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Cocaine-like Action of Hexylguanidine on Smooth Muscle Preparations[1]

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The actions of hexylguanidine on the smooth muscle preparations were compared with those of cocaine using guinea-pig hypogastric nerve-vas deferens, trachea, rat stomach, intestine and perfused rabbit ear preparations and the following results were obtained.

In each preparation, the responses induced by the electrical stimulation of the nerve were suppressed with hexylguanidine (10^{-3}—10^{-5}M). On the other hand, the responses were suppressed with a high dose of cocaine ($>10^{-5}$M), but were potentiated with a low dose (10^{-6}M).

In the smooth muscle preparations, giunea-pig vas deferens and perfused rabbit ear preparations which produced the contraction by catecholamines such as norepinephrine and epinephrine, the actions of catecholamines were potentiated by hexylgunidine and cocaine. On the other hand, on the smooth muscle preparations, guinea-pig trachea and rat stomach preparations, which produced the dilation by catecholamines, the actions of catecholamines were unaffected by hexylguanidine and cocaine. On the guinea-pig intestine, the contractions induced by tryptamine and 5-hydroxytryptamine were suppressed by hexylguanidine and cocaine, but those induced by acetylcholine were not affected.

From the results mentioned above, it is considered that the action of hexylguanidine may be neurotropic and the action is almost similar to the action of cocaine.

Authors have reported the effects and the structure-activity relationship of monoalkylguanidines on the smooth muscle preparations innervated by the sympathetic nerve, on the circulating system.[3] In the paper, we concluded that the actions of monoalkylguanidino compounds with a small substituent were different from those with a bulky substituent, and that the activity increased according to the carbon numbers.

Authors have also reported the effects of hexylguanidine, which has the most bulky carbon chain among these compounds, on the sympathetic nervous system using the cat *in situ* and demonstrated the catecholamine potentiating actions which were similar to those of cocaine.[4]

In the present paper, authors investigated in more detail the action of hexylguanidine on the smooth muscle preparations using the isolated organs to confirm the cocaine-like actions.

Methods and Materials

1. Guinea-pig Hypogastric Nerve-Vas Deferens Preparation——After the vas deferens was dissected out together with the hypogastric nerve from the guinea-pig, the preparation was suspended in a 10 ml bath containing 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 every 3 min at rectangular pulses (50 cps, 1 msec, supramaximal voltage) for 3 sec. The contractions of vas deferens were also induced by administrating norepinephrine, acetylcholine or histamine into the bath.

1) This work was reported at the 10th Annual Meeting of Japan Society of Smooth Muscle Research, Sendai, August 1968.
2) Location: *Aobayama, Sendai.*
3) H. Ozawa and K. Sugawara, *Chem. Pharm. Bull.* (Tokyo), **16**, 2376 (1968).
4) H. Ozawa and K. Sugawara, *Japan. J. Pharmacol.*, **19**, 343 (1969)

2. **Guinea-pig Trachea Preparation**——The experiment was carried out according to the method of Foster.[5] 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 cannula. The preparation was fitted into a 20 ml bath containing Tyrode solution at 37° and aerated with 95% O_2+5% CO_2. The other platinum electrode lay in the bath opposite to the tracheal muscle. The electrical stimulation was applied every 10 min at rectangular pulses (10 cps, 0.4 msec, 60 V) for 30 sec. Since the relaxant response was object of study, atropine (4×10^{-7} g/ml) was included in Tyrode solution which come into contact with trachea. The relaxation of trachea was also induced by the administration of epinephrine in the bath.

3. **Rat Stomach Preparation**——Rats of either sex were stunned and bled. The stomach and oesophagus of rat was dissected together with the vagi. The vagi were then separated from the oesophagus which was ligated near the stomach and cut away. Other tissues surrounding the vessels were removed. The stomach was cannulated through the pylurus, and the contents were washed out with Tyrode solution. The preparation was set up in a 50 ml bath filled with a Tyrode solution and aerated with 95% O_2+5% CO_2 at 37°. Intraluminal pressure was 20—30 mmH_2O and was recorded on polygraph. The nerve was stimulated at rectangular pulses (20 cps, 1 msec, 10—20 V) for 2 min. The solution always contained atropine (10^{-7} g/ml) to prevent contraction due to stimulation of cholinergic nerves.

4. **Rat Intestine Preparation**——Rat intestine was dissected and suspended in a 10 ml bath containing Tyrode solution and aerated with 95% O_2+5% CO_2 at 32°. The contraction was recorded on a smoked paper with an isotonic writing lever.

