Pharmacological Studies on Diphenylalkylamine Derivatives. (II)*

On the Actions of *l*-1, 2-Diphenyl-1-dimethylaminoethane Hydrochloride (SPA)

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The present report describes the further pharmacological studies of SPA, in which an attempt was made to differentiate its action from that of morphine. Although the actions of SPA resemble those of morphine in many respects, some of its actions are very differerent.

INTRODUCTION

The comparative pharmacological studies of the optical isomers of 1, 2-diphenyl-1-dimethylaminoethane hydrochloride in the previous report[1] have shown that though they were almost alike in their peripheral actions, the *l*-isomer showed the strong central depressant, analgesic and anti-tussive actions which the *d*-isomer did not or was markedly weak. The detailed studies of the *l*-isomer are described in this report. The clinical studies of the isomer are now in progress.

EXPERIMENTAL METHODS

1. Electroencephalogram in Cat

The cerebral cortex of a cat, weighing 2.2 to 4.3 kg, was exposed under ether anesthesia and the dura was removed at the site of the insertion of the induction- and stimulation-electrode and then the spinal cord was sectioned between CI and CII (*Encéphale isolé*). The experiment was carried out 2 to 3 hours after the termination of the anesthesia. As the induction-electrode on the cortex a monopolar Ag-AgCl electrode of the diameter of 0.5 mm was used. As the stimulation-electrode for the thalamus and the reticular formation two dental broaches which were insulated by enamel except at the tips, and placed a distance of 2 mm apart, were used. The indifferent electrode was fixed on the bone near the site of the induction-electrode.

As an amplifier the Universal-type Recorder MPA-204, Sanei Sokuki Co. was

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used. The head of the animal was fixed on the Johnson's stereotaxic instrument and the sites of stimulation were determined according to the stereotaxic atlas of Jasper. All the experiments were carried out at the room temperature of 29 to 31°C. All the drugs used were injected intravenously.

2. Transport Movement in Mouse Intestine

Mice, 12 to 14 g of body weight (male and female mixed) were fasted for 4 hours before the experiment. Then the animals were given 0.2 ml/10 g of the charcoal emulsion orally with a stomach tube. The charcoal emulsion was composed of charcoal powder 1.0 g, gum traganth 0.3 g and water 20 g. The animals were killed 15, 30 and 60 minutes after being given the emulsion, and the whole intestine including the duodenum was excised. After removing the mesentery the intestine was placed on a glass plate and the extent of charcoal transport from the pylorus in a given time was measured. The rate of transport was determined as a percent value of transport distance to the whole length of the intestine.

Drugs were given subcutaneously 30 minutes before the administration of the charcoal emulsion. The same procedures were taken as those in the control animals. The effects of the drugs were determined as a percent value of the transport distance of the animal received the drug to the same distance of the control animal.

3. Endplate potential of the Saritorius Muscle of Toad

When the skeletal muscle had fully relaxed 30 minutes after the intracardial injection of 3.0 mg of *d*-tubocurarine chloride in the toad, the spinal cord was destroyed, and the sartorious muscle with the sciatic nerve was isolated and immersed in Ringer solution for 30 minutes. Ozawa's isolation method[2] was used to measure the endplate potential of the muscle. In some of the experiments the action potential of the muscle, which in fact because of the use of the non-curarized muscle was summated with the endplate potential, was obtained by using the same technique. The action potential of the innervated muscle was induced directly from the nerve. For the electrical stimulation of the sciatic nerve, square wave stimuli of 0.5 msec in duration were used. All the potentials after amplification by 4 steps of R-C combination (time constant 0.3 sec), were recorded on the cathode oscillograph.

The other methods used in each experiment are described in detail in the experimental results.

EXPERIMENTAL RESULT

1. Toxicity

a) Acute toxicity. i) Mice: The toxic symptoms and LD 50 in mice by the intraperitoneal injection are already reported. The toxic symptoms of the drug by the oral and subcutaneous routes were similar to those previously reported symptoms. The LD 50 of the drug differed considerably according to the different routes of administration. Table 1 shows the intraperitoneal, subcutaneous and oral toxicities as LD 50 in mice.

Table 1. Toxicity and analgesic action (Haffner method) of SPA

Method of Administration	LD50 mg/10g. (95% limits)	ED50 mg/10g., (95% limits)	
		Single	with Morphine
Intraperitoneal	1.32 (1.18-1.48)	0.35 (0.25-0.48)	0.11 (0.07-0.18)
Subcutaneous	2.10 (1.81-2.44)	0.42 (0.22-0.81)	—
Oral	4.40 (3.83-5.06)	—	0.70 (0.47-1.05)

ii) Rats: Rats, weighing about 100 g, responded to the intraperitoneal injection of the drug similarly as mice did. The LD 50 by the peritoneal route was 100 (84.8 ~118) mg/kg.

b) Prolonged administration of SPA. i) Rats (administration for 60 days): Three groups of the test rats were used, one for control, one for morphine and the other for SPA. Each group consisted of 20 male rats of WK strain with body weight of 100±10 g. The drugs were administered subcutaneously once a day for 60 days and the growth curves of the test animals were compared as figures expressed in average body weight gain. Meanwhile, the analgesic actions of morphine and SPA were assayed in these animals every 5 or 6 days by use of D'Amour-Smith method. This interval was enough to prevent the tissue damage of the tail by stimulation of the infrared ray. The failure to respond to the stimulus within 10 seconds was regarded as an analgesic sign induced by the drugs.

