COCAINE-LIKE ACTIONS OF HEXYLGUANIDINE

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Ozawa *et al.* have reported the effects and the structure-activity relationship of monoalkylguanidines on the smooth muscle preparations innervated by the sympathetic nerve, on the cardiac activity and on the blood pressure (1). In the paper, they concluded that the actions of monoalkylguanidino compounds were different between the compounds with a small substituent and a bulky substituent, and that the activity increased according to the carbon numbers. These results coincide with the report by Ozawa *et al.* (2) on the effect and the structure-activity relationship of monoalkylguanidines in the skeletal muscle preparation.

In the present paper, authors investigated in more detail the action of hexylguanidine which has the most bulky carbon chain among these compounds and revealed a most striking pharmacological action resemble to cocaine.

METHODS AND MATERIALS

Cat blood pressure and nictitating membrane: The cats (2-4 kg) were anaesthetized with 1.4 g/kg urethane subcutaneously or were made spinal as described by Kumagai *et al.* (3). Blood pressure was recorded through the mercury manometer from one femoral artery. The contractions of the right nictitating membrane were recorded on a smoked paper with an isotonic writing lever. The injections of drugs were made into a femoral vein. The right preganglionic cervical sympathetic nerve was stimulated supramaximally by a square-wave stimulator for 10 seconds at a frequency of 20/sec with 1 msec duration.

Guinea-pig hypogastric nerve-vas deferens preparation: After the vas deferens was dissected together with the hypogastric nerve from guinea-pig, the preparation was suspended in a 10-ml bath containing a Tyrode solution (sodium chloride 137 mM, potassium chloride 2.7 mM, calcium chloride 1.8 mM, magnesium chloride 1.1 mM, sodium bicarbonate 11.9 mM, sodium dihydrogenphosphate 0.4 mM, and glucose 5.6 mM) at 32°C and aerated with 95% O₂+5% CO₂. 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 minutes at a frequency of 50/sec with 1 msec duration and at supra-maximal voltage for 3 seconds. The contractions of vas deferens were also induced by the administrations of norepinephrine, acetylcholine or histamine in the bath.

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°C from a Mariotte 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

FIG. 1. Effects of HG on the blood pressure and on the contraction of the nictitating membrane (cat, urethane anaesthesia). Stimulations were applied at dots on preganglionic fiber of the cervical sympathetic nerve (frequency 20/sec, duration 1 msec, at supramaximal voltage). HG: 5 mg/kg i.v. Vagotomy was made between (a) and (b).

FIG. 2. Effects of HG on the blood pressure and on the contraction of the nictitating membrane in spinal cat. N: norepinephrine 3 µg/kg i.v. (a) HG 5 mg/kg i.v. (b) Phentolamine 5 mg/kg was applied intravenously at arrow.

to the auricular nerve was applied at a frequency 20/sec with 1 msec duration and at supramaximal voltage for 20 seconds.

Intradermal test for local anaesthesia: A modification of the method of Bülbring *et al.* (4) 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 minutes after the injection.

Materials: The substances used in these experiments were following: hexylguanidine sulfate (HG), cocaine hydrochloride, *dl*-norepinephrine hydrochloride, *l*-epinephrine

FIG. 3. Effects of HG on the pressor actions of norepinephrine (i.v.) (a) and tyramine (i.v.) (b) in the blood pressure of spinal cats. Before (●) and after (○) HG 5 mg/kg i.v. Vertical bars represent standard errors calculated on non-paired basis. Probabilities were obtained by *t* test for paired data. N.S.= not significant ($P>0.05$).

hydrochloride, acetylcholine chloride (ACh), histamine hydrochloride, phentolamine methanesulfonate, tyramine hydrochloride, 2-(octahydro-1-azocinyl) ethylguanidine sulfate (guanethidine), atropine sulfate.

