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Effects of Indole Alkaloids from *Gardneria nutans* Sieb. et Zucc. and *Uncaria rhynchophylla* Miq. on a Guinea Pig Urinary Bladder Preparation *in Situ*

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The effects of six indole alkaloids on parasympathetic ganglionic transmission were studied in a preparation of the guinea pig urinary bladder *in situ*. The effect of hirsutine on spontaneous movement of the organ was also examined. Among these alkaloids, gardneramine and hirsutine most potently inhibited the contraction of the urinary bladder induced by electrical stimulation of the pelvic nerves. Their potency was about one-half of that of hexamethonium. The effect of gardneramine was of short duration. Both alkaloids depressed the contraction induced by intraarterial dimethylphenylpiperazinium, with no antagonizing action to the acetylcholine-induced contraction. Hirsutine showed a local anesthetic action in the isolated frog sciatic nerve preparation, while other alkaloids had only a weak effect. Hirsutine, isorhynchophylline, and gardnerine elevated the tone of the spontaneous movement of the organ and augmented its amplitude. The stimulating action of hirsutine was not affected by pretreatment with tetrodotoxin, atropine, diphenhydramine, or hexamethonium.

Keywords——frog sciatic nerve; ganglion blocking effect; gardneramine; guinea pig urinary bladder; hirsutine; indole alkaloids; local anesthetic effect; parasympathetic ganglia

We previously studied the effects of indole alkaloids from *Gardneria* genus and *Uncaria* genus on superior cervical ganglionic transmission in *in situ* preparations of the cat,[2)] rat,[3,4)] and rabbit[4)] and found that several of the alkaloids had a ganglion blocking effect. Garrett reported on regional differences in the effect of ganglion blocking agents, showing that hexamethonium was more effective in the sympathetic ganglia than in the parasympathetic ganglia and the reverse was the case for MG-624, while tetraethylammonium had no selective action.[5)] In the present study, the effects of these alkaloids on urinary bladder contraction induced by parasympathetic nerve stimulation and dimethylphenylpiperazinium (DMPP) in the guinea pig *in situ* were studied in order to examine their possible ganglion blocking action on the parasympathetic ganglia. In addition, the effect of hirsutine on spontaneous movement of the urinary bladder was studied, because this compound elevated its tone and augmented its amplitude.

Experimental

Method——1. Guinea Pig Urinary Bladder Preparation: Female guinea pigs weighing 280—480 g were anesthetized with 1.5 g/kg of urethane intraperitoneally and tracheal intubation was performed. A needle with a fine polyethylene tube was inserted into the brachial vein so that an additional anesthetic solution or an appropriate amount of 5% glucose solution could be supplied, if necessary. The lower abdominal cavity was opened along the midline for 3—4 cm and downward to both limbs by 1—2 cm to expose the urinary bladder. The cecum, the colon for 5—6 cm just posterior to the cecum, the ileum for 5—6 cm just anterior to the cecum, and a part of the rectum were removed. Since the pelvic nerves could be seen

1) Location: *1–33, Yayoi-cho, Chiba, 260, Japan.*
2) S. Murayama, M. Harada, Y. Ozaki, and T. Suzuki, *Jpn. J. Pharmacol.*, **23**, Suppl., 21 (1973).
3) M. Harada, Y. Ozaki, and M. Sato, *Chem. Pharm. Bull.* (Tokyo), **22**, 1372 (1974).
4) M. Harada and Y. Ozaki, *Chem. Pharm. Bull.* (Tokyo), **26**, 48 (1978).
5) J. Garrett, *Arch. Intern. Pharmacodyn.*, **144**, 381 (1963).

