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Some Observations on the Cardiovascular Effects of 9-Substituted Berberines

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A comparative study of the effect of berberine and its 9-substituted compounds (I, R=OH; II, berberine, R=OCH_3; III, R=OC_4H_9; IV, R=OC_5H_{11}; V, R=OC_8H_{17}; VI, R=$OC_{12}H_{25}$) on blood pressure of rats showed that substitution of OC_4H_9 or OC_5H_{11} for OCH_3 of berberine prolonged the transient hypotensive activity of berberine, whereas substitution by OH caused a slight hypertension.

The dose of III or IV, which caused hypotension, was found to inhibit cardiac function in rats, whereas II had no effect on it. II, III, and IV decreased the beating rate in isolated perfused toad heart. Only II was found to have acetylcholine-potentiating action in frog muscle as well as guinea-pig small intestine *in vitro*. In the same preparations III and IV, contrariwise, inhibited acetylcholine action. In spinal rats, any response in blood pressure was not observed after the injection of each of the test compounds. From these results, possible mechanism of action of the test compounds is discussed.

The transient hypotensive action of berberine, an active principle of *Coptis japonica* and various species of berberis plants, has been noticed, although the mechanism of its action is not well dissolved. The present study was undertaken to investigate the cardiovascular action of berberine and its 9-substituted compounds, and to clarify the relationship between 9-substitution in berberine molecule and the cardiovascular action.

Materials and Methods

The chemical structures of the test compounds are shown in Chart 1. Each compound was dissolved in isotonic glucose solution, as it was precipitated in Ringer's solution. Drugs used were acetylcholine chloride (Ovisot, Daiichi Seiyaku), hexamethonium bromide (Methobromin, Yamanouchi Seiyaku), atropine sulfate (Wako Pure Chem.), and barium chloride (Wako Pure Chem.).

Toxicity Test——Male mice of dd-strain (Shizuoka-farm) weighing from 15 to 25 g were used. Animals were injected with each of test compounds (2.5—30 mg/kg) intraperitoneally, and lodged in an observation cage. At least three animals were used for each dose.

Cardiovascular Experiments in Rats——Male rats of Wistar strain (Nippon Rat Co.) weighing from 150 to 250 g were anesthetized with urethane (1.2 g/kg, *i.p.*). Each of the compounds was administered into femoral vein *via* a polyethylene tube. a) Blood Pressure and Respiration: Arterial blood pressure in carotid artery was recorded by means of a mercuric manometer and a kymograph. Respiration was simultaneously recorded by means of a Marey's tambour. In some cases, spinalization was performed by in-

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serting a pin between the first cervical vertebra and os occipitale and destructing the brain, and respiration was maintained with an artificial respirator connected to a tracheal cannula.

b) Electrocardiogram and Blood Pressure: Electrocardiogram corresponding to human limb lead II was displayed on a Nihonkohden ink writing oscillograph (WI–180). Arterial blood pressure in carotid artery was simultaneously measured by means of a transducer (MP–4T).

Cardiovascular Experiments in Toads——a) Isolated, Perfused Heart: Heart of *Bufo vulgaris* was perfused according to Yagi's method.[2] Each of the compounds was administered directly into the perfusion cannula. Rate and amplitude of contractions were recorded by means of an isotonic lever and a kymograph.

b) Perfused Hind Limb Preparation: Perfused hind limb preparation of *Bufo vulgaris* was made according to Laewen–Trendelenburg's method.[3] The peripheral vascular resistance was measured by counting the number of Ringer's solution drops flowing out from cannula inserted into v. abdominalis. Each of the compounds was injected into a. abdominalis *via* a perfusion cannula.

Skeletal and Smooth Muscle Preparations——a) Skeletal Muscle: A strip of m. rectus abdominis of Rana nigromaculata was suspended in a 10 ml organ bath filled with amphibian Ringer's solution gassed with air. Movements of the muscle were recorded by means of an isotonic lever and a kymograph. After a constant control response to acetylcholine had been obtained, each experiment was performed. Acetylcholine was given to the preparation which had been exposed to each of the compounds for 5 min.

b) Smooth Muscle: A strip of male guinea-pig intestine about 3 cm long was suspended in a 30 ml organ bath filled with Tyrode's solution gassed with air. After a constant control response to acetylcholine had been obtained, each experiment was performed. Acetylcholine was given to the preparation which had been exposed to each of the compounds for 5 min. For testing anti-barium action, each of the test compounds was given after each contraction induced by barium chloride had reached a plateau.

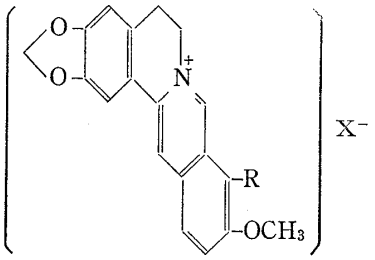

Chart 1

Compound	R	X
I	OH	Cl
II (berberine hydrochloride)	OCH_3	Cl
III	O-*n*-C_4H_9	Br
IV	O-*n*-C_5H_{11}	Br
V	O-*n*-C_8H_{17}	Br
VI	O-*n*-$C_{12}H_{25}$	Br

Results

Toxicity Test

The following is the commonly observed behavioural change of at least three mice after each test compounds. I (30 mg/kg, *i.p.*) inhibited spontaneous movement of mice for 10 min after the injection. Other signs were not observed. II (30 mg/kg, *i.p.*) soon inhibited spontaneous movement and respiration, and produced piloelection. Animals died 30 min after the injection. Administration of either III or IV (10 mg/kg, *i.p.*) inhibited spontaneous movement and respiration, and produced hypothermia and piloelection. Animals died next day. V and VI (2.5 mg/kg, *i.p.*) did not show any change in behaviour of mice.