5. **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 rectangular pulses (20 cps, 1 msec, supramaximal voltage) for 20 sec.

Materials——The drugs used in these experiments were following: hexylguanidine sulfate (HG), cocaine hydrochloride, *dl*- norepinephrine hydrochloride, *l*-epinephrine hydrochloride, acetylcholine chloride (ACh), histamine hydrochloride, tyramine hydrochloride, atropine sulfate, tryptamine hydrochloride, 5-hydroxytryptamine creatinine phosphate (5-HT).

Results

1. Guinea-pig Hypogastric Nerve-Vas Deferens Preparation

The contractions of the vas deferens induced by the electrical stimulation of the preganglionic fiber of the hypogastric nerve were suppressed with HG (10^{-5}—5×10^{-3}M) and a high dose of cocaine (>10^{-5}M), but were potentiated with a low dose of cocaine (10^{-6}M) (Fig. 1). Also, the contractions of the vas deferens induced by norepinephrine (5×10^{-6}M) were potentiated with HG and cocaine (Fig. 2), but those by ACh (5×10^{-6}M) and histamine (10^{-4}M) were little affected.

2. Guinea-pig Trachea Preparation

The dilations of the trachea induced by the transmural electrical stimulation were suppressed with HG (10^{-6}—10^{-4}M) and cocaine (10^{-5}—10^{-4}M), but were potentiated with the dose of cocaine 10^{-6}M (Fig. 3). Also, the dilations of the trachea induced by epinephrine (10^{-6}M) were unaffected with HG and cocaine.

3. Rat Stomach Preparation

The dilation of the stomach induced by the electrical stimulation of the vagus were suppressed with HG (10^{-4}—10^{-5}M) and cocaine (10^{-5}M), but were potentiated with the dose of cocaine 10^{-6}M (Fig. 4). Also, the dilations of the stomach induced by epinephrine (5×10^{-6}M) and the contractions by ACh (10^{-6}M) were little affected with HG and cocaine (Fig. 5).

4. Rat intestine Preparation

The contractions of the intestine induced by tryptamine (10^{-5}M) and 5-HT (10^{-7}M) were suppressed, but those induced by ACh (5×10^{-7}M) were unaffected with HG (10^{-5}M) and cocaine (10^{-5}M) (Fig. 6).

5) R. W. Foster, *J. Pharm. Pharmacol.*, **16**, 125 (1964).

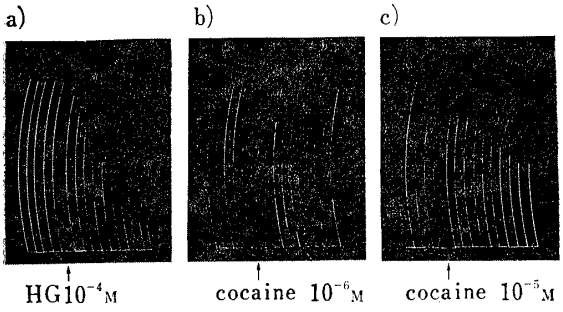

Fig. 1. Effects of HG and Cocaine on Hypogastric Nerve-Vas Deferens Preparation of Guinea-pig

Electrical stimulation to preganglionic fiber was applied at 50 cps, 1 msec, supramaximal voltage.
Drugs were administered at arrow.
a) HG 10^{-4}M b) cocaine 10^{-6}M c) cocaine 10^{-5}M

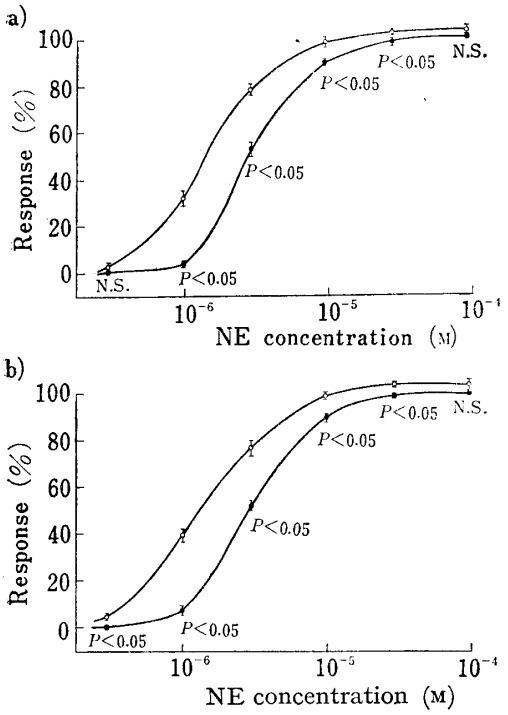

Fig. 2. Effects of HG and Cocaine on Contractions of Norepinephrine in Vas Deferens Preparation of Guinea-pig