on the bladder and emerging from muscle fascia and connective tissues surrounding the ureters, the pelvic nerves, together with the ureters, were freed from the adjacent tissues, and cut or crushed at the side of the kidney.[6] The left external iliac artery was freed from the adjacent tissues and a fine polyethylene tube was proximally inserted into this artery up to the branching point of the left internal iliac artery for drug administration. The abdominal aorta just above its branching point to the right and left common iliac arteries and the right external iliac artery were freed from the adjacent tissues for temporary occlusion during periods of drug injection. A polyethylene tube 2.5 mm in diameter was inserted into the urinary bladder through the urethral orifice and the urinary bladder was filled with saline through the tube after removal of urine. The tube was connected with a water manometer so that contraction of the bladder could be isotonically recorded on a kymograph. Finally a paraffin pool at 37° was prepared for protection against dryness of the operated area. Contraction of the urinary bladder was induced either by electrical stimulation of the pelvic nerve on both ureters[6] or by applying DMPP and acetylcholine. The pelvic nerves were bilaterally stimulated with a square pulse of 0.6 msec duration, supramaximal voltage, at a frequency of 15 Hz, and for 6 sec every 3.5 min by means of platinum electrodes using an electronic stimulator (3F-31, Sanei). Doses of DMPP and acetylcholine were 2.5 and 0.5—1 μg per animal, respectively. The test solution was injected 2 min before nerve stimulation and the agonistic agents were applied 15 min before, and 3, 15, 30, and 60 min after injection of the test solution. Each of these solutions (0.1—0.2 ml) and a flushing saline containing heparin (150 units/ml) were administered *via* the intraarterial (*i.a.*) cannula with a total volume of 0.4 ml and for 15—20 sec.

2. Isolated Frog Sciatic Nerve Preparation: A bath composed of 3 compartments connected in series was used. The compartments were separated by agar walls. The middle compartment was filled with 2.7 ml of frog Ringer solution and the other 2 compartments with liquid paraffin. Stimulating and recording bipolar platinum electrodes were placed in the paraffin pools. Frogs (*Rana nigromaculata*) weighing 18—25 g were decapitated and the sciatic nerve trunk was dissected from the cord to the knee carefully. Both ends of the nerve trunk were placed on the electrodes with the middle part in the pool of Ringer solution. The nerve trunk was stimulated with a square pulse of 0.1 msec duration, supramaximal voltage, at a frequency of 1 Hz. The action potential was visualized on an oscilloscope and recoded on film (Biophysiograph 130 system, Sanei). As the action potential had several peaks, and the amplitude of the main peak having 1 msec latency was the largest, changes in the largest peak were adopted as an index of changes in the action potential. The test solution (0.3 ml) was applied to the middle compartment. The pH value of all solutions tested was approximately 7.0.

Test Compounds and Standard Drugs——The alkaloids used were gardneramine (GA), gardnerine (GI), gardnutine (GT), hydroxygardnutine (HGT) (from *Gardneria nutans* Sieb. *et* Zucc.), hirsutine (HS), and isorhynchophylline (IR) (from *Uncaria rhynchophylla* Miq.). They were dissolved in phosphoric acid solution and the pH was adjusted to 6.2 or 4.2 using NaOH. Doses of these compounds are expressed in terms of free base per animal. The standard drugs were as follows: acetylcholine chloride, atropine sulfate, 1,1-dimethyl-4-phenylpiperazinium iodide, diphenhydramine hydrochloride, hexamethonium bromide, histamine dihydrochloride, and tetrodotoxin. These drugs were dissolved in saline and the doses refer to those of the salt per animal.

Results

1. Effects of the Test Compounds on Urinary Bladder Contraction induced by Electrical Stimulation of the Pelvic Nerves in Guinea Pigs

Saline and the phosphoric acid solution of pH 6.2 which served as a control showed no activity. The control solution of pH 4.2 produced only a slight and transient inhibitory action. Tetrodotoxin, 0.5 μg, completely abolished the contraction for 20 min. Typical recordings of the effects of the test compounds and hexamethonium are presented in Figs. 1 and 2 and the results are summarized in Table I. Hexamethonium, 100 μg, produced a maximal inhibitory action of 20% at 2—5.5 min and the depressed contraction recovered to 90% of the original level by 30—60 min. A higher dose, 250 μg, produced 50% inhibition and recovery to 90% of the original level was not observed within 60 min. GA, GI, and GT at a dose of 500 μg depressed the contraction by 50, 30, and 20%, respectively. The inhibitory effect of HGT (500 μg) was very weak. HS, 500 μg, depressed the contraction by 50% at 2 min and the recovery time was more than 30 min. IR, 250 μg, showed a weak

6) D.F. Weetman, *Arch. Intern. Pharmacodyn.*, **196**, 383 (1972).

inhibitory effect. Its effect at higher doses was not evaluated because of the occurrence of a marked acceleration of movement of the urinary bladder, as described below.