RESULTS

1. Cat blood pressure and nictitating membrane

In anaesthetized cats, HG (5 mg/kg) produced a temporary fall followed by a rise in blood pressure. This was not abolished after vagotomy (Fig. 1). The contraction of the nictitating membrane induced by the electrical stimulation on preganglionic fiber of the cervical sympathetic nerve was not affected by HG. In spinal cats, a temporary

FIG. 4. Effects of cocaine on the pressor actions of norepinephrine (i.v.) (a) and tyramine (i.v.) (b) in the blood pressure of spinal cats. Before (●) and after (○) cocaine 2 mg/kg i.v. Vertical bars represent standard errors calculated on non-paired basis. Probabilities were obtained by *t* test for paired data. N.S. = not significant ($P > 0.05$).

fall of the blood pressure induced by HG was abolished and only a rise was observed and the nictitating membrane went into a contracture (Fig. 2a). The rise of blood pressure and the contracture of the nictitating membrane were antagonized by phentolamine 5 mg/kg i.v. (Fig. 2b). However, bilateral adrenalectomy and pretreatment with reserpine (2 mg/kg, s.c., for two days) failed to abolish the rise of blood pressure and the contracture of the nictitating membrane produced by HG 5 mg/kg i.v.

The pressor action of norepinephrine was potentiated and that of tyramine was inhibited by HG and cocaine. The dose-response curves of there results were shown in Figs. 3 and 4. But the stimulant action of tyramine on the nictitating membrane was never abolished by HG.

FIG. 5. Effects of HG pretreatment on the pressor actions of norepinephrine (i.v.) (a) and tyramine (i.v.) (b) in the blood pressure of spinal cats. HG (20 mg/kg) was injected subcutaneously before 24 hours of testing. Before (●) and after (○) HG. Vertical bars represent standard errors calculated on non-paired basis. Probabilities were obtained by *t* test for paired data. N.S.=not significant ($P>0.05$).

The pressor actions of norepinephrine and tyramine on the preparation pretreated with 20 mg/kg HG (injected subcutaneously before 24 hours of testing) could not confirm a significant difference as compared with that untreated (Fig. 5).

Low doses of guanethidine have a marked pressor action in spinal cats (5). The pressor actions of guanethidine (2 mg/kg, i.v.), also, were suppressed by HG 5 mg/kg i.v. (Fig. 6).

In spinal cat, the depressor response to ACh was unaltered after HG 5 mg/kg i.v. Nicotinic action of ACh on preparation injected with 2 mg/kg atropine intravenously, also, was not abolished by HG 5 mg/kg i.v. (Fig. 7).

FIG. 6. Effect of HG (5 mg/kg, i.v.) on the pressor action of guanethidine (2 mg/kg, i.v.) in the blood pressure of spinal cat. HG was administered between (a) and (b).

FIG. 7. Effect of HG on the action of ACh in the blood pressure of spinal cat. HG (5 mg/kg, i.v.) was administered between (i) and (ii). (a) The action of ACh 10 µg/kg, i.v. (b) The nicotinic action of ACh 4 mg/kg, i.v., in cat pretreated with atropine 2 mg/kg, i.v.

2. Guinea-pig hypogastric nerve-vas deferens preparation

The contractions of the vas deferens induced by the electrical stimulation of the pre-ganglionic fiber of the hypogastric nerve were suppressed by HG (10^{-5} – 5×10^{-3} M) and a high dose of cocaine ($>10^{-5}$ M), but were potentiated by a low dose of cocaine (10^{-6} M) (Fig. 8). The contractions of the vas deferens induced by norepinephrine (5×10^{-6} M) were potentiated by HG and cocaine, but those by ACh (5×10^{-6} M) and histamine (10^{-4} M) were little affected (Fig. 9).

3. Perfused rabbit ear

On the rabbit ear perfusion experiments, the vasoconstrictions induced by the elec-

FIG. 8. Effects of HG and cocaine on hypogastric nerve-vas deferens preparation of guinea-pig. Electrical stimulation to preganglionic fiber was applied at 50/sec, 1 msec, supra-maximal voltage. Drugs were administered at arrow. (a) HG 10^{-4} M (b) cocaine 10^{-6} M (c) cocaine 10^{-5} M.

