[Chem. Pharm. Bull. 26(1) 48—52 (1978)]

UDC 547.944.09 : 615.322.015.4.076.9

Effect of Gardneria Alkaloids on Ganglionic Transmission in the Rabbit and Rat Superior Cervical Ganglia *in Situ*

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(Received May 4, 1977)

Effect of 6 Gardneria alkaloids, namely, gardneramine, gardnerine, gardnutine, hydroxygardnutine, 18-demethylgardneramine, and Alkaloid I on ganglionic transmission was examined in the rabbit and rat superior cervical ganglionic *in situ* preparations. In general, no marked, if at all, difference between both preparations with respect to their maximal response to each of the first 4 compounds was observed. Among these compounds, the most potent ganglion blocking effect was found in gardneramine, gardnerine, and Alkaloid I. Their effect was short-acting compared with that of hexamethonium. The activity of gardneramine and gardnerine was about 1/2 (rabbit), and about 1/4 (rat), as potent as that of hexamethonium. On the other hand, the effect of hydroxygardnutine and 18-demethylgardneramine was very weak.

Keywords——Gardneria alkaloid; hexamethonium; ganglion blocking effect; rabbit superior cervical ganglion; rat superior cervical ganglion; *Gardneria nutans* Sieb. et. Zucc.; *Gardneria multiflora* Makino

Concerning the pharmacology of Gardneria alkaloids which have chemically been investigated by Sakai, *et al.*,[2)] general pharmacological screening of gardneramine (GA) and gardnerine (GI),[3)] effect of GA on ganglionic transmission in the cat superior cervical ganglion,[4)] and effect of GA, GI, gardnutine (GN), and hydroxygardnutine (HG) on neuromuscular transmission in the rat limb[5)] have been reported. In the present study, as GA showed a hexamethonium (C6)-like action on the cat superior cervical ganglion[4)] and as the ganglion blocking effect of C6 on the dose basis was more potent in the cat than in the rat,[6)] effect of 6 Gardneria alkaloids on ganglionic transmission in the rabbit and rat superior cervical ganglia *in situ* was examined for comparison of the activity among the compounds and from the viewpoint of species difference.

Experimental

Materials[2)]——The alkaloids tested were gardneramine, gardnerine, gardnutine, and hydroxygardnutine from *Gardneria nutans* Sieb. et. Zucc. and 18-demethylgardneramine (DG) and Alkaloid I (AI) from *Gardneria multiflora* Makino (Fig. 1). All of them were dissolved in phosphoric acid and their pH was set at the fixed value with sodium hydroxide solution. The pH of the solutions of GA, GI, DG, and AI was 6.2 and that of GN and HG was 4.2. The control solution was prepared according to same procedure without materials. Doses of these compounds are expressed as the free base and are based on per animal. Hexamethonium bromide dissolved in saline was used as a referential drug.

Animals——Male rabbits (2.5—3.5 kg) and male Wistar rats (300—350 g) were used.

Methods——1. Rabbit Superior Cervical Ganglionic Preparation: Under anesthesia of the animal with 1.2—1.8 g/kg of intraperitoneal urethane and after intubation of the trachea, the upper part of the trachea and of esophagus was removed. The postganglionic nerve running along the internal carotid artery and the preganglionic sympathetic trunk running along the common carotid artery were made free from the

1) Location: *Yayoi-cho, Chiba, 280, Japan.*
2) S. Sakai, *Heterocycles*, **4**, 131 (1976).
3) M. Harada, Y. Ozaki, S. Murayama, S. Sakai, and J. Haginiwa, *Yakugaku Zasshi*, **91**, 997 (1971); M. Harada and Y. Ozaki, *ibid.*, **92**, 1540 (1972).
4) S. Murayama, M. Harada, Y. Ozaki, and T. Suzuki, *Jpn. J. Pharmacol.*, **23**, Suppl, 21 (1973).
5) M. Harada and Y. Ozaki, *Chem. Pharm. Bull.* (Tokyo), **24**, 211 (1976).
6) M. Harada, Y. Ozaki, and M. Sato, *Chem. Pharm. Bull.* (Tokyo), **22**, 1372 (1974).