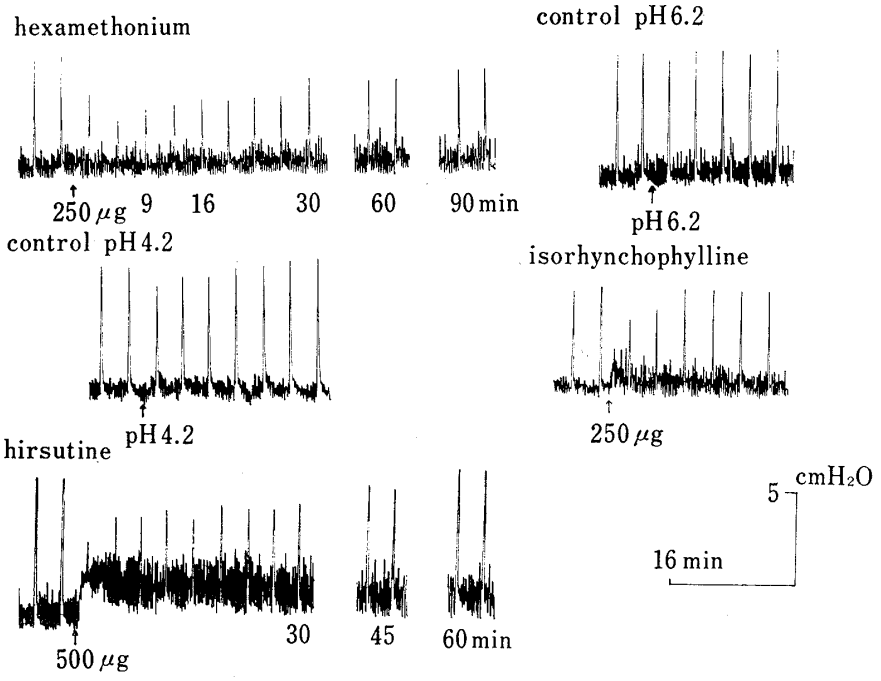

Fig. 1. Effects of Hirsutine, Isorhynchophylline, and Hexamethonium on the Urinary Bladder Contraction induced by Electrical Stimulation of the Ureters in Guinea Pigs