FIG. 9. Effects of HG (a) and cocaine (b) on the contractions of vas deferens of guinea-pig induced by norepinephrine, ACh and histamine. (i) control (ii) after administrations of HG (10^{-4} M) and cocaine (10^{-6} M).

trical stimulation of the auricular nerve, by epinephrine, and by tyramine were blocked by HG at a concentration of 10^{-5} g/ml, which had been included in the perfusion fluid for more than 1 hour. When the ear was replaced with normal Tyrode solution, the vaso-

FIG. 10. Effects of HG and cocaine on the perfused rabbit ear. Electrical stimulation was applied for 20 seconds at S on the auricular nerve (frequency 20/sec, duration 1 msec, at supramaximal voltage). Drugs were injected into the arterial cannula. A: epinephrine 0.01 and 0.02 μ g T: tyramine 10 and 50 μ g. HG 10^{-5} g/ml (a), 10^{-4} g/ml (b), and cocaine 10^{-5} g/ml (c) were present in the perfusion fluid between the arrows.

FIG. 11. Local anesthetic actions of HG and cocaine by intradermal weal test in guinea-pig.

constrictor action of epinephrine was potentiated compared with the response to the initial injections of this substance, but that of tyramine remained antagonized (Fig. 10a). Fig. 10b shows the example of HG 10^{-4} g/ml, which produced vasodilation. Whereas cocaine at a concentration of 10^{-5} g/ml, as shown in Fig. 10c, potentiated the vasoconstrictor action of epinephrine and inhibited that of tyramine.

4. Intradermal test for local anaesthesia

As shown in Fig. 11, the activity of HG (1%) in a local anaesthesia was the same degree with cocaine (1%) and longer duration than that of cocaine.

DISCUSSION

As described in the introduction, the actions of monoalkylguanidino compounds were different between the compounds with a small substituent and a bulky substituent on the blood pressure of cat and rat (1). Namely, on anaesthetized cats, guanidine produced a rise in the blood pressure, and HG produced a temporary fall followed by a rise. The temporary fall in the blood pressure produced by HG was not abolished by vagotomy and treatment of atropine. In spinal cats, however, a temporary fall of the blood pressure induced by HG was abolished and only a rise was observed. Therefore, it is considered that the temporary fall would be perhaps due to the reflex of carotid sinus or the effect to central nervous system. But since guanidine derivatives does not pass through a blood brain barrier because of the strong base, the former possibility is expected.

HG potentiated the actions of epinephrine and norepinephrine on blood pressure of the cat, on the isolated guinea-pig vas deferens and on the perfused rabbit ear. Also, HG inhibited the action of tyramine. These effects were similar to those of cocaine. The inhibition of tyramine by HG was compared with the anti-tyramine actions of cocaine and guanethidine (6). Doses of these drugs producing a 50% inhibition of the pressor action of tyramine in spinal cats were following; HG 2.7 mg/kg, cocaine 1 mg/kg, guanethidine 7.2 mg/kg. Thus, the action of HG was slightly weak than that of cocaine, but strong than that of guanethidine. Furthermore, on guinea-pig vas deferens and on guinea-pig skin, the actions of HG were more prolonged than those of cocaine.

It was recognized, generally, to explain the mechanism of the potentiation of catecholamines by cocaine that the inhibition of the uptake of catecholamines into nerve terminals by cocaine leads to a greater concentration of catecholamine in the immediate locale of the receptor (7). Also, cocaine would inhibit the action of tyramine since it inhibit the fixation into the tissue and would decrease its ability to release tissue-bound catecholamines. If one accepts this assumption, the effect of HG on the pressor and the vasoconstrictor actions of catecholamines and tyramine can be explained in this way.

Reiffenstein (8) reported the potentiating action of catecholamine by cocaine analyzing the relation between the rate of rise of and the height of the contraction using the cat spleen strip. According to the report, it is demonstrated that though the inhibition of uptake is probably sufficient to explain 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*, and that the main mode of potentiation by cocaine may be due to a direct effect on the adrenergic receptor to allow increased utilization of receptors. Considering from that the experiment carried out by Iversen (7) used the isolated heart, one should not neglect the inhibition of uptake. In addition to the inhibition of uptake, the direct action of cocaine would be considered. In the case of HG, also, it is considered that HG has the direct action on the smooth muscle.

