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Effect of Indole Alkaloids from *Gardneria* Genus and *Uncaria* Genus on Neuromuscular Transmission in the Rat Limb *in Situ*

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Effect of six indole alkaloids and one synthetic compound on neuromuscular transmission was examined in the rat limb preparation *in situ*. Gardneramine inhibited the gastrocnemius contractions elicited by electrical stimulation of the sciatic nerve, but exerted little or no inhibition on the contractions elicited by direct stimulation of the muscle. The inhibitory effect of gardneramine was a little stronger than that of hexamethonium and was very weak when compared with that of *d*-tubocurarine. Gardnerine augmented both contractions elicited by nerve and muscle stimulation. Gardnutine, hydroxygardnutine, hirsutine, and one synthetic compound showed a long-lasting depressive effect on both contractions, while isorhynchophylline was little effective.

These results indicate that only gardneramine affects neuromuscular transmission chiefly.

Previously, we examined the ganglion-blocking effect of indole alkaloids contained in *Gardneria* genus, *Uncaria* genus, and several related synthetic compounds on superior cervical ganglion *in situ* preparations of the cat[2] and rat[3] and it was found that several compounds including gardneramine and hirsutine have a ganglion-blocking action. It is well known that ganglion-blocking agents, in large doses, exert a curare-like action on the neuromuscular junction, and *d*-tubocurarine has a ganglion-blocking action which is attributed to the blockade of the nicotinic receptor on the ganglion.[4,5] In order to find if these alkaloids may also exert a blocking action on neuromuscular transmission, we studied their effect on it in the rat limb *in situ*.

Experimental

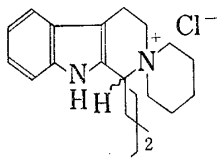
Method——Wistar male rats weighing 300—350 g were anesthetized with urethane (1.2—1.5 g/kg *i.p.*). The trachea was cannulated and a fine polyethylene tube was inserted up to the branching point of the abdominal aorta through the left femoral artery for the drug administration. The right gastrocnemius muscle was freed from the adjacent muscles leaving the vessels intact. The sciatic nerve was separated and cut at a position as proximal as possible. All branches of the sciatic nerve except the branch which innervates the gastrocnemius muscle were cut. Finally a paraffin pool was made for protection against dryness of the operated area. The muscle was alternately contracted by electrical stimulation of the nerve and the muscle itself. In the case of stimulation of the nerve, the sciatic nerve was stimulated with a rectangular pulse of 10—30 msec duration, supramaximal voltage, and in a frequency of 0.2 Hz by means of platinum electrodes using an electronic stimulator (3F—31, Sanei). The muscle was stimulated with a rectangular pulse of 1—10 msec duration, supramaximal voltage, and in a frequency of 0.2 Hz through a pair of silver needle electrodes set between the distal tendon of the muscle and the muscle body. The muscle contractions were recorded on a pen-writing recorder (W-809, Sanei) *via* a force displacement transducer (SB-1T, Nihonkohden). Since there is a possibility that the nerve may also be stimulated when the muscle is stimulated, responses of each preparation to both directly (*via* the muscle) and indirectly (*via* the nerve) applied stimulations were examined using *d*-tubocurarine in advance. Then each test was carried out under the condition where *d*-tubocurarine inhibited only the indirectly elicited contraction and not the directly elicited one. In such a case, influence of

1) Location: *1-33, Yayoi-cho, Chiba, 280, Japan.*
2) S. Murayama, M. Harada, Y. Ozaki, and T. Suzuki, *Japan. J. Pharmacol.* Suppl., **23**, 21 (1973).
3) M. Harada, Y. Ozaki, and M. Sato, *Chem. Pharm. Bull.* (Tokyo), **22**, 1372 (1974).
4) R.M. Eccles and B. Libet, *J. Physiol.* (London), **157**, 484 (1961).
5) C. Takeshige and R.L. Volle, *J. Pharmacol. Exp. Ther.*, **138**, 66 (1962).

the nerve on the muscle contractions elicited by direct stimulation of the muscle, if at all, can virtually be excluded.

In all the experiments, 0.2 ml of the test solution and 0.2 ml of an additional saline solution containing 100—150 units of heparin per 1 ml were injected *via* the femoral artery for 15—20 sec. In order to compare the relative potency of the neuromuscular blocking action of the test compounds, activity of *d*-tubocurarine was evaluated in each experiment.