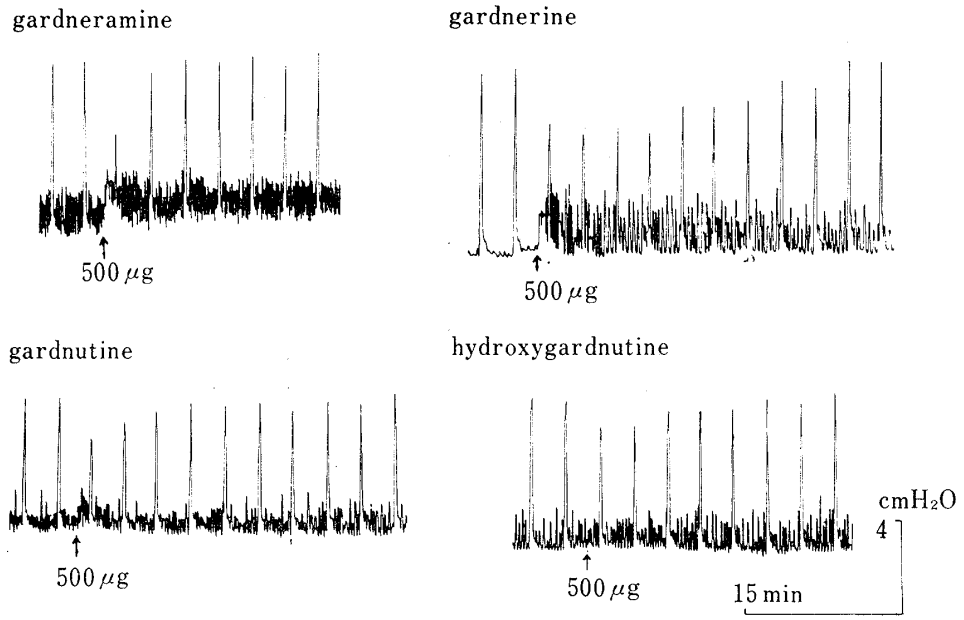

Fig. 2. Effects of Gardneramine, Gardnerine, Gardnutine, and Hydroxygardnutine on the Urinary Bladder Contraction induced by Electrical Stimulation of the Ureters in Guinea Pigs

2. Effects of the Test Compounds on Urinary Bladder Contraction induced by DMPP and Acetylcholine in Guinea Pigs

The results are summarized in Table II. The control solutions of pH 6.2 and 4.2 either depressed the contraction at 3 min or not, depending on the preparations. Hexamethonium,

Table I. Effects of the Test Compounds on Urinary Bladder Contraction induced by Electrical Stimulation of the Ureters in Guinea Pigs

Compound	pH of solution	Dose (μg, *i.a.*)	Maximal inhibitory action (%)[a]	Period until 90% recovery (min)	No. of animals
Gardneramine	6.2	250	27.1	5.5—30	3
Gardneramine	6.2	500	49.9	5.5—30	3
Gardnerine	6.2	250	17.7	12.5	3
Gardnerine	6.2	500	34.0	30	3
Gardnutine	4.2	250	20.3	12.5—16	3
Gardnutine	4.2	500	31.8±4.4	12.5—16	4
Hydroxygardnutine	4.2	250	5.0		2
Hydroxygardnutine	4.2	500	16.7	5.5— 9	2
Hirsutine	6.2	250	24.4±4.4	5.5—16[b]	5
Hirsutine	6.2	500	52.7±7.5	60	4
Isorhynchophylline	4.2	100	12.2	5.5—12.5[b]	3
Isorhynchophylline	4.2	250	19.2±4.8	9—12.5[b]	4
Hexamethonium		100	23.5±2.7	30—60	4
Hexamethonium		250	46.4±4.0	>60	7
Control	6.2		1.4±3.1		5
Control	4.2		8.9		3

a) Mean ±S.E.
b) Period until recovery to the original level.

Table II. Effects of the Test Compounds on Urinary Bladder Contraction induced by Dimethylphenylpiperazinium (2.5 μg) in Guinea Pigs

Compound	Dose (μg. *i.a.*)	Inhibitory action after injection (%)[a]				No. of animals
		3 (min)	15 (min)	30 (min)	60 (min)	
Control pH 6.2		9.7	0.2	7.2		3
Gardneramine	250	38.7	31.0	20.5	26.4	3
Gardneramine	500	38.6±6.5	31.6±7.4	22.9±3.4	21.2±6.9	5
Gardnerine	250	5.7	10.0	16.6	12.3	2
Gardnerine	500	21.1	14.6	20.1	11.6	3
Hirsutine	250	41.3±11.3	24.8±7.3	13.1±6.6	11.0±6.7	4
Control pH 4.2		24.5±12.5	7.5±6.5	5.4±5.6		4
Gardnutine	250	5.8	4.2			2
Gardnutine	500	10.9±10.2	17.1±1.0	11.3±9.0		4
Isorhynchophylline	250	45.6±7.6	29.2±7.9	12.1±6.4	5.4±5.5	4
Hexamethonium	100	62.3±11.9	50.3±9.5	37.1±10.1	23.0±5.5	4

a) Mean ±S.E.
The original contraction corresponded to approximately 5—7 cm H_2O.

100 μg, depressed the DMPP-induced contraction by 60% at 3 min, and the depressed contraction recovered to 80% of the original level within 60 min. GA (500 μg), HS and IR (250 μg) depressed the contraction by 30, 30, and 20% at 3 min, respectively. The inhibitory effects of GI and GT, 500 μg, were very weak. On the other hand, none of these compounds in the doses described here gave protection against acetylcholine-induced contraction.

