actions of tyramine are suppressed by HG.

The contraction of isolated guinea-pig atria and that of isolated rabbit papillary muscle were slightly decreased by HG. On isolated perfused rabbit ear, also, the vasodilation was produced by a high concentration of HG. Thus it is considered that the effects of HG on the heart tissue and the ear vessel are a direct action to the muscle. Therefore, the rise of blood pressure by HG cannot be attributed to the heart tissue and the ear vessel.

HG raised the blood glucose concentration of rabbit and potentiated the hyperglycaemic action by epinephrine. Judging from that the mechanisms of the decrease of blood glucose concentration by guanidine derivatives such as phenetylbiguanide are due to whether the increase of glucose uptake in the muscle or the decrease of glyconeogenesis by the inhibition of oxidative enzyme such as cytochrome oxidase or succinate dehydrogenase,[17] it is considered that the mechanism of hyperglycaemia by HG is due to whether the decrease of glucose uptake

12) M.L. Tainter, *J. Pharmacol. Exptl. Therap.*, **36**, 569 (1929).
13) J.E. Moore and N.C. Moran, *J. Pharmacol. Exptl. Therap.*, **136**, 89 (1962).
14) L.L. Iversen, "The Uptake and Storage of Noradrenaline in Sympathetic Nerves," at the University Press, Cambridge, 1967.
15) J.W. McCubbin, Y. Kaneko and I.H. Page, *J. Pharmacol. Exptl. Therap.*, **131**, 346 (1961).
16) R.F. Furchgott, S.M. Kirkepar, M. Rieker and A. Schwab, *J. Pharmacol. Exptl. Therap.*, **142**, 39 (1963).
17) R.H. Williams, *Metabolism*, **6**, 311 (1957).

in the muscle or the increase of glyconeogenesis. However, as regards the matter it is not obvious and it is necessary to investigate for the future studies. Bartley[6a] described that the mechanisms of the rising of the blood glucose concentration and that of the potentiation of the hyperglycaemic action of epinephrine by 3-phenoxypropylguanidine would be perhaps due to the potentiation of the actions of circulating catecholamines. Considering the matter in this way, also the potentiation of the hyperglycaemic action of epinephrine by HG would be explained by the potentiating action of catecholamine as well as the effect of HG on the pressor action of catecholamine.

It was concluded that the pressor action of HG appeared mainly to be due to a direct sympathomimetic action on the α-adrenergic receptors, but part of its action was connected with catecholamine stores in the adrenergic nerve terminals or with some other peripheral site except the adrenal medulla.