Material——*d*-Tubocurarine chloride and hexamethonium bromide were used as standard drugs. Doses of these drugs refer to those of the salt per animal. The test compounds were as follows: Gardneramine, gardnerine, gardnutine, hydroxygardnutine (contained in *Gardneria nutans* Sieb. et Zucc., Loganiaceae), hirsutine, isorhynchophylline (contained in *Uncaria rhynchophylla* Miq., Rubiaceae), and compound V (synthetic compound). All compounds were dissolved in phosphoric acid–saline solution. The pH value of the solution of these compounds was set at either 4.2 or 6.2. Doses of these compounds are based on per animal and expressed as a free base.



compound V

Results

1. Effect of *d*-Tubocurarine and Hexamethonium

The saline solution which served as a control was free from any activity for 1 hr. Ten μg of *d*-tubocurarine depressed the indirectly elicited contraction without having any influence on the directly elicited one. Its maximal depressive action was about 90% and the recovery time was about 20 min. When the dose was reduced to 3 μg, only a very slight and transient depression was observed. Hexamethonium, in a dose of 4 mg, also depressed only the indirectly elicited contraction. Its depressive action was less than 50% and very transient, and the depressed contraction recovered to the original level within 5 min (Fig. 1 and 2).

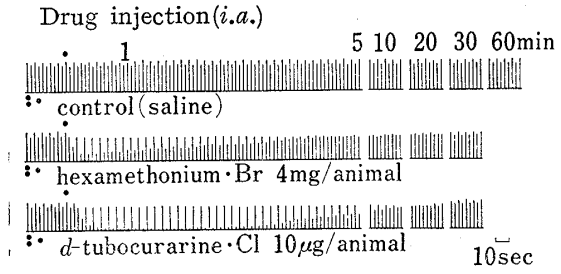


Fig. 1. Tracing of the Directly and Indirectly Elicited Muscular Contraction affected by the Intra-arterial (*i.a.*) Administration of Hexamethonium and *d*-Tubocurarine

: (large contraction): indirectly elicited contraction
· (small contraction): directly elicited contraction

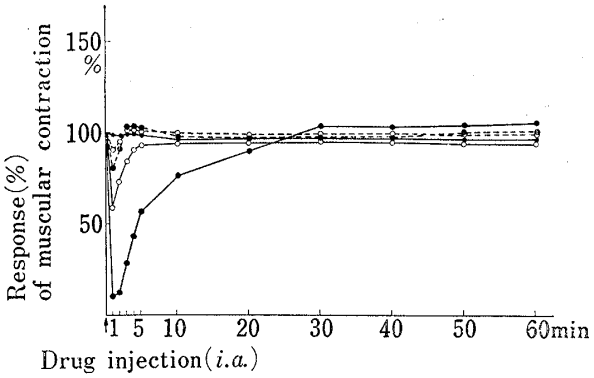


Fig. 2. Effect of Hexamethonium and *d*-Tubocurarine on the Indirectly Elicited Muscular Contraction

—·—: control (saline) ($N=5$)
---○---: hexamethonium bromide 2 mg/animal ($N=5$)
—○—: hexamethonium bromide 4 mg/animal ($N=6$)
---●---: *d*-tubocurarine chloride 3 μg/animal ($N=2$)
—●—: *d*-tubocurarine chloride 10 μg/animal ($N=5$)

2. Effect of Test Compounds

The phosphoric acid–saline solutions of pH 4.2 and 6.2 which served as control were free from any activity for 1 hr. Four mg of gardneramine depressed the indirectly elicited contraction, with little or no influence on the directly elicited one. The maximal depressive effect was about one-half of that induced by 10 μg of *d*-tubocurarine and the recovery time was about 20 min (Fig. 3 and 4).