SPA was administered in a daily dose of 20 mg/kg throughout the experimental period, but the dose of morphine started from 10 mg/kg daily was increasd by 10 mg/kg every time the rats were found to be non-responsive to the former analgesic dose. The rats which received SPA usually showed increased spontaneous movements and some signs of excitation for 10 to 20 minutes after every administration. But no other remarkable toxic symptoms could be seen throughout the experimental period. The body weight curve gradually went upward and at the end of the experimental period the increase of the body weight of animals which received SPA was 106 g in average, while the increase of body weight of the control group was 110 g in average. The average final body weight in the SPA group was 213±5.4 g., and that of the control group 218±5.9 g. These results clearly show that there is no significant difference of body weight gain between the control and SPA groups. The responses of the animals which received morphine daily changed somewhat from about the seventh day of the administration. The animals showed signs of excitement for about five minutes and then turned to increased spontaneous movements for 20 to 30 minutes. Especially during the 10 th to 14 th days of the experiment they showed a violent grawing action after morphine. From the 14 th day of the administration the dose of morphine was increased to 20 mg/kg and thereafter two animals died on 27 th and 28 th days respectively. On the 36 th day, the dose of morphine was increased again to 30 mg/kg. There occured a distinct excitation which appeared 5 to 10 minutes after the administration, and was followed by a marked sedation. Some animals continued the foreleg-licking action even if the external stimuli had been applied. The other animals lay down in a drunken state and did not respond any external stimuli. Such a

toxic state usually lasted for 3 to 4 hours. At this state, the clamping of the limb of the animal caused the considerable rigor of the whole skeletal muscles and the spasm of the muscles made the body and tail of the animal horizontal. On the other hand the body weight of the animals which received morphine gradually increased and attained a gain of 100 g at 60 th day. Although the precise comparison of the body weight gain of the morphine treated animals was impossible because of the early deaths of some animals and because of two steps of increased dosages, it looked like the body weight gain of the morphine group was significantly smaller than that of the control group. On the other hand the changes of the analgesic responses to SPA and morphine during the prolonged administration are worth, mentioning. The number of animals which showed analgesic response to SPA increased from the initial 5% to 15% on 35 th day and 10% on 50 th day, while in the morphine group the number of animals which showed the analgesic response to the initial dose of 10 mg/kg of morphine was 50%, but annuled at 13 th day. When the dose of morphine was increased to 20 mg/kg on the 14 th day, the number of the responsive animals increased to 35%, but annuled again at 35 th day. The increase of the dose of morphine to 30 mg/kg from 36 th day was followed by 45% of the number of the responsive animals at 36 th day and this decreased continuously to about 5% at 60 th day. These results clearly show that the repeated injection of morphine could readily produce tolerance, while the injection of SPA was not likely to induce tolerance (Fig. 1).

ii) Dogs (administration for 30 days): Six adult dogs were divided into two groups, and one group was administered subcutaneously 20 mg/kg of SPA daily for 30 days and another group 5 mg/kg of morphine for the same period. During that time the general signs of the behaviour of the animal and the analgesic effect of the drug on the withdrawal reflex of the limb in response to the pinching of the toe-pad by an artery clamp were observed.

SPA group: No.1 (Female, 7.5 kg); During the early period of the administration there occurred a sedation which lasted for several hours, and after the 10 th day slight salivation and the dull movements in response to the injection of the drug were observed. However, the animal did not show a clear-cut excitation. Throughout the period the withdrawal reflex of the foot to painful stimulus remained unchanged and sometimes the animal barked at the stimulus without biting movement.

No. 2 (Male, 6.3 kg): The animal showed sedation for about 3 to 4 hours in response to every injection throughout the experiment. No other sign of toxicity was observed. The animal similarly behaved to the painful stimulus as in the case of No. 1.

No. 3 (Male, 7.0 kg): The animal showed the similar pattern of the signs as the No. 2 did. All three animals had almost no change of their body weights during the experimental period.

Morphine group: No. 4 (Female, 14 kg); Within 5 minutes after the injection of the drug defecation and vomiting followed by salivation and inactivity appeared, and the animal could not respond to any external stimuli. The withdrawal reflex of the foot to painful stimulus remained unchanged but the animal did not bark to the same stimulus. On 14 th day though the animal still vomited and salivated

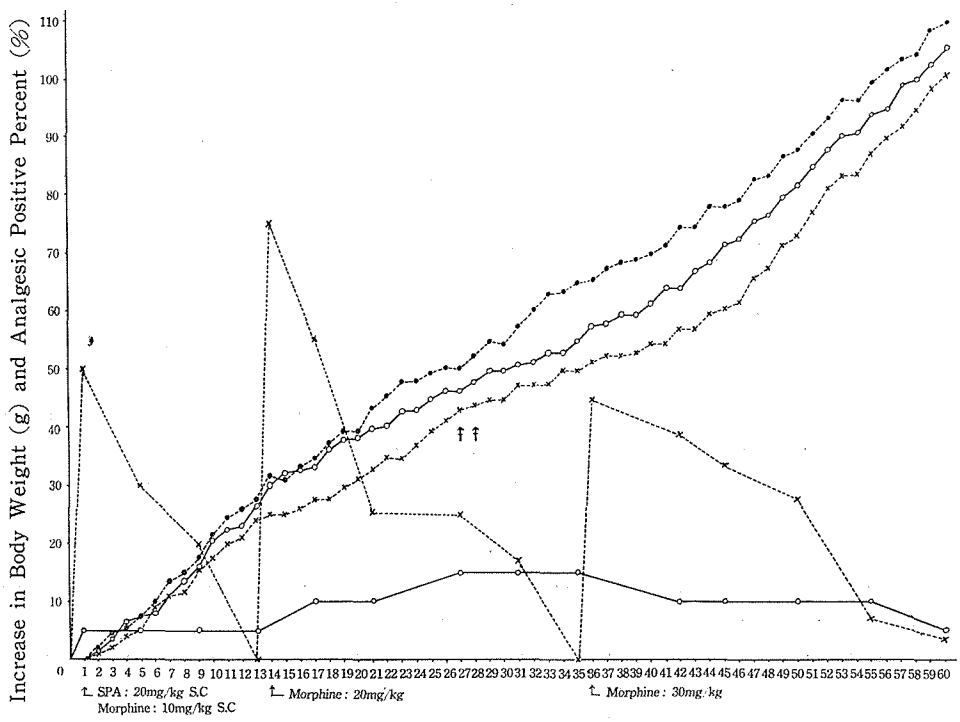

Fig. 1 Body weight and analgesic action curves by prolonged administration in rats
Spa ○-○-○, Morphine ×-×-×, Control ●-●-●
‡ indicates an animal died here in the morphine group. The ordinate indicates increase in body weight (g) as well as analgesic activity (%).

considerably in response to morphine, it showed some response to the external stimuli. From about the 20 th day of the experiment the animal became weak and dull and no longer vomited in response to morphine. On 30 th day the body weight had decreased to 9.5 kg.

No. 5 (Male, 7.0 kg) : From 4 th day vomiting and from 12 th day salivation disappeared. The withdrawal reflex of the foot to the painful stimulus was absent during the initial 15 days, but reappeared from the 20 th day. There were some dull movements followed by sedation. There was no decrease of body weight throughout the period. There were some signs which showed the developed tolerance to morphine.

