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Ganglion Blocking Effect of Indole Alkaloids contained in *Uncaria* Genus and *Amsonia* Genus and Related Synthetic Compounds on the Rat Superior Cervical Ganglion *in Situ*

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Effects of some standard drugs, indole alkaloids contained in *Uncaria* Genus and *Amsonia* Genus and several related synthetic compounds on transmission were examined in the rat superior cervical ganglionic preparation *in situ*.

In this preparation, standard drugs could produce typical actions to be expected. It was confirmed that this preparation could be routinely used and an arterial injection of drugs was preferable in order to examine their direct action on the ganglion.

All indole compounds, administered arterially, showed a ganglion blocking action except one. Among these, hirsutine, 2,3-seco-yohimbine, I and V exerted a relatively strong inhibitory effect comparable with that of hexamethonium.

Haginiwa, *et al.* and Sakai, *et al.* isolated four indole alkaloids from *Gardneria nutans* SIEB. et ZUCC. (Loganiaceae) and determined their chemical structure, namely, gardnerine, gardnutine, hydroxygardnutine and gardneramine.[2] The pharmacological studies on gardnerine and gardneramine have already been reported by Harada, *et al.*[3] and a specific ganglion blocking activity has especially been found in gardneramine by Murayama, *et al.*[4] Further, Haginiwa, *et al.* have isolated and identified many analogous alkaloids from *Uncaria rhynchophylla* MIQ., *Uncaria kawakamii* HAYATA, *Uncaria florida* VIDAL (Rubiaceae) and *Amsonia elliptica* ROEM. et SCHULT. (Apocynaceae).[5] In the present study, we examined the ganglion blocking effect of 4 indole alkaloids, one derivative and 5 related synthetic compounds[6] in order to make a comparison with that of gardneramine. These are presented in Chart 1.

Cats are an animal of the first choice for the pharmacological study on ganglia *in situ*. Rabbits and rats are used less frequently.[7,8] The merit of the rat as an experimental model depends on much saving in amounts of test chemicals, high availability of individual definite strains and its easy handling if an operation is not difficult. From these reasons we selected rats. As pharmacological data on the rat superior cervical ganglion *in situ* are not sufficient to be referred to, representative standard drugs were tested, to begin with.

Experimental

Experimental Method——The experimental procedure was based upon the method reported by Volle, *et al.*[8] Wistar rats of either sex weighing 300—400 g were anesthetized with 1.2 g/kg of urethane intraperito-

1) Location: *Yayoi-cho, Chiba.*

2) J. Haginiwa, S. Sakai, A. Kubo, and T. Hamamoto, *Yakugaku Zasshi*, **87**, 1484(1967); S. Sakai, N. Aimi, A. Kubo, M. Kitagawa, M. Shiratori, and J. Haginiwa, *Tetrahedron Letters*, **1971**, 2057.

3) M. Harada, Y. Ozaki, S. Murayama, S. Sakai, and J. Haginiwa, *Yakugaku Zasshi*, **91**, 997 (1971); M. Harada, Y. Ozaki, *ibid.*, **92**, 1540 (1972); M. Harada, Y. Ozaki, and M. Sato, Abstracts of Papers, The 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, April, 1972, p. 117.

4) S. Murayama, M. Harada, Y. Ozaki, and T. Suzuki, Proceedings, 46th General Meeting of Japanese Pharmacological Society, Kumamoto, April, 1973, p. 21.

5) J. Haginiwa, S. Sakai, K. Takahashi, M. Taguchi, and S. Seo, *Yakugaku Zasshi*, **91**, 575 (1971); J. Haginiwa, S. Sakai, N. Aimi, E. Yamanaka, and N. Shinma, *ibid.*, **93**, 448 (1973); S. Sakai, H. Ohtani, H. Ido, and J. Haginiwa, *ibid.*, **93**, 483 (1973).

6) S. Sakai, N. Aimi, K. Kato, H. Ido, and J. Haginiwa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1503 (1971).

7) S.O. Kayaalp and R.J. McIsaac, *J. Pharmacol. Exptl. Therap.*, **173**, 193 (1970).