Mean values of 6 experiments. Vertical bars represent standard errors calculated on nonpaired basis.
Probabilities were obtained by t test for paired data.
N.S.=not significant ($P>0.05$)
a) effect of HG10^{-4}M ○:before ●:after HG^{-4}M
b) effect of cocaine 10^{-6}M ●:before ○:after Cocaine 10^{-6}M

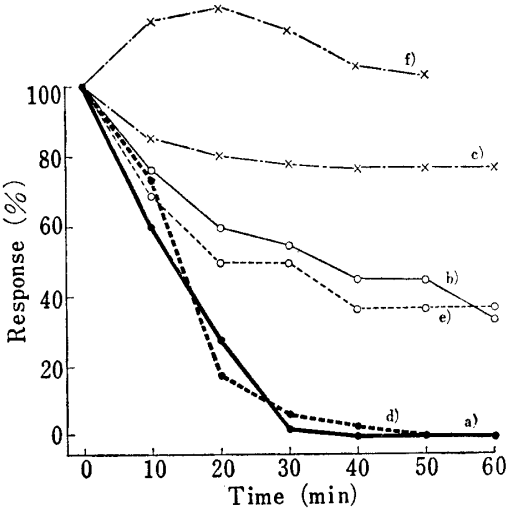

Fig. 3. Effects of HG and Cocaine on Trachea Preparation of Guinea-pig

Electrical stimulation was applied transmurally at 10 cps, 0.4 msec, 60 V. Mean values of 5 experiments.
a)—c) HG 10^{-4}M, 10^{-5}M and 10^{-6}M, respectively
d)—f) cocaine 10^{-4}M, 10^{-5}M and 10^{-6}M, respectively

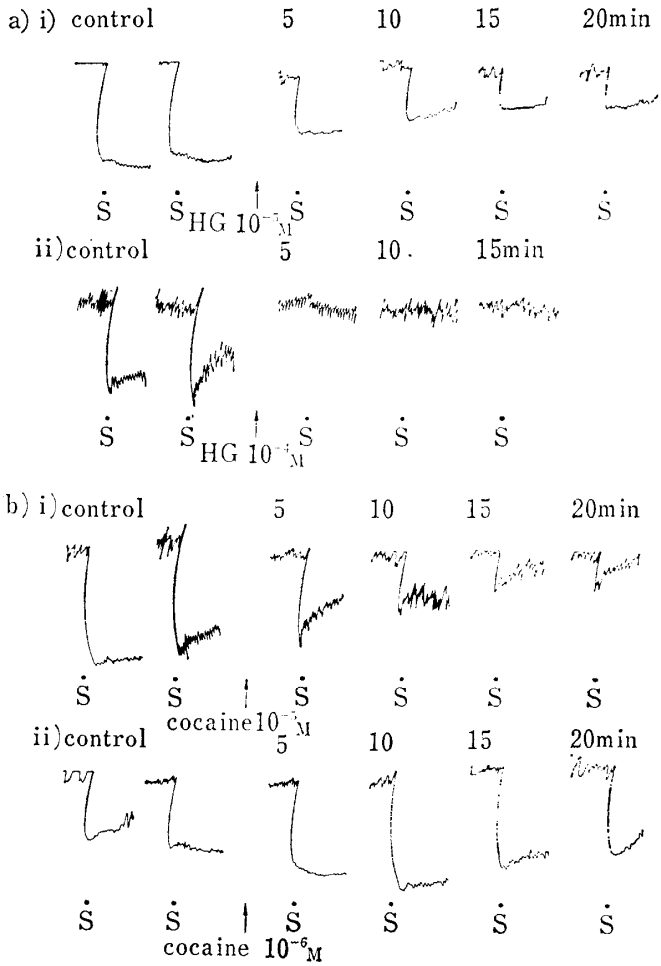

Fig. 4. Effects of HG and Cocaine on Rat Stomach Preparation

Electrical stimulation was applied at S on vagal nerve (20 cps, 1 msec, 10—20 V, for 2 min). Drugs were administered at arrow.
a) effect of HG (i) 10^{-5}M, ii) 10^{-4}M)
b) effect of cocaine (i) 10^{-5}M, ii) 10^{-6}M)