No. 6 (Female, 8 kg) : The animal responded to morphine as No. 5 did.

2. Analgesic Action

a) Haffner method. The subcutaneous ED50 obtained by this method was almost the same as the intraperitoneal ED50. Although the peroral dose of SPA could not give an ED50, the peroral application of SPA together with a threshold dose of morphine gave the oral ED50 of SPA, which was 6 times larger than the intraperitoneal one (Table 1).

b) Stretching suppression method[3]. It has been reported that "stretching" of

the mouse caused by intraperitoneal injection of 0.1 ml/10 g of 0.6% acetic acid was inhibited by a number of analgesics and that the extent of the inhibition was in parallel to the degree of analgesia. By use of this method the 50% inhibition doses (ED50) of some drugs on the stretching response were estimated. The values were for SPA 7.5 (4.7 to 11.8) mg/kg, for morphine 0.5 (0.3 to 0.9) mg/kg, for codeine 5.0 (3.9 to 6.5) mg/kg, and for *d*-methamphetamine 0.09 (0.06 to 0.13) mg/kg (all subcutaneous dose). These results showed that the suppressing activity of SPA was almost equal to that of codeine, while the activity of *d*-methamphetamine was much stronger than that of morphine. The latter results, it is believed, are worth reexamination.

c) Prolongation of the jumping and fatality time of mice in higher temperature. According to the method of Mineshita *et al*[43]. the duration of the active jumping and the time until death of mice placed in a room at 42°C was prolonged by a number of analgesics. In this experiment the subcutaneous injection of 25 mg/kg of SPA prolonged both jumping and death times as much as the subcutaneous injection of 2.5 mg/kg of morphine did (Fig. 2).

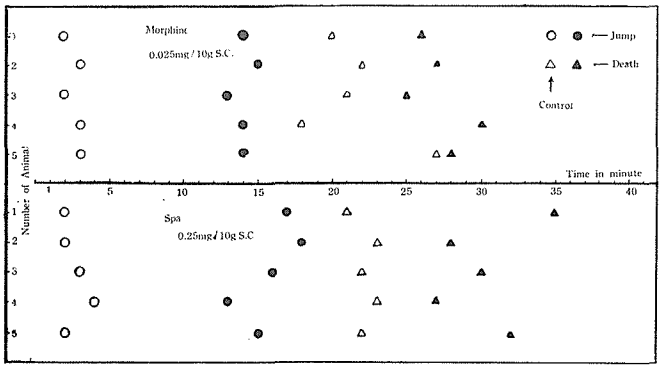

Fig. 2 Prolongations of jumping and fatality periods in mice at 42°C. An experimental group consists of 5 mice. ○ : Jumping appeared in the control group ; △ : Death took place in the control group ; ● : Jumping appeared in drug administered group ; ▲ : Death took place in drug administered groups.

3. Effects of SPA on EEG in Cat

a) Effects on the spontaneous EEG. The intravenous injection of 1.0 mg/kg of SPA did not affect the spontaneous EEG in cats, but several minutes after the administration of 2 to 3 mg/kg of the drug the spontaneous spindle bursts markedly decreased or disappeared completely, so that the EEG pattern shifted toward the low potential and fast waves. The EEG pattern in response to 4 mg/kg of SPA showed only the fast waves of low voltage which resembled to the so-called arousal pattern in awakening or intention,and lasted for several hours. The injection of 8 to 10 mg/kg of the drug caused disturbances of the EEG patterns which were caused by the twitching of the skeletal muscles. When the twitching of the muscle was abolished by the injection of 0.3 mg/kg of *d*-tubocurarine the EEG showed an arousal pattern. When 15 to 20 mg/kg of SPA was administered to the curarized animal

the EEG showed an immediate flattening of the pattern which turned to the arousal pattern within several minutes. The arousal pattern did not recover within 8 hours after the drug injection. Only the flattening of the EEG in response to the dose above 30 mg/kg of the drug did not turned to the arousal pattern. The *d*-isomer of SPA is weaker in this regard and a dose of 8 to 10 mg/kg was required to complete the abolition of the spindle bursts.

The administration of morphine in the dose of 2 mg/kg also decreased the spindle bursts and gave a pattern rich in fast wave component. Especially, in doses above 4 mg/kg the arousal pattern consisting of the fast waves of low voltage appeared. In doses of 30 to 35 mg/kg the slow wave patterns reappeared, but in 60 mg/kg of the drug the flattening of EEG appeared within few minutes after the administration. The flattened EEG turned to the slow wave pattern in 10 minutes. When 100 mg/kg of morphine was given only the flattening of EEG was observed (Fig. 3-A).

b) **Effects on the EEG arousal response.** The effects of SPA or morphine on the arousal responses of the EEG recorded from the cerebral cortex following repeated stimulation of the brain stem reticular formation could not be determined because of the shift of EEG pattern toward the fast wave of low voltage by the drug action (Fig. 3-B).

c) **Effects on the recruiting response.** The recruiting response recorded from the cerebral cortex following repeated stimulation of the thalamic diffusely projecting nuclei was slightly inhibited by 2 mg/kg of SPA. The same inhibition was more distinct in the dose of 4 to 5 mg/kg. In the dose of 8 to 10 mg/kg the same inhibition became much marked and in some experiments the recruiting response disappeared, and however, 10 minutes thereafter, the response reappeared. When 15mg/kg of SPA was injected, the recruiting response was blocked, but the application of the increased intensity of stimuli could evoked a slight response. Thereafter, the recovery of the recruiting response was parallel with that of the flattened EEG. The administration of 2 to 4 mg/kg of *d*-isomer of SPA potentiated somewhat the recruiting response induced by stimulation of low intensity, but had no effect on the same response induced by stimulation of the higher intensity. Ten to 15 mg/kg of *d*-isomer distinctly inhibited the same response, too. These results may show that the central action of *d*-isomer is weaker than that of *l*-isomer (SPA). On the other hand the administration of 2 mg/kg of morphine inhibited the recruiting response to some extent, and the inhibition by morphine was marked in the dose of 4 to 6 mg/kg. In the dose of 10 to 15 mg/kg the inhibition was very marked, yet an increased intensity of stimulation could reveal the response. In the dose of 100 mg/kg the recruiting response disappeared even if the intensity of the stimulation was maximal (Fig. 3-C).