8) J.C. Hancock and R.L. Volle, *J. Pharmacol. Exptl. Therap.*, **169**, 201 (1969).

neally. The trachea was canulated and the upper part of the trachea and the esophagus were removed. The superior cervical ganglion is located at the branch point of the common carotid artery to the internal and external carotid artery. The ganglion is supplied with blood chiefly from the internal carotid artery. The postganglionic nerve running along the internal carotid artery from the ganglion and the preganglionic nerve running along the common carotid artery were made free from adjacent tissues and were cut long enough to be set on a recording and a stimulating bipolar platinum electrode, respectively. The external carotid artery was proximally canulated with a thin polyethylene tube for the drug administration. In this respect Volle, *et al.* made an injection through a needle inserted distally in the common carotid artery. Ligation of extra branches of the main vessels between the canulated site and the ganglion is preferable if possible in order to keep efficient delivery of a drug solution to the ganglion. Finally a paraffin pool was made for protection against dryness in the operated part and both electrodes were set to the respective nerves. The activity of drugs was evaluated in terms of the voltage change of the biggest peak of the action potential which was elicited by an electrical stimulation of the preganglionic nerve and recorded from the postganglionic nerve on an oscilloscope or a pen-writing recorder. The nerve was stimulated with square wave pulses of 0.5 msec duration, just suprathreshold intensity and in a frequency of 1 cps. Drugs were administered *via* the external carotid artery or *via* the femoral vein. In the case of an arterial 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 for 15—20 sec. The action potential was measured 0.5, 1, 2, 3, 4 and 5 min after the drug administration and every 5 min thereafter. Prior to the succeeding injection, at least an interval of 15 min was allowed to elapse after the action potential resumed the original level or a sequence of the steady voltage.

The following standard drugs were used: hexamethonium bromide; atropine sulfate; atropine methylbromide; procaine hydrochloride; nicotine sulfate; adrenaline hydrochloride; noradrenaline hydrochloride; isoproterenol hydrochloride; acetylcholine chloride; potassium chloride; *d*-tubocurarine chloride. All drugs were dissolved in 0.9% saline solution. Doses of the drugs given refer to the weights of the salt. All indole alkaloids and related synthetic compounds were dissolved in phosphoric acid except V which was dissolved in saline. The pH value of these solutions which were prepared to an appropriate volume with saline falls between 4.2 and 6.8. Doses given refer to the weights of the free base.