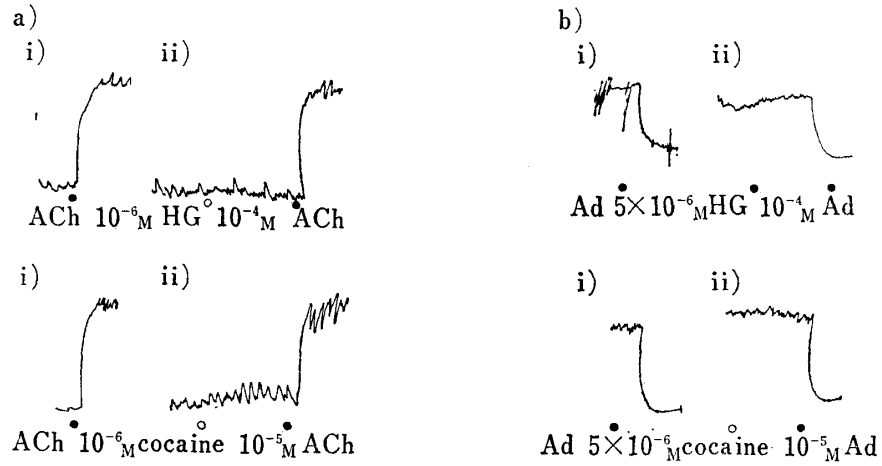

Fig. 5. Effects of HG and Cocaine on Rat Stomach Preparation

Drugs were administered at dots.
a) effects of HG and cocaine on contraction by ACh 10^{-6}M
i) control
ii) after daministration of HG (10^{-4}M) and cocaine (10^{-5}M)
b) effects of HG and cocaine on dilation by epinephrine 5×10^{-6}M
i) control
ii) after administration of HG (10^{-4}M) and cocaine (10^{-5}M)

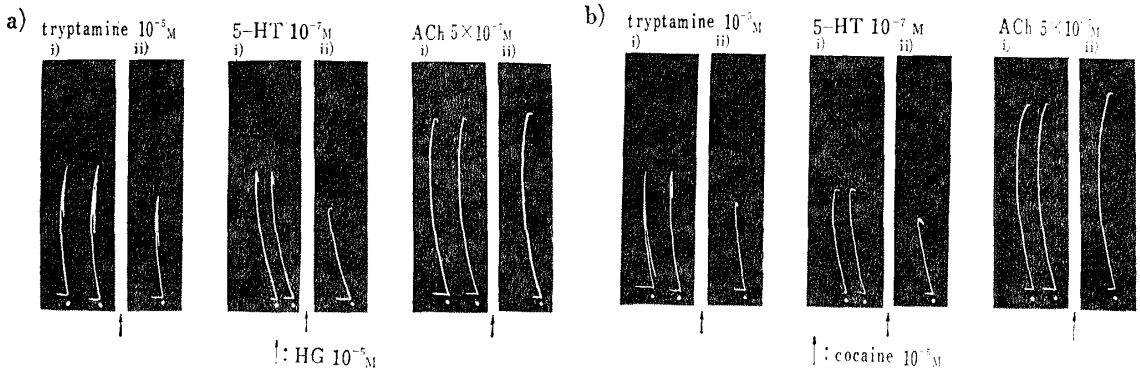

Fig. 6. Effects of HG (a) and Cocaine (b) on Rat Intestine Preparation

Contractions of intestine were produced by tryptamine (10^{-5}M), 5-HT (10^{-7}M) and ACh (5×10^{-7}M).
i) control
ii) after administration of HG (10^{-5}M) and cocaine (10^{-5}M)

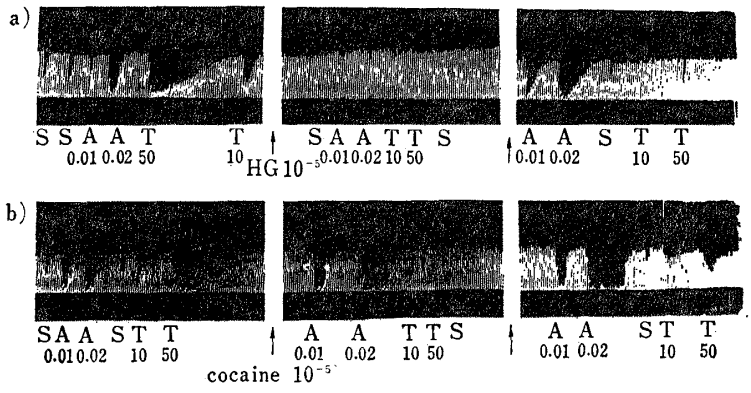

Fig. 7. Effects of HG and Cocaine on Perfused Rabbit Ear

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 cocaine 10^{-5} g/ml (b) were present in the prefusion fluid between the arrows.
A: epinephrine 0.01 and 0.02 μg T: tyramine 10 and 50 μg