4. Effects on the Body Temperatue in Rats

It has been shown that morphine raised the body temperature of rats[8]. The subcutaneous injecton of 5 to 20 mg/kg of SPA, however, did not affect the rectal temperature of rats. On the contrary the subcutaneous injection of morphine increased the rectal temperature about 0.8 to 1.2°C preceded by a slight decrease (Fig. 4).

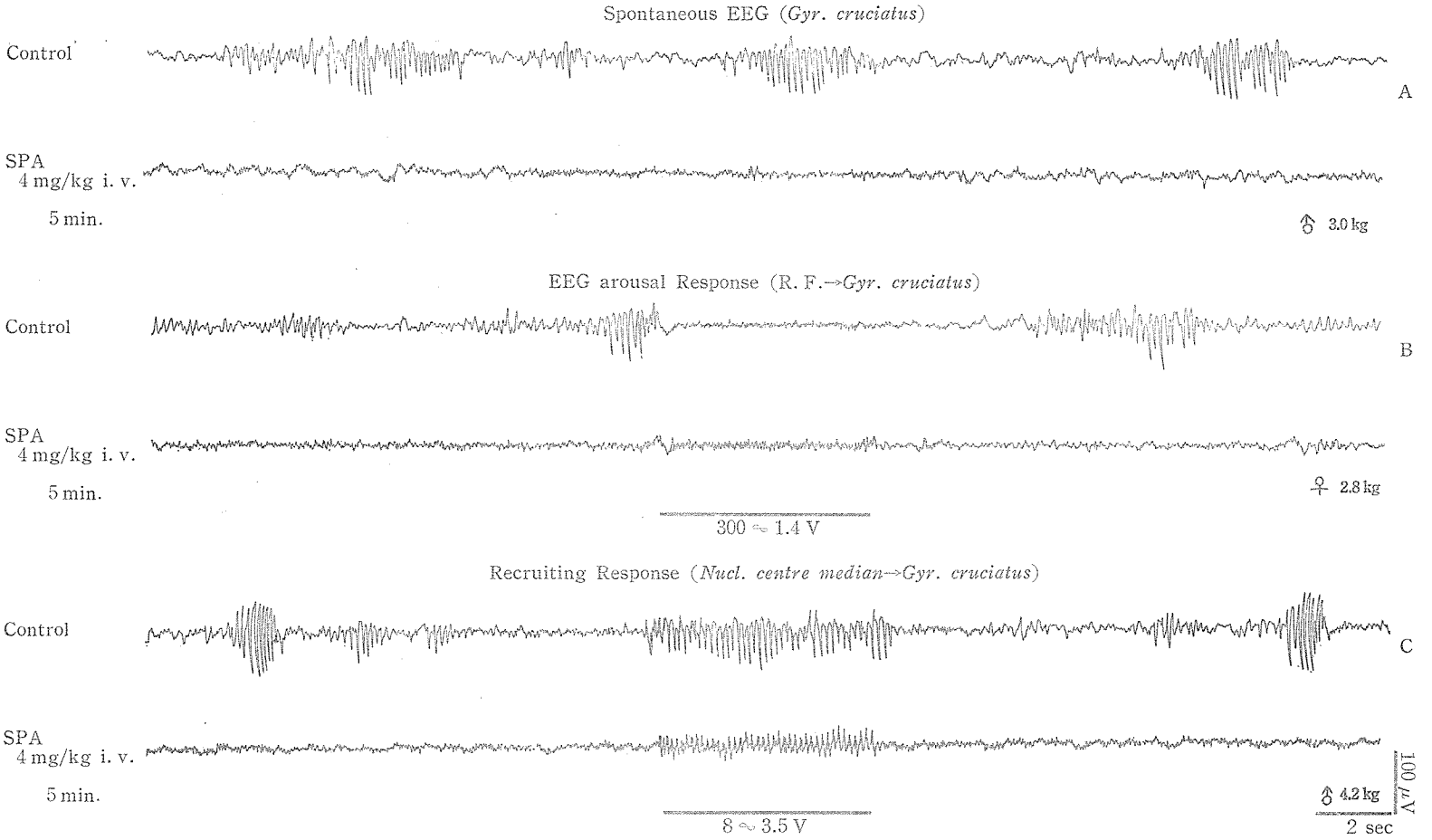

Fig. 3 Effects of spa on the E.E.G. in cats (Encéphale isolé preparation).

A : Effects of spontaneous E.E.G (Cerebral cortex).

B : Effects on the arousal response in E.E.G. recorded from the cerebral cortex by repeated stimulation of the brain stem reticular formation (F+0.2, L+3.0, H−1.0).

C : Effects on the recruiting response recorded from the cerebral cortex by repeated stimulation of the thalamic diffusely projecting nuclei.

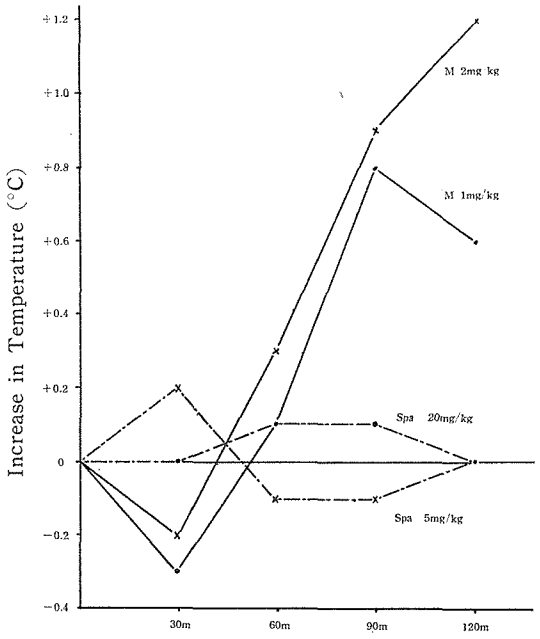

Fig. 4 Effect of morphine (M) and spa on the rectal temperature of rats.

5. Effects on the Convulsive Death of Mice by CNS Stimulants and Electroshock

The intraperitoneal administration of 20 mg/kg of SPA did not affect either the convulsions or the convulsive death induced by the intraperitoneal injections of 50 mg/kg of metrazol, 0.5 mg/kg of strychnine and 200 mg/kg of coramine. Though the same dose of SPA could not prevent the extensor tetanus induced by the electroshock, some reduction of the mortality of the animal was obtained.