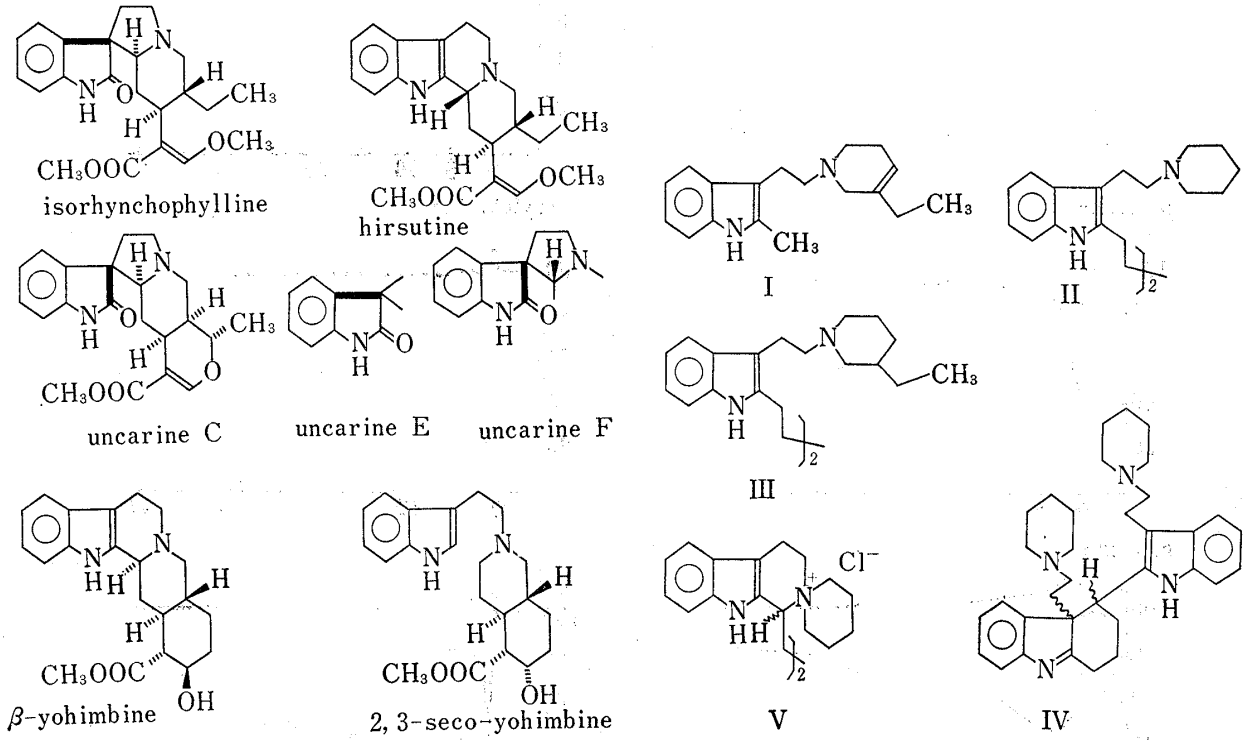

Chart 1. The Chemical Structure of the Indole Alkaloids and the Related Synthetic Compounds

Uncarine mixture (C: E: F=6: 3: 1) was used.

Result

1. Effect of the Standard Drugs

i) Arterial Administration (*i.a.*)——Effect of hexamethonium depicted by a pen-writing recorder is illustrated in the lowest row of Fig. 2. All results are given in Fig. 1 and Table I.