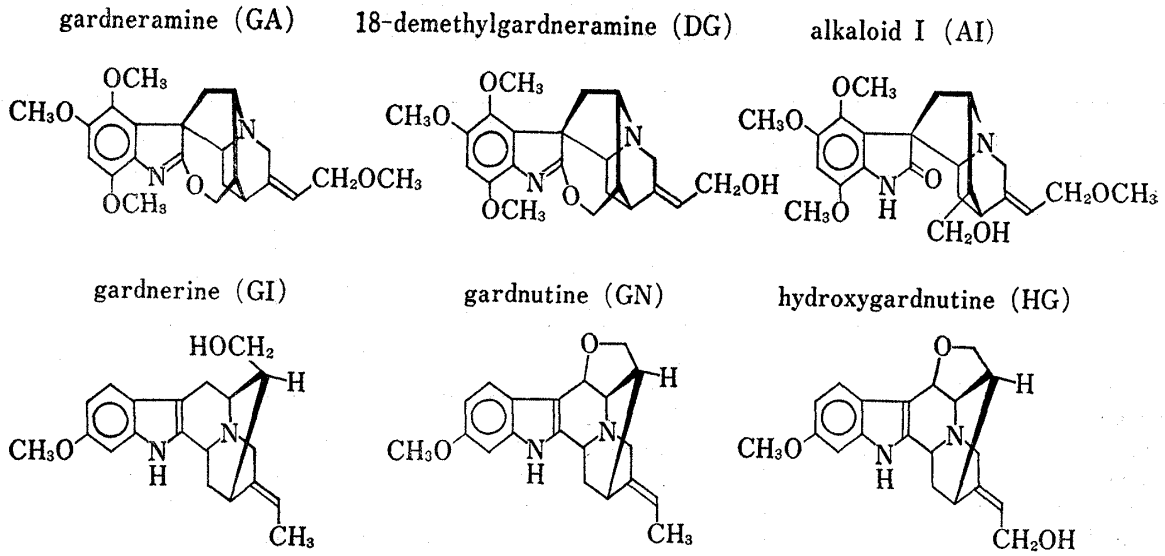

Fig. 1. Chemical Structure of the Gardneria Alkaloids

adjacent tissues and were cut long enough to be set on recording and stimulating bipolar platinum electrodes, respectively. All major extra branches of the common carotid artery except the internal and external carotid arteries were ligated and a fine polyethylene tube was proximally inserted in the lingual artery in a manner similar to that described by Murayama and Unna[7] for the drug administration. Finally a paraffin pool was made for protection against dryness in the operated area and both electrodes were set to the respective nerves. The nerve was stimulated with a supramaximal rectangular pulse of 0.5 msec duration and at a rate of 1 Hz by means of an electronic stimulator (3F-31, Sanei) and the action potentials were recorded on an oscilloscope (130 system, Sanei) and on a pen-writing recorder (Type 8s, Sanei). As the action potential elicited by one shot of stimuli did not necessarily give one single peak in the present experiment, which has been reported by Kosterlitz and Wallis,[8] the change of the largest peak was adopted as index of the change of the action potential. For the drug administration, 0.2 ml of a test solution and 0.2 ml of an additional saline solution containing 100—150 unit of heparin per 1 ml were injected *via* the lingual artery for 15—20 sec under simultaneous occlusion of the common carotid artery and the external carotid artery distal to the origin of the lingual artery for an efficient delivery of a solution to the ganglion.

2. Rat superior Cervical Ganglionic Preparation: The operative procedure and the method for obtaining the action potentials and for an intraarterial drug injection were identical with those described by us previously.[6]

Result

1. Rabbit Superior Cervical Ganglionic Preparation

The effect of 6 compounds and C6 on ganglionic transmission is given in Table I and effect of GA and C6 on the action potentials recorded by a pen-writing recorder is illustrated in Fig. 2. The control solution of pH 6.2 and 4.2 did not practically show any activity. C6 in a dose of 1 mg inhibited the action potentials by 66% in 1 min and its inhibitory effect gradually decreased to 26% in 30 min. All of the test compounds inhibited the action potentials in a dose-dependent manner and the inhibitory effect was short-acting in each of the compounds. The most effective materials were GA, GI, and AI in a dose of 2 mg and their inhibitory effect was about 60% in 1 min and decreased to 0—20% in 10—20 min. GN was less effective and the activity of HG and DG was so weak that 2 mg of them inhibited the action potentials only by less than 30%. The maximal inhibitory effect elicited by 2 mg of GA, GI, and AI was approximately equal to that of 1 mg of C6. In some preparations treated with GA and GN, the action potentials were completely abolished in 1 min and their recovery was rapid and complete in GA (Fig. 2).