3. Effect of Hirsutine on Spontaneous Movement of the Urinary Bladder in Guinea Pigs

The action of test compounds on spontaneous movement of the urinary bladder is shown in Figs. 1 and 2. HS, IR, and GI elevated the tone of the spontaneous movement and augmented its amplitude. HS-induced responses were not antagonized by hexamethonium, atropine, diphenhydramine, or tetrodotoxin. These results are shown in Fig. 3.

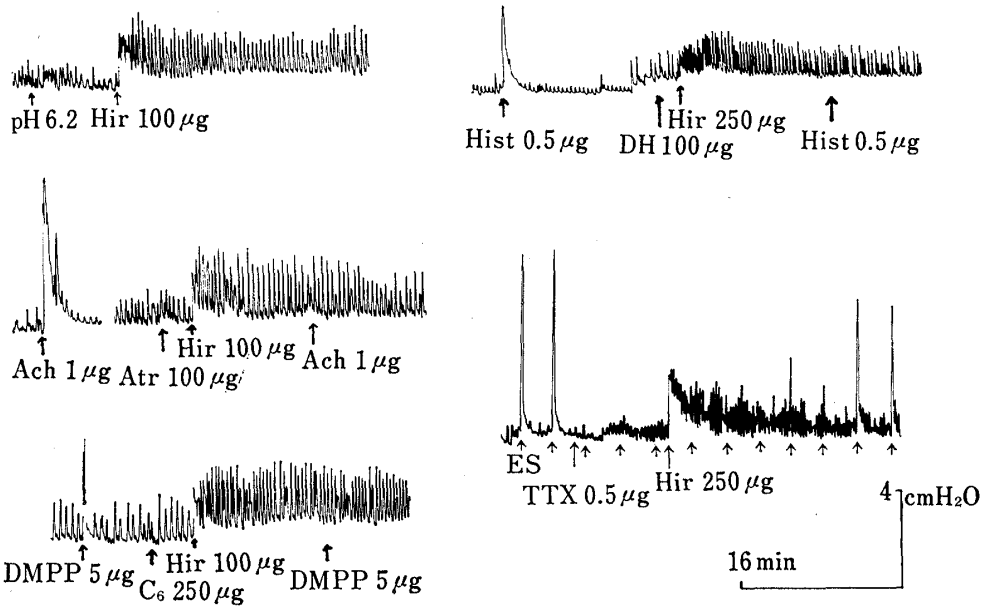

Fig. 3. Effect of Hirsutine on the Spontaneous Movement of the Urinary Bladder in Guinea Pigs

Ach, acetylcholine; Atr, atropine; C_6, hexamethonium; DH, diphenhydramine; DMPP, dimethylphenylpiperazinium; ES, electrical stimulation; Hir, hirsutine; Hist, histamine; TTX, tetrodotoxin.

4. Effects of the Test Compounds on Conduction of the Isolated Frog Sciatic Nerve

The control solutions of pH 6.2 and 4.2 essentially did not affect the action potential for 30 min. HS reduced the action potential by 30 and 70% at 1×10^{-3} and 3×10^{-3} g/ml in 30 min, respectively. GA, GI, IR, and GT reduced the action potential by approximately 15—30% at 3×10^{-3} g/ml in 30 min. Complete recovery of the action potential from the suppressing action of these compounds was obtained by washing the nerve with Ringer solution. Procain reduced the action potential by 35 and 70% at 3×10^{-4} and 1×10^{-3} g/ml, respectively, in 30 min. HS was about 1/3 as potent as procaine in local anesthetic activity, while the other 4 alkaloids were 1/10 as potent or less. These results are shown in Table III.