Gardnerine, in doses of 1 and 2 mg, augmented both indirectly and directly elicited contractions. The effect lasted for 1 hr in the case of 2 mg (Fig. 3 and 4). Two mg of gardnutine augmented both indirectly and directly elicited contractions during the initial 5 min period

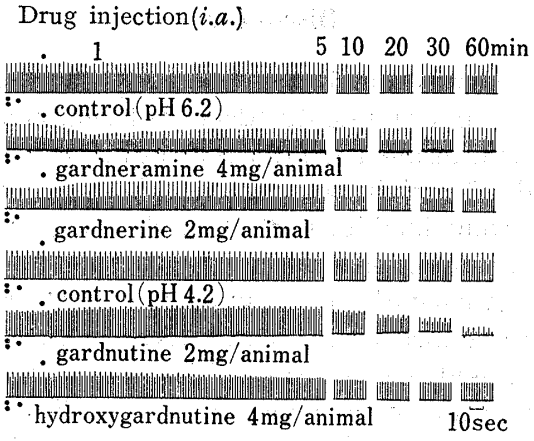


Fig. 3. Tracing of the Directly and Indirectly Elicited Muscular Contraction affected by the *i.a.* Administration of *Gardneria* Alkaloids

: (large contraction): indirectly elicited contraction
• (small contraction): directly elicited contraction

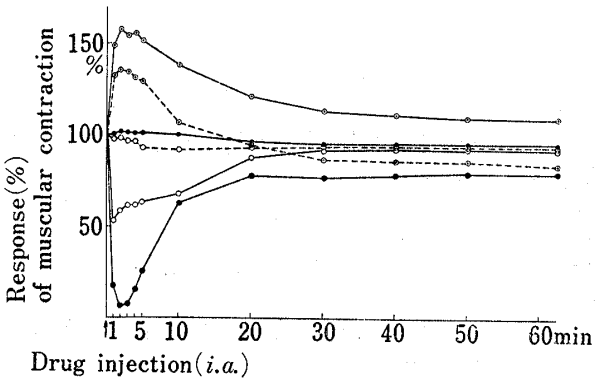


Fig. 3. Effect of Gardneramine and Gardnerine on the Indirectly Elicited Muscular Contraction

—•—: control (pH 6.2) (N=6)
---○---: gardneramine 2 mg/animal (N=5)
—○—: gardneramine 4 mg/animal (N=7)
---⊙---: gardnerine 1 mg/animal (N=5)
—⊙—: gardnerine 2 mg/animal (N=4)
—●—: *d*-tubocurarine chloride 10 μg/animal (N=11)

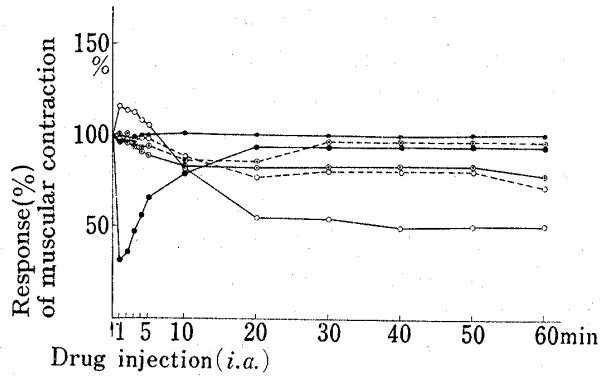


Fig. 5. Effect of Gardnutine and Hydroxygardnutine on the Indirectly Elicited Muscular Contraction

—•—: control (pH 4.2) (N=6)
---○---: gardnutine 1 mg/animal (N=4
—○—: gardnutine 2 mg/animal (N=5)
---⊙---: hydroxygardnutine 2 mg/animal (N=2)
—⊙—: hydroxygardnutine 4 mg/animal (N=3)
—●—: *d*-tubocurarine chloride 10 μg/animal (N=7

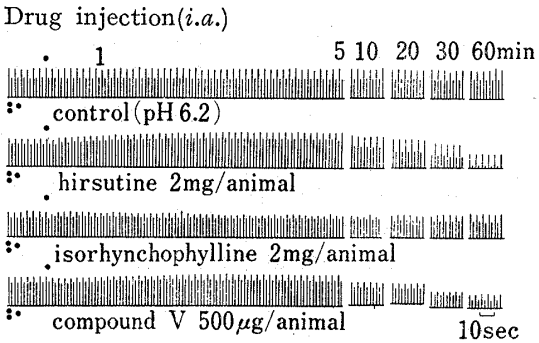


Fig. 6. Tracing of the Directly and Indirectly Elicited Muscular Contraction affected by the *i.a.* Administration of *Uncaria* Alkaloids and a Synthetic Compound

: (large contraction): indirectly elicited contraction
• (small contraction): directly elicited contraction

and gradually depressed both contractions thereafter. The depressed state never returned to the original level. In a dose of 1 mg, the recovery of the depressed state was also hardly observed and the initial enhancement was not induced (Fig. 3 and 5). Four mg of hydroxygardnutine gradually depressed both indirectly and directly elicited contractions. Its depressive action at 4 mg was comparable with that induced by 1 mg of gardnutine (Fig. 3 and 5).