5. Perfused Rabbit Ear

Vasoconstriction was recorded after the stimulation of the auricular nerve and injections of tyramine and epinephrine. When HG 10^{-5} g/ml had been included in the perfusion fluid vasoconstriction induced by the stimulation of the nerve, tyramine and epinephrine became inactive. However, when the ear was again perfused with fresh Tyrode solution, the vasoconstrictor action of epinephrine was potentiated as compared with the response to the initial injections of this substance, and that of tyramine was remained antagonized (Fig. 7a). While, when cocaine 10^{-5} g/ml had been included in the perfusion fluid vasoconstrictions induced by the stimulation of the nerve and by tyramine were inhibited and that induced by epinephrine was potentiated (Fig. 7b).

Discussion

HG suppressed the contraction and dilation of the smooth muscles induced by the electrical stimulation of nerve in guinea-pig hypogastric nerve-vas deferens, guinea-pig trachea, rat vagal nerve-stomach, and perfused rabbit ear preparations. While a high dose of cocaine suppressed this response, its low dose potentiated it. Also, HG potentiated the effect of catecholamines on the smooth muscles, such as guinea-pig vas deferens and perfused rabbit ear preparations, which undergo contraction by catecholamines such as norepinephrine and epinephrine. However, HG did not affect the effect of catecholamines on the smooth muscles, such as guinea-pig trachea and rat stomach preparations, which undergo dilation by catecholamines. These results were similar to those with cocaine. Therefore, it can be considered that the action of HG may be a neurotropic which is probably similar to that of cocaine.

The hypothesis widely used to explain the phenomenon of potentiation of the responses of smooth muscle to norepinephrine by cocaine is that inhibition of the uptake of norepinephrine into nerve terminals by cocaine leads to a greater concentration of norepinephrine in the immediate locale of the receptor.[6] According to this hypothesis, it would be supposed that HG inhibits the uptake of catecholamines into the tissue. Furthermore, the vasoconstrictor action of tyramine on the perfused rabbit ear was suppressed by HG and cocaine. It is generally known that cocaine inhibits the action of tyramine because cocaine inhibits the fixation of tyramine into the tissue and abolish its ability to release endogenous catecholamines. The suppression of tyramine by HG can also be explained in this way. Though a low dose of cocaine potentiated the response induced by electrical stimulation, HG did not. Thus, a difference was observed between cocaine and HG but since even the potentiation of cocaine which inhibits the uptake strongly was not so marked, it is considered that the action of HG would be weaker than cocaine. Therefore, even though HG inhibited the uptake of catecholamines, it would not be observed as the potentiation of the contraction.

In the smooth muscles which were dilated by catecholamines, a low dose of cocaine also potentiated the response induced by electrical stimulation. On the other hand, a low dose of cocaine did not affect the action of exogenous catecholamines in these preparations. These results suggest that there are some differences between the action of endogenous catecholamines and that of exogenous catecholamines.

Reiffenstein[7] reported the potentiating action of catecholamine by cocaine from the analysis of a relation between the speed (the rate of rise) and height of the contraction using a cat spleen strip. His report demonstrated that though the inhibition of uptake is probably sufficient to explain the potentiation of catecholamine *in vivo*, where uptake could materially reduce circulating levels of catecholamine, it does not seem to be sufficient for potentiation *in vitro*,

6) L. L. Iversen, "The Uptake and Storage of Noradrenaline in Sympathetic Nerves," at the University Press, Cambridge, 1967.
7) R. J. Reiffenstein, *Brit. J. Pharmacol.*, 32, 591 (1968).

and that the main mode of potentiation by cocaine may be due to its direct effect on the adrenergic receptor to allow increased utilization of receptors. The possibility of a direct muscular action of cocaine was also suggested by Bevan and Verity.[8] They stated that cocaine increased the maximum response of both normal and nerve-free strips of rabbit aorta to norepinephrine. Furthermore, Maxwell, *et al.*[9] have concluded that cocaine enhanced the response of aortic strips to norepinephrine by its direct action on the smooth muscle cells. In the case of HG, there still remains the problem of whether HG has a direct action on the smooth muscle, togheter with its inhibition of the uptake of catecholamines.

8) J. A. Bevan and M. A. Verity, *J. Pharmacol. Exptl. Therap.*, **157**, 117 (1967).
9) R. A. Maxwell, W. B. Wastila and S. B. Eckhardt, *J. Pharmacol. Exptl. Therap.*, **151**, 253 (1966).