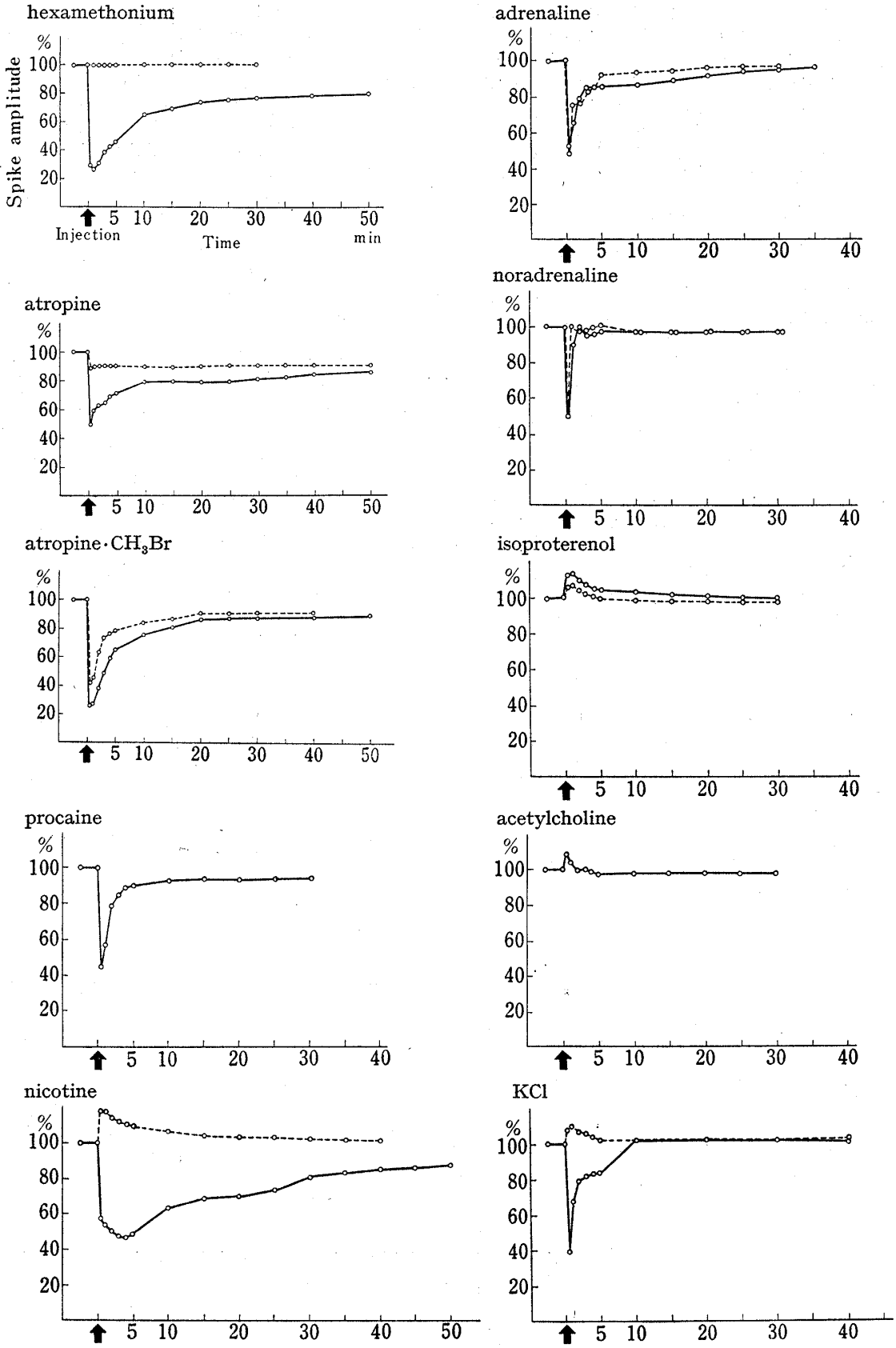

Fig. 1. Time Course of the Action Potential after the Administration of Standard Drugs

hexamethonium: ○---○; saline control, ○—○; 500 μg *i.a.*
atropine: ○---○; 200 μg *i.a.*, ○—○; 400 μg *i.a.*
atropine·CH_3Br: ○---○; 200 μg *i.a.*, ○—○; 400 μg *i.a.*
procaine: ○---○; 500 μg *i.a.*
nicotine: ○---○; 2 μg *i.a.* ○—○; 20 μg *i.a.*
adrenaline: ○---○; 2 μg *i.a.*, ○—○; 5 μg *i.a.*
noradrenaline: ○---○; 2 μg *i.a.*, ○—○; 5 μg *i.a.*
isoproterenol: ○---○; 1 μg *i.a.*, ○—○; 2 μg *i.a.*
acetylcholine: ○—○; 10 μg *i.a.*
KCl: ○---○; 1 mg *i.a.*, ○—○; 2 mg *i.a.*

The maximal effect of drugs was mostly displayed 30—40 sec after the drug administration but the time to the recovery was different respectively. In hexamethonium, the manifestation of the maximal inhibitory effect was shown 40—70 sec after the drug injection, being delayed compared with other drugs. In the case of 500 μg the recovery of more than 90% as the average value was not obtained even in 60 min. Atropin exerted a mild inhibition in 200 μg but a complete recovery was not obtained in 60 min. In procaine, the recovery was rapidly accomplished. Nicotine augmented the action potential in 2 μg and inhibited it in 20 μg. In the latter case, the augmentation prior to the inhibition was occasionally observed. In noradrenaline, the maximal inhibitory effect was elicited 20—30 sec after the administration, occurring faster than in adrenaline and the recovery was also more rapid. On the other hand, isoproterenol displayed only an augmentative effect in 1 to 2 μg. In acetylcholine, a transient augmentative effect was obtained in 10 μg. Like nicotine, potassium chloride showed an augmentative effect in a low dose of 1 mg and an inhibitory effect in a high dose of 2 mg. As to *d*-tubocurarine, death occurred in a dose of 30 μg at which no definite effect was displayed.

Table I. Effect of Standard Drugs on the Action Potential in the *in Situ* Preparation of the Rat Superior Cervical Ganglion