Table III. Effects of the Test Compounds on the Action Potential elicited by Electrical Stimulation of the Isolated Frog Sciatic Nerve

Compound	Dose (g/ml)	Inhibitory action after application (%)[a]			No. of animals
		5 (min)	10 (min)	30 (min)	
Control pH 6.2		2.2±2.0	3.8± 2.8	4.5± 5.9	4
Gardneramine	3×10^{-3}	3.9±2.4	11.9± 4.0	30.4±10.5	7
Gardnerine	3×10^{-3}	11.2±8.7	15.3±11.2	26.2±20.1	4
Hirsutine	1×10^{-3}	9.8	15.4	31.7	3
Hirsutine	3×10^{-3}	19.6±7.3	33.5± 7.9	69.0±11.3	8
Control pH 4.2		2.5±1.7	2.1± 3.2	8.2± 7.7	5
Gardnutine	3×10^{-3}	12.7±4.7	14.9± 5.1	15.4± 5.6	4
Isorhynchophylline	3×10^{-3}	9.1±6.9	13.1± 9.0	29.4±13.2	5
Procaine	3×10^{-4}	15.0±7.1	22.7±10.4	34.9±13.5	8
Procaine	1×10^{-3}	24.5±5.6	37.7± 7.1	72.0± 6.9	17

a) Mean ±S.E.

Discussion

Weetman reported that in an isolated guinea pig urinary bladder preparation which was suspended in an organ bath, the contraction induced by electrical stimulation of the pelvic nerves with a train of short pulses was blocked by hexamethonium or tetrodotoxin, while atropine produced only a 10% blockade and guanethidine produced little, if any, effect.[6] It is generally accepted that the contraction of the urinary bladder induced by parasympathetic nerve stimulation is not blocked by conventional doses of atropine.[7] Chesher and Thorp observed that in an isolated guinea pig urinary bladder preparation, nicotine produced a prompt contraction and this contraction was blocked by hexamethonium,[8] then Chesher and James concluded that the nicotinic receptors were confined to the ganglion cells of the urinary bladder, since denervation by cooling or anoxia completely blocked the nicotine-induced contraction without any protection against the acetylcholine-induced contraction.[9] Taira *et al.* reported that in a preparation of the dog urinary bladder *in situ*, the contraction induced by pelvic nerve stimulation and *i.a.* DMPP was blocked by hexamethonium and tetrodotoxin.[10] These findings indicate that the contraction of the organ induced by electrical stimulation of the pelvic nerves and by DMPP was due to activation of the parasympathetic ganglia. In the present study, the pelvic nerves were stimulated with a pulse of short duration, such as 0.6 msec, and the resulting contraction of the urinary bladder was blocked by hexamethonium and tetrodotoxin, while the DMPP-induced contraction was also blocked by hexamethonium. Such findings indicate that these contractions were induced through selective excitation of the parasympathetic ganglia.

Among the alkaloids, GA and HS most potently inhibited both the nerve-stimulated and DMPP-induced contractions. Their potency in depressing the former contraction was about 1/2 of that of hexamethonium. This potency ratio approximately corresponds to that obtained in rat[3,4] or rabbit[4] superior cervical ganglion preparation. The effect of HGT was very weak, which was also the case in the rat or rabbit preparation.[4] None of these alkaloids showed protection against the acetylcholine-induced contraction. HS showed local anesthetic action in the isolated frog nerve preparation, while the other alkaloids had a weak effect. The local anesthetic activity of these alkaloids did not necessarily parallel their activity to depress the contraction of the urinary bladder. We have noted an inability of procaine, even in large doses, to depress the DMPP-induced contraction in a preparation of the dog urinary bladder *in situ* (unpublished observation). GA and HS inhibited superior cervical ganglionic transmission, as mentioned above. From these findings, it is reasonable to conclude that GA and HS inhibited the contraction of the organ through an inhibition of the parasympathetic ganglionic transmission and that a blockade of the nicotinic receptors played a role in this effect.

HS, IR, and GI exerted a stimulating action on the spontaneous movement of the urinary bladder. The HS-induced effect was not affected by pretreatment with tetrodotoxin, atropine, diphenhydramine, or hexamethonium, which indicated that HS affected a pharmacological active site(s) in the musculature other than the acetylcholine- and histamine-sensitive sites.

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