Hirsutine, in a dose of 2 mg, depressed both indirectly and directly elicited contractions, with the initial slight augmentation. Its depressive effect of about 50% lasted longer than 1 hr (Fig. 6 and 7). Two mg of isorhynchophylline depressed both indirectly and directly elicited contractions slightly (Fig. 6 and 7).

Compound V, in doses of 250 and 500 μg, gradually and long-lastingly depressed both indirectly and directly elicited contractions. The depressive effect of 250 and 500 μg on the indirectly elicited contraction was 50% and 75% respectively (Fig. 6 and 7).

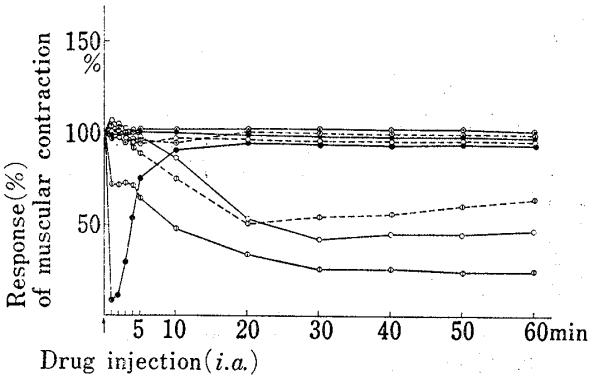


Fig. 7. Effect of Hirsutine, Isorhynchophylline, and Compound V on the Indirectly Elicited Muscular Contraction

—•—: control (pH 6.2) ($N=6$)
---○---: hirsutine 1 mg/animal ($N=4$)
—○—: hirsutine 2 mg/animal ($N=4$)
---⊙---: isorhynchophylline 1 mg/animal ($N=4$)
—⊙—: isorhynchophylline 2 mg/animal ($N=4$)
---◐---: compound V 250 μg/animal ($N=4$)
—◐—: compound V 500 μg/animal ($N=3$)
—●—: *d*-tubocurarine chloride 10 μg/animal ($N=5$)

Discussion

It was found that hexamethonium slightly and transiently depressed neuromuscular transmission in the present preparation and this activity was very weak compared to its strong inhibitory effect on the ganglionic transmission observed in the rat.[3]

Among the test compounds, gardneramine seemed to be characteristic in that it depressed the indirectly elicited contraction, with little or no influence on the directly elicited one and its depressive effect was the most marked at an initial stage and completely disappeared thereafter, like *d*-tubocurarine and hexamethonium. Since gardneramine has a hexamethonium-like ganglion-blocking action affecting the nicotinic receptor and lacks a local anesthetic activity,[2] the site of its action might be the end-plate on the neuromuscular junction. The inhibitory effect of gardneramine was similar to that of hexamethonium in magnitude and was more long-lasting in duration, which indicates that this alkaloid has a higher affinity to the end-plate than hexamethonium. Gardnerine displayed only an augmentation of both indirectly and directly elicited contractions in contrast to gardneramine. A high dose of gardnutine also showed a similar effect at an initial stage. Gardnutine, hydroxygardnutine, hirsutine, and compound V showed a sustained depressive effect in both indirectly and directly elicited contractions, indicating a long-lasting direct influence on the muscle itself. The effect of compound V was the strongest. The activity of gardnutine was more marked than that of hydroxygardnutine, which is comparable with the case observed in the experiment concerning their effect on the ganglion of the rabbit and rat (unpublished observations), and this fact shows that a hydroxyl group attached to the side chain of hydroxygardnutine molecule lessens the activity of gardnutine. Isorhynchophylline showed little effect in the present preparation, which corresponds to its very weak ganglion-blocking effect.[3]

Concerning all compounds tested except gardneramine and isorhynchophylline, we could not appreciate their effect on neuromuscular transmission in the present experimental method, because they all influenced the muscle directly.

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