Drug	Dose (μg)	Route	Maximal action[a] mean ± S.E. (%)	Time to recovery of 90% (min)	No. of animals
Hexamethonium	500	*i.a.*	−74±5.8	>60	10
Atropine	200	*i.a.*	−12±2.6	3	5
Atropine	400	*i.a.*	−51±8.1	>60	4
Atropine·CH_3Br	200	*i.a.*	−58±10.7	20	4
Atropine·CH_3Br	400	*i.a.*	−74±8.8	60	5
Procaine	500	*i.a.*	−55±15.0	10	4
Nicotine	2	*i.a.*	+19±6.4	20[b]	4
Nicotine	20	*i.a.*	−52±20.9	>60	5
Adrenaline	2	*i.a.*	−52±9.9	5	5
Adrenaline	5	*i.a.*	−47±12.7	20	5
Noradrenaline	2	*i.a.*	−38±4.8	<1	4
Noradrenaline	5	*i.a.*	−50±2.7	2	4
Isoproterenol	1	*i.a.*	+7±3.3	4[b]	4
Isoproterenol	2	*i.a.*	+13±6.3	15[b]	6
Acetylcholine	10	*i.a.*	+8±2.1	2[b]	5
KCl	1000	*i.a.*	+10±1.1	5[b]	4
KCl	2000	*i.a.*	−60±20.0	10	5
Hexamethonium	5 mg/kg	*i.v.*	−50±11.2	>60	5
Atropine	4 mg/kg	*i.v.*	−8±1.5	—	4
Atropine·CH_3Br	4 mg/kg	*i.v.*	−48±6.0	10	4

a) −: inhibition, +: augmentation
b) time to recovery to the control value

ii) Venous Administration (*i.v.*)——Results as to 3 drugs are shown in Table I. The manifestation of the maximal inhibitory effect of these drugs took place 40—60 sec after the administration, being delayed than in the arterial application. Atropine did not exert a definite inhibitory effect in 4 mg/kg, whereas atropine methylbromide revealed a marked inhibitory one in the same dose. Adrenaline displayed a transient inhibitory effect of 22% and 2 min in a dose of 20 μg/kg (n=3). As for the duration period of the inhibition, hexamethonium showed the most potent action.

2. Effect of Indole Alkaloids and Related Synthetic Compounds

The administration of compounds was all made *i.a.* The phosphoric saline solution of pH 4.2 which served as control was free from any activity. The representative figures and

all results are given in Fig. 2 and Table II. All compounds showed only a ganglion blocking effect. Effect of uncarine and isorhynchophylline was weak and short-acting. Effect of hirsutine in 1 mg lasted longer than that of hexamethonium in 500 μg. Although β-yohimbine gave the effect in 250 μg, it was accompanied with occasional death in more doses. 2,3-Seco-yohimbine also displayed a prolonged inhibitory effect. I showed an effect comparable with that of hexamethonium. Death sometimes occurred in 25 μg of II prior to appearance of any effect. III displayed a weak effect in 250 μg accompanied with occasional death and the effect was hardly observed in less doses. The effect of IV was relatively weak. V showed a characteristic feature that a maximal effect was obtained 10—15 min after the injection and its duration was very long. The effect of V was comparable with that of hexamethonium.

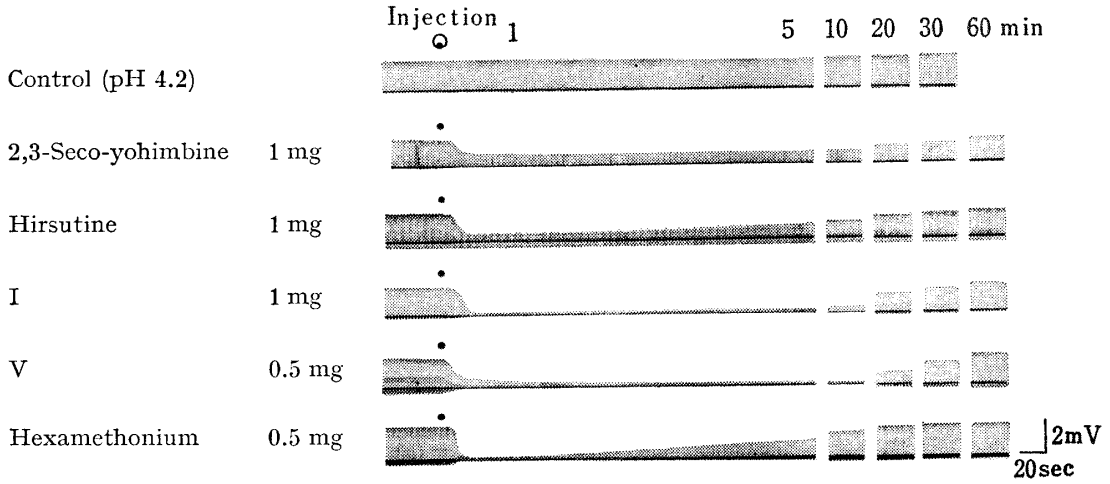

Fig. 2. Tracing of the Action Potential affected by the *i.a.* Administration of Hexamethonium and Indole Compounds