6. Effects on the Pupil of Rabbit

The subcutaneous injection of the doses above 10 mg/kg of SPA always caused a mydriasis. Though the extent of the mydriasis depended on the dose applied, the peak effect was usually obtained 30 minutes after the injection. On the contrary the injection of morphine above the dose of 5 mg/kg always caused a distinct miosis (Fig. 5).

7. Effects on the Blood Pressure

The effects of the intravenous injection of SPA on the carotid artery blood pressure in the anethetized dog and cat, and spinal cat were already described in the previous report.

a) The intravenous injection of 1 mg/kg of SPA did not affect the pressor response to stimulation of the splanchnic nerve in dog. b) Neither the pressor response to the intravenous injection of 1 m micro g/kg of adrenaline nor the depressor response to the same procedure of 0.5 micro g/kg of acetylcholine was affected by the intravenous dose of 1 mg/kg of SPA in dog. c) In the cat or dog neither the depressor response to stimulation of the peripheral end of the cervical

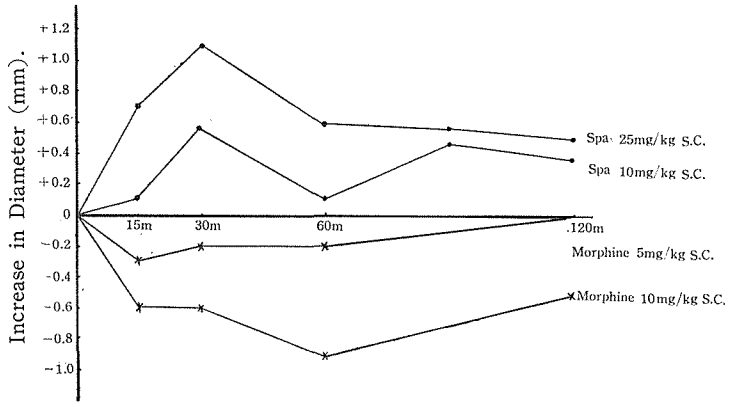

Fig. 5 Effects on the rabbit pupil.

An experimental group consisted of 3 male rabbits of body weights of 2.1-2.3 kg.; the average diameter of the pupils before the drug administration was 6.8 mm±1.07.

vagus nerve not the pressor response to stimulation of the central end of the same nerve was affected by the intravenous dose of 1 to 2 mg/kg of SPA.

8. Effects on the Nictitating Membrane of Cat

The effects of the injection of SPA on the nictitating membrane of the cat anesthetized with amytal sodium.

a) The injection of 0.1 to 1 mg/kg of SPA caused no change or only a slight contraction of the normal nictitating membrane.

b) The contraction of the normal membrane to stimulation of the preganglionic fiber of the cervical sympathetic nerve was not affected by the injection of 1 to 2 mg/kg of SPA.

c) The contraction of the normal and the denervated membrane to adrenaline or noradrenaline was not affected by 1 mg/kg of SPA.

9. Effects on the Salivary Secretion in Dog

According to the method of Inoue[5] the cannula was inserted to the Wharton duct of the amytal sodium anesthetized dog and the effect of the intravenous injection of SPA on the salivary secretion induced by the electrical stimulation of

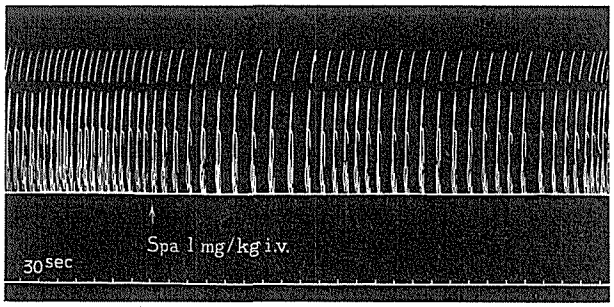

Fig. 6 Effects in the salivation under Stimulation on the tympanum cord
Male amytal-anesthesized dog with body weight of 6 kg. Electric stimulation: frequency 10/sec., duration 1 msec.

the chorda tympani was studied. The injection of 1 mg/kg of SPA slightly inhibited the secretion for approximately 10 minutes. (Fig. 6)

10. Effects on the Foot Oedema of Rats (Antiphlogistic Effect)

According to the method of Shimamoto and Kanemitsu[6] the effects of the previous subcutaneous injection of SPA (30 minutes before the injection of the phlogistic agents) in the dose of 50 mg/kg on the foot oedema induced by the injection of 0.1 ml of 0.75% formalin, 0.05 ml of 6% dextran or 0.1 ml of 10% egg-white into the foot pad of the intact rat were tested. The results revealed that SPA considerably inhibited the oedema formation by formaline and eggwhite, but was almost without effect on the oedema by dextran. The extent of the inhibition obtained by 50 mg/kg of SPA was comparable to that obtained by 50 mg/kg of aminopyrine or 10 mg/kg of morphine, as are shown in Fig. 7.

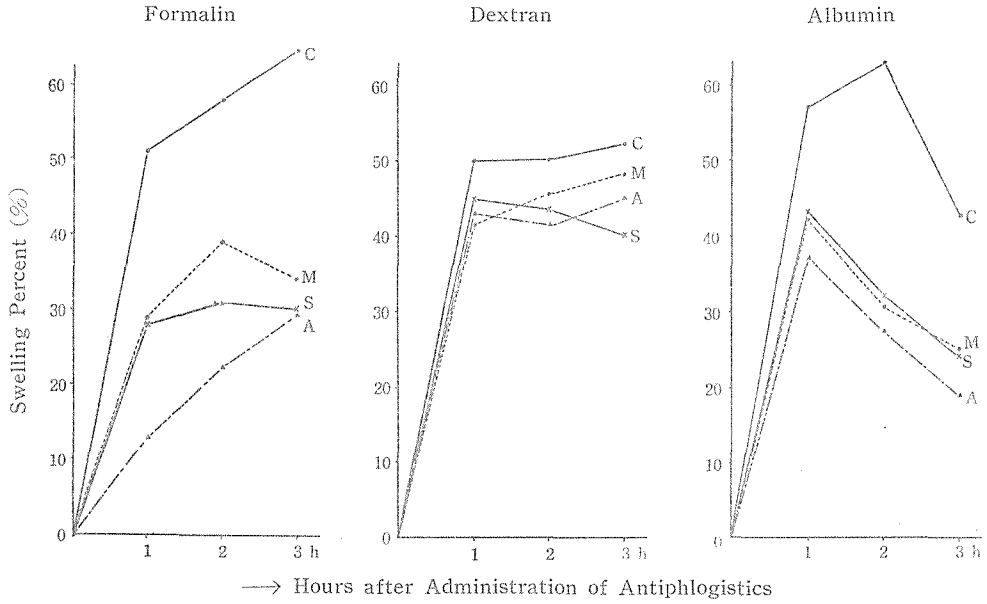

Fig. 7 Inhibiting action on the swelling of rat hind paw (Antiphlogistic action).
C : Control ; S : Spa 50 mg/kg. s. c. ; M : morphine 10 mg/kg. s. c. ;
A : Aminopyrin 50 mg/kg. s. c.