7) S. Murayama and R.K. Unna, *J. Pharmacol. Exp. Ther.*, **140**, 183 (1963).
8) H.W. Kosterlitz and D.I. Wallis, *Brit. J. Pharmacol. Chemother.*, **26**, 334 (1966).

Table I. Effect of Gardneria Alkaloid and Hexamethonium on the Ganglionic Transmission in the Rabbit Superior Cervical Ganglion *in Situ*

Compound	Dose mg/head	No of animals	% of action potential to control value 1	5	10	20	30 (min)
Gardneramine	1	9	62.6±12.1	81.7± 6.1	90.6± 4.1	97.0± 3.0	
Gardneramine	2	11	38.7± 9.4	63.8± 8.0	85.8± 4.2	98.3± 3.0	
Gardnerine	1	4	63.5±17.3	81.9± 8.2	89.0± 4.8	98.9± 1.4	
Gardnerine	2	4	42.0±22.8	70.8±12.4	81.7± 7.6	96.0± 2.1	
Gardnutine	1	7	68.0±17.0	80.7±11.0	89.4± 5.3	94.0± 3.3	98.0±2.0
Gardnutine	2	6	57.8±12.4	72.8±15.1	77.5±15.7	83.0±11.9	86.2±9.4
Hydroxygardnutine	1	6	93.5± 5.9	97.0± 2.6	98.8± 2.6		
Hydroxygardnutine	2	5	72.8±10.0	92.8± 2.4	96.0± 1.8		
18-Demethylgardneramine	1	5	87.7± 4.7	92.7± 3.2	95.1± 2.5		
18-Demethylgardneramine	2	5	80.0±12.5	85.6± 4.9	91.2± 2.5	100.0± 0.9	
Alkaloid I	1	4	76.1± 8.2	85.3± 3.3	87.8± 1.7	96.6± 3.7	
Alkaloid I	2	6	44.2± 9.6	64.8± 5.6	74.5± 4.2	86.3± 4.0	95.5±2.8
Hexamethonium bromide	1	16	33.9± 6.8	43.6± 7.5	52.4± 8.4	67.1± 8.5	74.1±8.5
Control (pH 6.2)		9	106.2± 2.0	99.8± 0.8	100.9± 0.9		
Control (pH 4.2)		12	97.9± 3.1	100.8± 1.3	101.3± 2.1		

Drugs were intraarterially administered at 0 min.
Results are expressed as mean±S.E.