According to McCubbin *et al.* (5), it is suggested that a small dose of guanethidine raises blood pressure through the release of endogenous catecholamines and that the action is similar to that of tyramine. In the present experiment, it appears to be the proof that the pressor action of guanethidine 2 mg/kg was suppressed by HG.

The nicotinic action of ACh was not abolished by HG on the atropinized spinal cat. Also, the contraction of the nictitating membrane induced by the electrical stimulation of the cervical sympathetic nerve was not suppressed by HG. These results might be suggest that there would be some difference between the actions of endogenous catecholamine and those of exogenous catecholamine. Furthermore, the contraction of the nictitating membrane was unaffected on the cat even when the cardiovascular actions of tyramine had been abolished by HG in these preparations.

It was concluded that the sympathomimetic actions of HG were produced either by a potentiation of the actions of circulating catecholamines or by a release of catecholamine from some peripheral sites other than adrenals. Neither of these explanations can be excluded, but the similarity with cocaine supports the former possibility.

SUMMARY

The cocaine-like actions of hexylguanidine were investigated using cat blood pressure and nictitating membrane, guinea-pig hypogastric nerve-vas deferens preparation, perfused rabbit ear and guinea-pig skin reflex and the following results were obtained.

1. In anaesthetized cats, hexylguanidine produced a temporary fall followed by a rise in the blood pressure, and the temporary fall was unaffected by vagotomy and treatment of atropine. In spinal cats, the initial fall in blood pressure was abolished and only a rise was observed. The pressor actions were suppressed by phentolamine, but were not affected by adrenalectomy and by previous treatment with reserpine. Also, hexylguanidine potentiated the pressor action of norepinephrine and inhibited those of tyramine and guanethidine, but did not affected to the muscarinic and nicotinic actions of acetylcholine. These results were similar to those of cocaine. Moreover, the contractions of the nictitating membrane induced by the electrical stimulation of the cervical sympathetic nerve and by tyramine were unaffected by hexylguanidine.

2. On the guinea-pig vas deferens preparation and the perfused rabbit ear, the responses induced by the electrical stimulation of the nerve were suppressed with hexylguanidine. The contractions of vas deferens and of ear vessel produced by catecholamines such as norepinephrine and epinephrine were potentiated with hexylguanidine, but the contractions of vas deferens produced by acetylcholine or histamine were not affected, and

the contractions of ear vessel produced by tyramine were suppressed. Also these results were almost similar to those of cocaine.

3. Hexylguanidine had local anaesthetic action as potent as cocaine, and its action was more lasting than that of cocaine.

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

REFERENCES

- 1) OZAWA, H. AND SUGAWARA, K.: *Chem. Pharm. Bull.* **16**, 2376 (1968)
- 2) OZAWA, H. AND TAKEDA, M.: *Yakugaku Zasshi* **85**, 991 (1965)
- 3) KUMAGAI, H., YUI, T., OGAWA, K. AND OHGA, H.: *Seitai no Kagaku* **5**, 132 (1953)
- 4) BÜLBRING, E. AND WAJDA, I.: *J. Pharmac. exp. Ther.* **85**, 78 (1945)
- 5) McCUBBIN, J.W., KANEKO, Y. AND PAGE, I.H.: *J. Pharmac. exp. Ther.* **131**, 346 (1961)
- 6) MAXWELL, R.A., PLUMMER, A.J., POVALSKI, H. AND SCHNEIDER, F.: *J. Pharmac. exp. Ther.* **129**, 24 (1960)
- 7) IVERSEN, L.L.: *The Uptake and Storage of Noradrenaline in Sympathetic Nerves*, Cambridge: at the University Press (1967)
- 8) REIFFENSTEIN, R.J.: *Br. J. Pharmac. Chemother.* **32**, 591 (1968)