11. Effect on the Transport Movement in Mouse Intestine

The subcutaneous injection of 20 to 30 mg/kg of SPA had no effect on the transport movement of the charcoal in the intestine of mouse. The administration of 0.4 mg/kg significantly inhibited the transport of the charcoal. In contrast to SPA the administration of 2.5 mg/kg of morphine and 20 mg/kg of codeine significantly decreased the transport movements of the charcoal (Fig. 8).

12. Effects on the Neuromuscular Junction

a) Sciatic-gastrocnemius preparation of rats

According to the method of Inoue[5] the sciatic-gastrocnemius preparation of the rat, anesthetized with 1 g/kg of urethane was used. The intravenous injections of 1 to 10 mg/kg of SPA were almost without effect on the contraction of the muscle

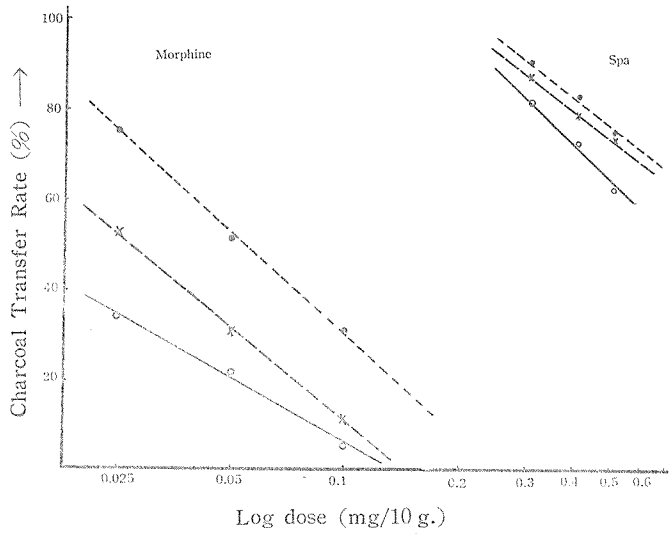

Fig. 8 Effects on the transfer movement of mouse intestine.

○-○ Transfer rate 15 minutes after charcoal powder administration; ×-× transfer rate 30 minutes after; ◍-◍ transfer rate 60 minutes after.

to indirect stimulation. But the injection of the doses above 10 mg/kg potentiated the contraction. Though the *d*-isomer showed a similar action, it was much weaker than SPA. The injection of 5 to 10 mg/kg of SPA was without effect on the curarizing action of 30 to 50 micro g/kg of *d*-tubocurarine, given intravenously.

b) Effects on the contraction of the frog's rectus abdominis muscle by acetylcholine

The contraction of the extirpated rectus abdominis muscle of frog by 10^{-6} of acetylcholine was inhibited about 50% by the previous treatment of the preparation with 10^{-5} of SPA. Morphine did not show any effect on the same contraction of the muscle. As is shown in fig. 9, the comparisons of 50% inhibition concentration of *d*-tubocurarine, cocaine and xylocaine on the contraction of the muscle by 10^{-6} of acetylcholine showed that SPA was weaker than *d*-tubocurarine (2×10^{-7}) and nupercaine (5×10^{-6}) and was stronger than cocaine (2×10^{-5}) and xylocaine (8×10^{-5}).

c) Sciatic-sartorius preparation of toad

i) Endplate potential: The application of SPA in the concentration of 10^{-4} markebly depressed the endplate potential and blocked it 30 minutes after the application of 10^{-3}. The applicaiton of morphine or xylocaine was without effect in the concentration of 2×10^{-4} and depressed the potential above 10^{-4} (Fig. 10).

ii) Action potential of the muscle: The application of SPA in the concentration of 10^{-4} depressed the action potential. Morphine and xylocaine depressed the potential above the concentration of 2×10^{-4} (Fig. 11).

iii) Action potential of the nerve: The application of SPA in the concentration of 10^{-3} was without effect on the nerve action potential, whereas the application of the same concentration of xylocaine markedly depressed it (Fig. 12).

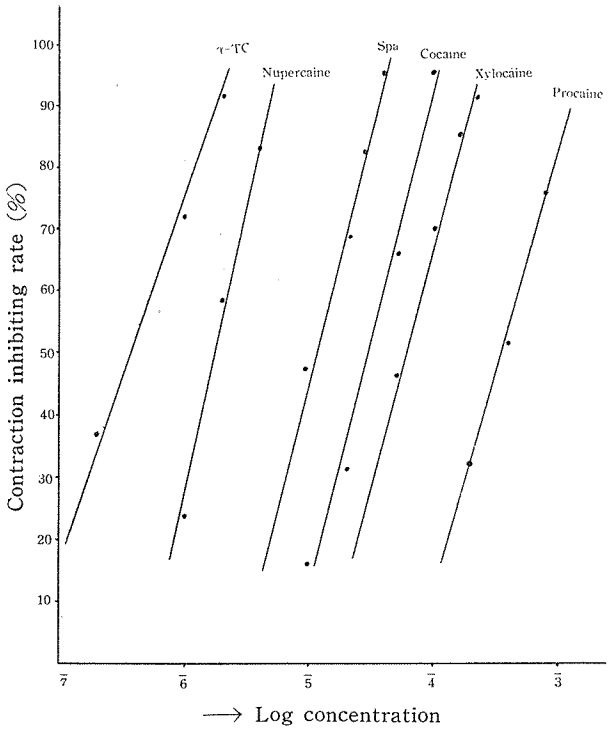

Fig. 9 Inhibiting action on the contraction of the isolated rectus abdominis muscle of frog by acetylcholine.