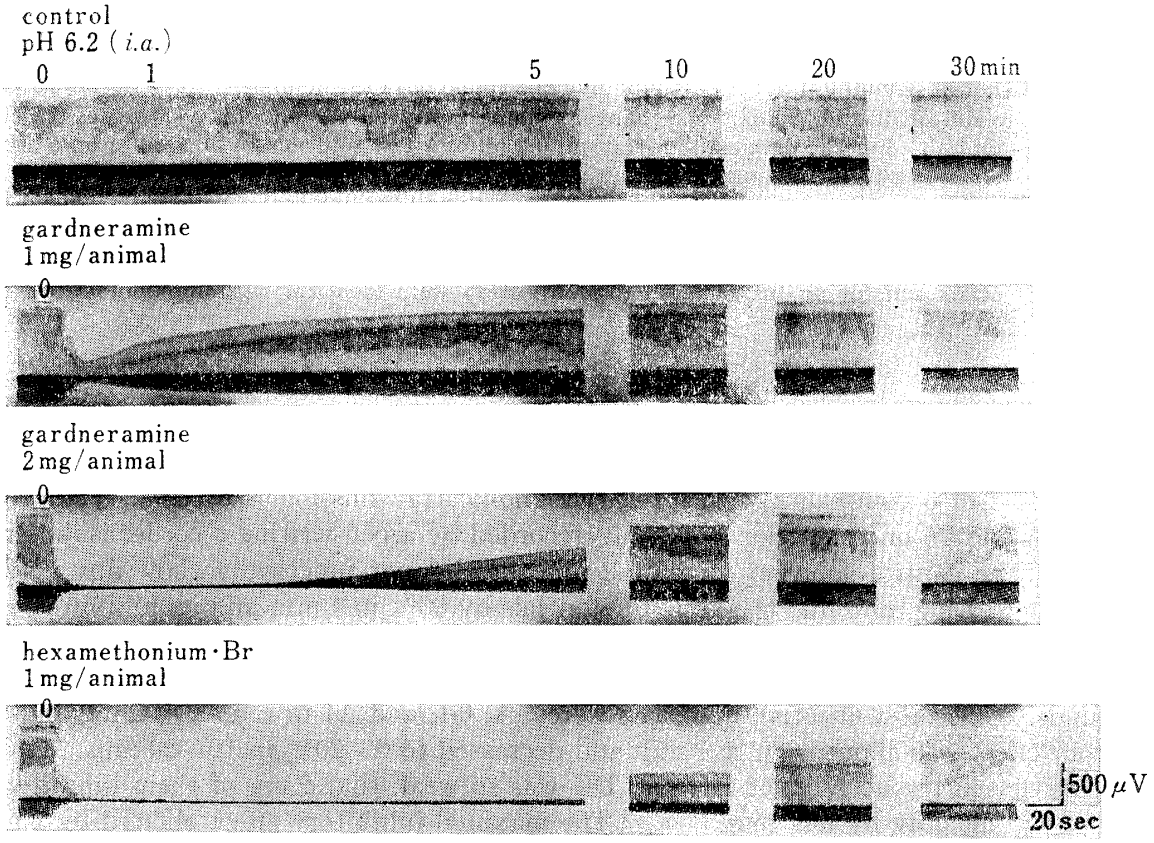

Fig. 2. Effect of Intraarterial Gardneramine and Hexamethonium on the Action Potentials of the Superior Cervical Ganglia of a Rabbit

2. Rat Superior Cervical Ganglionic Preparation

Effect of 4 compounds and C6 on ganglionic transmission is given in Table II and effect of GA and C6 on the action potentials recorded by a pen-writing recorder is illustrated in

Fig. 3. The control solution of pH 6.2 and 4.2 was free from any activity. C6 inhibited the action potentials by 59% and 92% in 1 min in a dose of 0.5 mg and 1 mg, respectively. The inhibitory effect induced by both doses gradually decreased, still being potent more than 20% in 20 min. GI, GA, and GN elicited a dose-dependent inhibitory effect and their potency decreased in that order. The effect of GA and GN was short-acting, whereas that of GI was

TABLE II. Effect of Gardneria Alkaloid and Hexamethonium on the Ganglionic Transmission in the Rat Superior Cervical Ganglion *in Situ*

Compound	Dose mg/head	No of animals	% of action potential to control value				
			1	5	10	20	30 (min)
Gardneramine	1	5	92.3± 2.2	96.4± 2.8	96.4± 3.1		
Gardneramine	2	4	38.4±14.6	78.5±14.1	86.1±10.9	90.6± 8.9	92.1± 7.4
Gardnerine	1	4	61.8±10.9	75.0± 7.4	81.4± 8.7	85.3± 7.7	93.0± 8.8
Gardnerine	2	3	38.7± 1.2	37.6± 1.1	51.8±11.2	63.2± 9.3	78.6±12.8
Gardnutine	1	5	88.7± 6.6	92.5± 5.2	96.8± 2.4		
Gardnutine	2	3	53.9±23.8	80.5±13.3	89.9± 6.6	95.3± 2.4	
Hydroxygardnutine	1	3	95.8± 1.9	94.9± 0.4	96.9± 1.7		
Hydroxygardnutine	2	3	95.1± 4.0	89.0± 3.7	89.1± 2.7	88.9± 2.5	91.6± 7.7
Hexamethonium bromide	0.5	9	40.7± 8.3	47.1± 7.7	63.8± 6.9	76.9± 8.7	84.2± 8.3
Hexamethonium bromide	1	4	8.2± 1.3	24.6±10.0	53.8±15.8	76.7±19.5	
Control (pH 6.2)		5	99.4± 0.9	95.7± 2.2	100.0± 1.3		
Control (pH 4.2)		8	96.6± 3.0	99.2± 1.2	100.1± 1.0		

Drugs were intraarterially administered at 0 min.
Results are expressed as mean ± S.E.