TABLE II. Effect of the Indole Alkaloids and the Related Synthetic Compounds on the Action Potential in the *in Situ* Preparation of the Rat Superior Cervical Ganglion

Drug	Dose (mg, *i.a.*)	Maximal action[a] mean ± S.E. (%)	Time to recovery of 90% (min)	No. of animals
Isorhynchophylline	0.5	−7±2.0	—	5
Isorhynchophylline	1	−25±7.3	3	6
Hirsutine	0.5	−36±9.0	30	5
Hirsutine	1	−57±14.3	>90	5
Uncarine	0.5	−6±3.2	—	4
Uncarine	1	−15±8.4	3	5
β-Yohimbine	0.25[b]	−33	10	3
2,3-Seco-yohimbine	0.5	−24±10.3	>60	4
2,3-Seco-yohimbine	1	−44±9.6	>90	7
I	0.5	−65±18.4	40	5
I	1	−74±10.8	90	6
II	0.025[b]	0		2
III	0.25[b]	−18	20	3
IV	0.5	−25±5.4	10	4
V	0.5	−80±7.6	90	5
Hexamethonium	0.5	−73±6.4	80	9

a) −: inhibition
b) Death occurred in this dose.

Discussion

It was found that the almost steady action potential can be maintained for several hours in the preparation of the rat superior cervical ganglion *in situ*. In the case of an arterial injection from the external carotid artery, the speed of the injection should be slow enough to prevent an antidromic flow of a drug solution through the common carotid artery.

In the present rat preparation *in situ*, the mode of action of each standard drug was almost similar to that elicited in the cat and rabbit preparation. Kayaalp and McIsaac examined the effect of various drugs on the rabbit superior cervical ganglion *in situ*.[7] They used hexamethonium, atropine, procaine, noradrenaline, nicotine, potassium chloride and other drugs. Their results as to the relation of doses and voltage changes of the action potential resembled our results obtained in the rat preparation. In regard to the sensitivity of the drug action, the cat preparation seems to be superior to that of the rat.

Hexamethonium exerted a prolonged inhibitory effect of more than 50% in an *i.a.* dose of 0.5 mg and an *i.v.* dose of 5 mg/kg, respectively. While atropine inhibited the action potential in an *i.a.* injection, it showed only a slight inhibition in an *i.v.* administration. Such a phenomenon has been observed in the test in rabbits. This fact indicates that an effective amount of atropine poorly reaches the ganglion if given systemically. As one explanation for this phenomenon, it can be assumed that an effective concentration in the blood is decreased through the transfer of the drug from the blood stream into many tissues or the rapid decomposition of the compound to an inert substance(s). Consequently, an *i.a.* application of drugs is preferable in order to examine its direct action on the ganglion *in situ*. On the other hand, atropine methylbromide displayed an inhibitory effect in both methods of injection, being similar to hexamethonium. This fact indicates the presumable easy reaching to the ganglion of systemically applied quarternary ammonium salts.

All indole alkaloids and synthetic compounds displayed only an inhibitory effect. Uncarine and isorhynchophylline have an oxyindole skeleton which is not present in other compounds. The inhibitory effect of these alkaloids was not potent and of showing a complete recovery. Rats were observed to move on the injection of both alkaloid. Hirsutine, 2,3-seco-yohimbine, I and V exerted a relatively strong inhibitory effect. Among these were I and V most effective, expressing a comparable potency with that of hexamethonium. V is characteristic as compared with other substances in that it is a quarternary ammonium salt, its action gradually proceeded and its potent maximal inhibition was obtained 10—15 min after the administration. β-Yohimbine, II and III all occasionally induced death even below a dose enough to exert an inhibitory effect. This indicates their higher toxicity by way of the *i.a.* application.

From these findings it is concluded that in an *in situ* preparation of the rat superior cervical ganglion, several indole compounds, either natural or synthetic, which exert a ganglion blocking effect comparable with that of hexamethonium were found among the tested compounds which are not a quarternary ammonium substance except V.

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