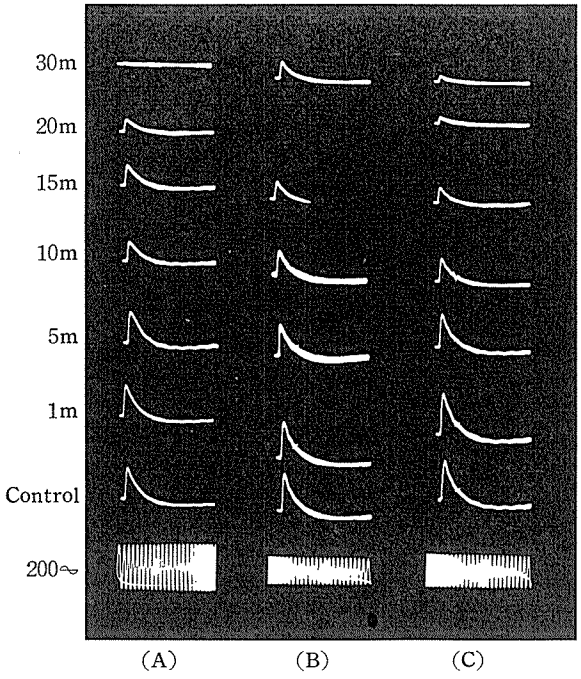

Fig. 10 Effects of drugs on the end plate potential.
(A) Spa (10^{-3}), (B) Morphine (10^{-3}), (C) Xylocain(10^{-3}).

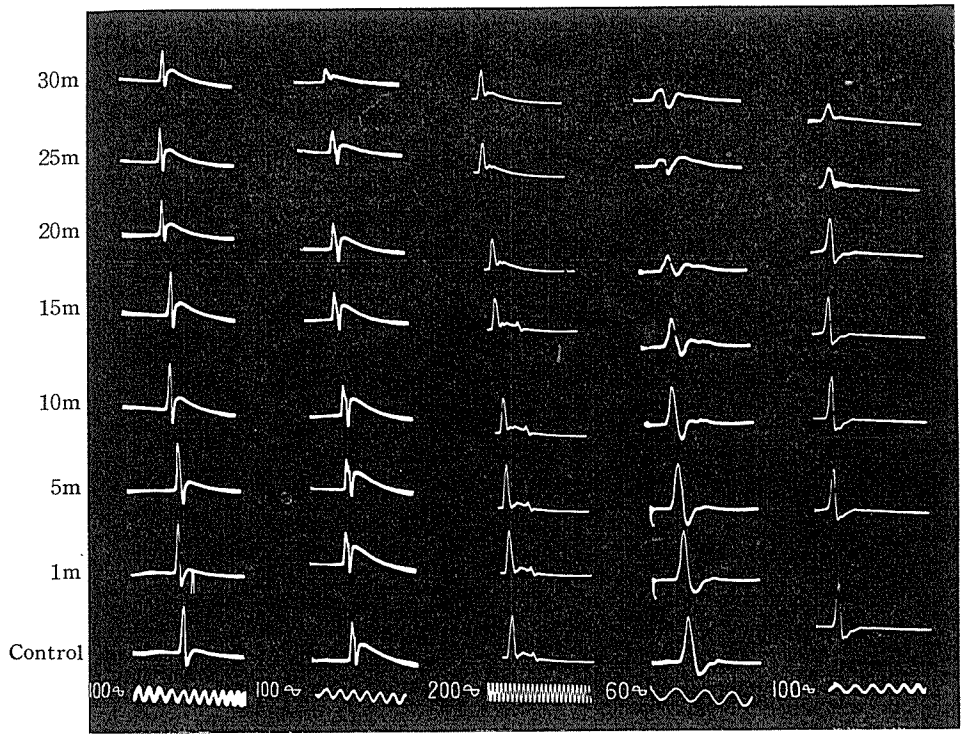

Fig. 11 Effects of drugs on the muscle action potential.
(A) Spa(10^{-4}), (B) Spa (2×10^{-4}, the same concentration for the following), (C) Morphine, (D) Xylocain, (E) Procaine.

DISCUSSION AND SUMMARY

The results in the present report are summarized and are discussed together with the results obtained in the previous experiment.

1) Toxicity: The subcutaneous LD50 in mice was 210 (181～244) mg/kg, the oral LD50 in mice 440 (383～506) mg/kg, and the intraperitoneal LD50 in rats 100 (84.8～118) mg/kg. The toxic symptoms of SPA were closely related in rats and mice by several routes of the administration. The LD50 was almost alike in rat and mouse except of oral route.

The prolonged subcutaneous administration of 20 mg/kg of SPA a day for 60 days did not cause any pathological signs and the average body weight gain did not show a significant difference. The analgesic tests of SPA during the prolonged administration period shows that the tolerance of SPA action did not develop. Though the daily administration of 5 mg/kg of morphine for 30 days caused a severe loss of body weight, the same procedure with 20 mg/kg of SPA did not cause a marked change in the dog. These results and the results obtained in the previous report show that the general toxicity of SPA in rats is comparatively low.

2) Analgesic action: The analgesic action of SPA in mice by use of Haffner method was almost the same in potency (ED50, 42 (22～81 mg/kg s. c.) by the subcutaneous or the intraperitoneal route of administration. The inhibitory potency

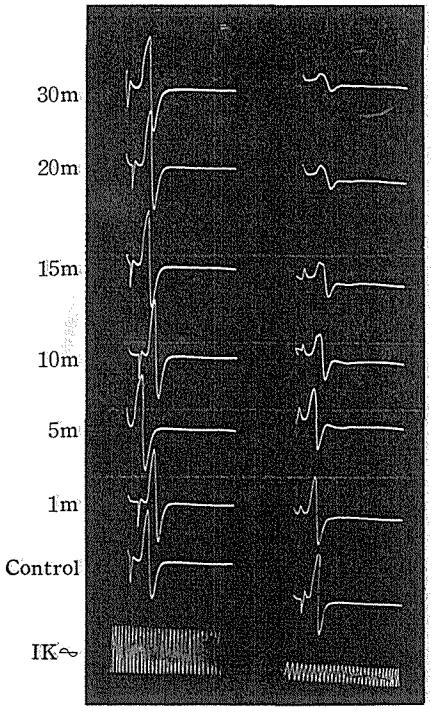

Fig. 12 Effects on the nerve action potential.
(A) Spa (10^{-3}), (B) Xylocain (10^{-3}).

of SPA on the "stretching" response of mice to the injection of 0.1 ml of 0.6% acetic acid was approximately that of codeine. The prolongation of the jumping and lethal times of mice in high temperature by 25 mg/kg of SPA was equivalent to that obtained by 2.5 mg/kg of morphine.