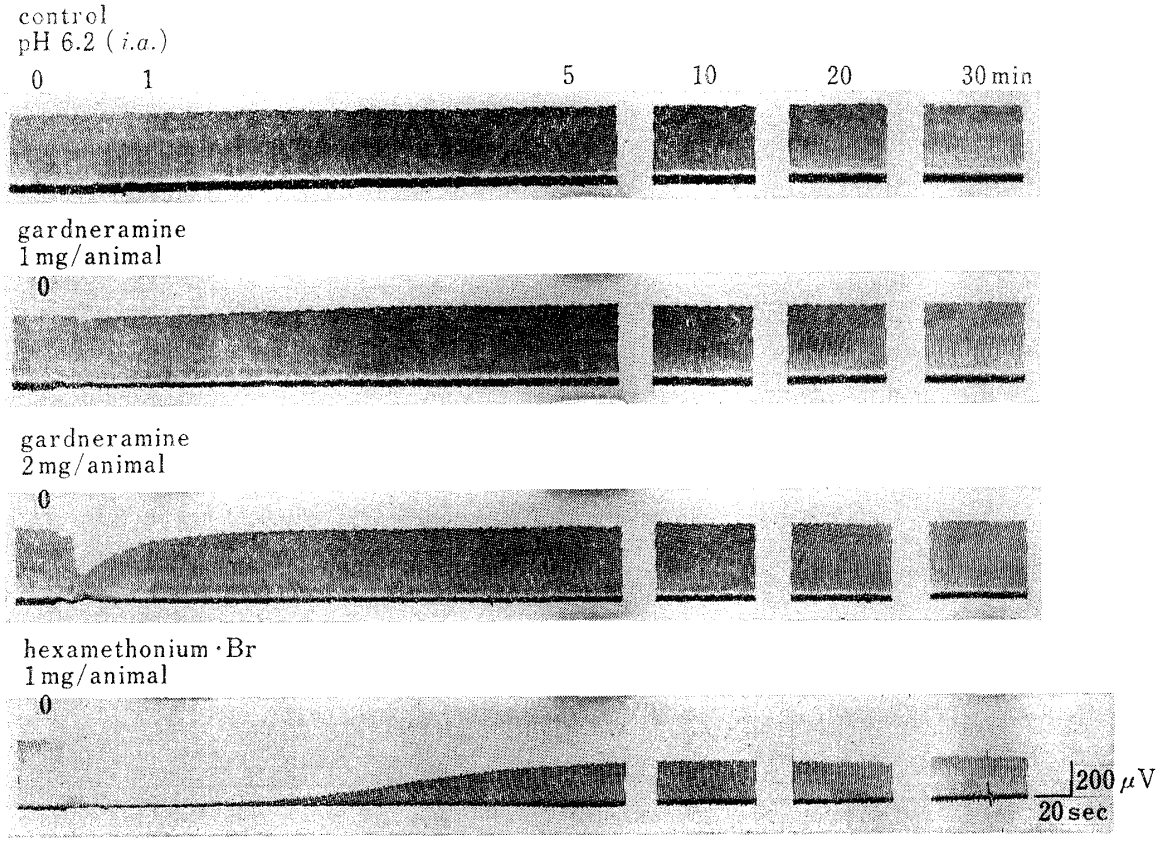

Fig. 3. Effect of Intraarterial Gardneramine and Hexamethonium on the Action Potentials of the Superior Cervical Ganglia of a Rat

rather long-lasting. The magnitude of the maximal inhibition by 2 mg of GA and GI approximately corresponded to that induced by 0.5 mg of C6. On the contrary, HG, even in a dose of 2 mg, only slightly affected ganglionic transmission.

Discussion

Intraarterial GA produced a complete and short-acting abolition of the postganglionic action potentials and their subsequent complete recovery in the cat superior cervical ganglionic preparation *in situ*.[4] GA, in the present rabbit and rat preparations, inhibited ganglionic transmission in a similar manner. The order of potency was as follows: GI$\geqq$GA, AI$>$GN$\gg$HG, DG. Among them, AI is a compound in which opening of an ether ring of GA molecule is made, and HG and DG have a hydroxyethylidene group, that is, HG is a compound in which a methyl group in the ethylidene part of GN is replaced by a hydroxymethyl group and DG is a demethylated compound of GA molecule at the terminal methyl group. In the rat limb preparation, GN also affected the muscle contraction more markedly than HG.[5] Concerning the response of both preparations to the alkaloids, the rabbit preparation was somewhat more sensitive than, or approximately equal to, the rat preparation in the initial maximal inhibitory activity and as to C6, its ganglion blocking effect in the rat preparation was about twice as potent as that in the rabbit preparation. Accordingly, potency of GA and GI corresponded to about 1/2 (rabbit) of, and about 1/4 (rat) of, that of C6, based on the inhibitory degree of about 60% in the present study. The response of both preparations to C6 and GA was inferior to that of the cat preparation.

Acknowledgement We are grateful to Prof. Shinichiro Sakai for his kind supply of the test compounds.