The fact that SPA showed almost the same ED50 of analgesia via the subcutaneous or intraperitoneal route suggests that the compound is quite stable in the body. This is a marked difference from morphine. It is worth mentioning that morphine and the other analgesic drugs were more sensitive to D'Amour Smith method than to Haffner method, while the circumstance was reversed to SPA. Though the reliability of the stretching response or the jumping and letheal time in high temperature as an evaluation method of the analgesic drug has not been established, the results in these experiments showed the method to have some usefulness.

3) Electroencephalogram: The effect of SPA on the spontaneous EEG, the EEG arousal response induced by stimulation of the brain stem reticular formation, and the recruiting response induced by stimulation of the thalamic diffusely projecting nuclei were studied in the encéphale isolé preparation of cats. SPA above the doses of 2 to 4 mg/kg reduced or almost eliminated the spindle bursts and induced low voltage fast waves. The effect of SPA on the EEG arousal response was difficult to recognize because of the marked arousal pattern by the drug, but the administration of SPA above the doses of 4 to 5 mg/kg distinctly inhibited the

recruiting response.

The effects of SPA on the EEG so far obtained were quite similar to those of morphine. However, the present experiments were not sufficient to determine or to compare the sites of action of morphine and SPA. In reference, Morita et al[7]. have shown that the intravenous injection of SPA in the dose of 2 mg/kg caused the low voltage fast waves and the same procedure of the higher doses caused a convulsive wave in the EEG of the intact rabbit.

4) The subcutaneous injection of 5 to 20 mg/kg of SPA had no effect on the rectal temperature of the rat. The same procedure of 1 to 2 mg/kg of morphine increased it about 0.8 to 1.2°C preceded by a transient fall. The responses of the rectal temperature of the rat to morphine and SPA seems somewhat different from those of the mouse.

5) The administration of SPA in mice had no preventive effect on the convulsive death by the CNS stimulants or the electroshock. The results are reasonable because SPA in toxic doses induced convulsions by itself.

6) The pupil of rabbit was dilated by the intravenous injection of 20 mg/kg of SPA. This result was a sharp contrast to the effect of morphine in which a marked constriction of the pupil was observed.

7) The changes of the blood pressure in the dog or the cat in response to stimulation of the cervical vagus or the splanchnic nerves, and to intravenous injection of acetylcholine or adrenaline were not affected by 1 to 2 mg/kg of SPA. It was reported in the previous paper that though the administration of SPA caused a slight persistent rise of the blood pressure in the anesthetized dog and cat, the same procedure in the spinal cat caused a transient decrease. It is believed that some parts of the pressor response to the drug are central in origin, and that other parts are peripheral in origin. It has weak vasoconstrictive action on the perfused ear vessels of rabbit.

8) The administration of SPA had no effect on the contractions of the normal and sympathetically denervated nictitating membrane of cat in response to adrenaline, noradrenaline and to stimulation of the cervical sympathetic nerve.

9) The salivary response of the submaxillary gland in response to stimulation of the chorda tympani was slightly inhibited by the administration of 1 mg/kg of SPA. But as was described above, during the prolonged administration of SPA some of the dogs showed a profuse salivary secretion in response to the daily dose of 20 mg/kg.

10) The foot oedema induced by the injection of formalin or eggwhite was considerably inhibited by SPA, but the same oedema induced by the injection of dextran was slightly affected. The inhibition obtained by 50 mg/kg of SPA corresponded to that obtained by the same dose of aminopyrine or 10 mg/kg of morphine. Since SPA had no marked effects on the blood pressure and the body temperature, the mechanism of the antiphlogistic effect appears to arise the peripheral origin, but rather from the central origin.

11) The inhibitory effect of SPA on the transport movement of the charcoal in the intestine of mice was very weak and the distinct inhibition was obtained above the doses of 40 mg/kg. Green[9] showed that the inhibition of the transport

of the charcoal in the intestine of rat by the analgesic drugs was in parallel with the analgeic petencies of the drugs. In these experiments morphine and codeine showed a markedly strong inhibition. The results in the previous report that though SPA had no marked effect on the blood glucose of rabbit, morphine greatly affected it corresponded to the results in this experiment.

12) The higher concentration of SPA inhibited the contraction of the rectus abdominis muscle of the frog induced by the application of acetylcholine. In the sciatic-sartorius preparation of the toad the higher concentration of SPA depressed the endplate potential and muscle action potential, but not the nerve action potential.

From the clinical use of SPA comes the question of whether the drug acts on skeletal muscle in a manner similar to the local anesthetics. The inhibitory effect of SPA on the contration of the rectus abdominis muscle of frog was stronger than those of the local anesthetics including cocaine, xylocaine and procaine. The depressive effect on the endplate potential, and the muscle action potential of the sartorius muscle of toad was also stronger than those of the local anesthetic agents. But SPA lacked the effect on the nerve action potential whereas xylocaine greatly depressed it.

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REFERENCES

(1) H. Fujimura and K. Kawai, *Folia pharmacol. Japon.*, **56**, 514 (1960).
(2) S. Shibata, T. Fujimoto and M. Yasuda, *Ibid*, **55**, 683 (1959).
(3) R. Koster *et al*, *Fed. Proc.*, **18**, 412 (1959) ; H. Fujimura, Abstracts 18th Kinki Meeting, Japan. Pharmacol. Soc.
(4) T. Mineshita, K. Hirose and T. Oota, *Shionogi Kenkyusho Nempo*, **5**,196 (1955).
(5) K. Inoue, *Folia pharmacol. japon.*, **53**, 797 (1956).
(6) K. Simamoto and O. Kanamitsu, *Ibid.*, **56**, 575 (1960).
(7) M. Morita and M. Yasuhara, *Ibid.*, **56**, 3§ (1960).
(8) J. B. Herrmann, *J. Pharmacol.*, 76, 309 (1942).
(9) A. F. Green, *Brit. J. Pharmacol.*, **14**, 26 (1959).