Cardiovascular Experiments in Rats

a) Effects on Blood Pressure and Respiration——Arterial blood pressure after each suitable dose of the test compounds in anesthetized rats and the averaged change of blood pressure of at least four animals after various doses of each compound are shown in Fig. 1 and Fig. 2, respectively. I (R=OH) (2.5×10^{-6} mole/kg) caused a transient hypertension, whereas II (R=OCH_3, berberine) (2.5×10^{-6} mole/kg) caused a transient but marked hypotension which returned to the original level within 5 min, the minimum dose of II required to cause hypotension being 3.1×10^{-7} mole/kg (about 0.1 mg/kg). III and IV caused a prolonged hypotension in doses ranging from 3.1×10^{-7} to 2.5×10^{-6} mole/kg. V (6.2×10^{-7}

2) K. Takagi and H. Ozawa, "Yakubutsugaku Jikken," 5th ed., Nanzando, Tokyo, 1969, pp. 118—121.
3) P. Trendelenburg, *Arch. Exptl. Path. Pharmakol.*, **63**, 161 (1910).

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mole/kg) or VI (3.1×10^{-7} mole/kg) did not show any significant effect. In each case, primary effect on respiration was not observed.

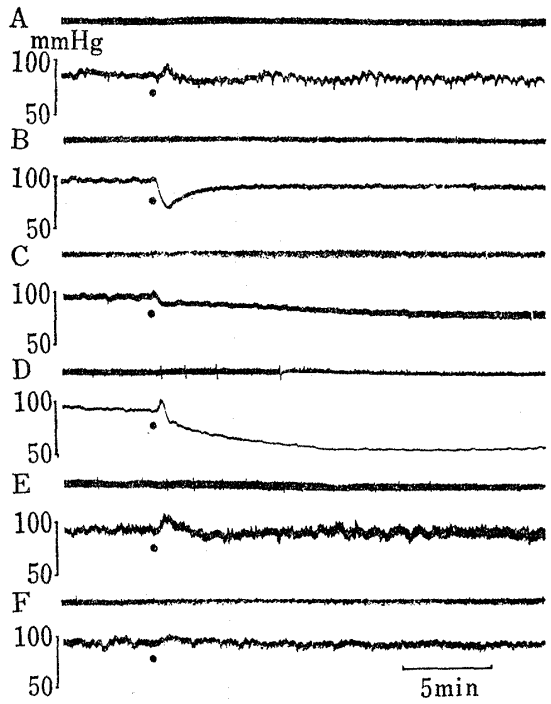

Fig. 1. Arterial Blood Pressure recorded from Anesthetized Rats weighing between 150 and 250 g, before and after Intravenous Injection of Each Suitable Dose of 9-Substituted Berberines

Each compound was administered at the point indicated by the dot.
A: I, 2.5×10^{-6} mole/kg; B: II, 2.5×10^{-6} mole/kg,
C: III, 2.5×10^{-6} mole/kg; D: IV, 2.5×10^{-6} mole/kg;
E: V, 6.2×10^{-7} mole/kg; F: VI, 3.1×10^{-7} mole/kg

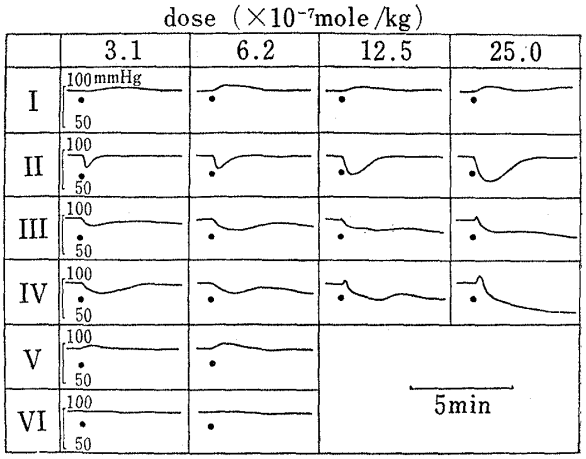

Fig. 2. Typified Changes in Arterial Blood Pressure recorded from Anesthetized Rats, after Intravenous Injection of Various Doses of 9-Substituted Berberines

Each response is the mean for at least four animals.

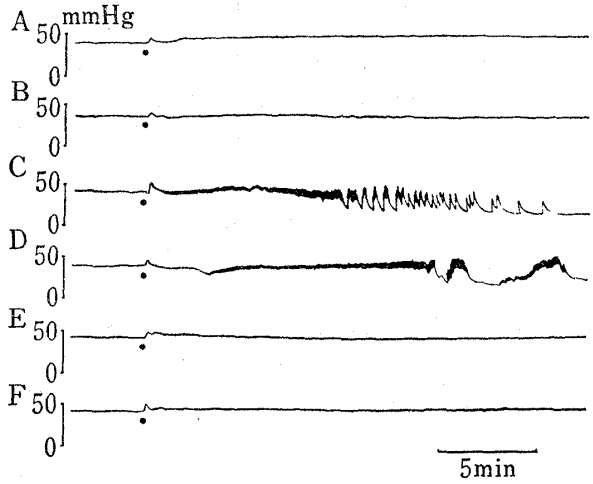

Fig. 3. Arterial Blood Pressure recorded from Spinal Rats, before and after Intravenous Injection of Each Suitable Dose of 9-Substituted Berberines

A: I, 2.5×10^{-6} mole/kg; B: II, 2.5×10^{-6} mole/kg;
C: III, 6.2×10^{-7} mole/kg; D: IV, 2.5×10^{-6} mole/kg;
E: V, 3.1×10^{-7} mole/kg; F: VI, 3.1×10^{-7} mole/kg

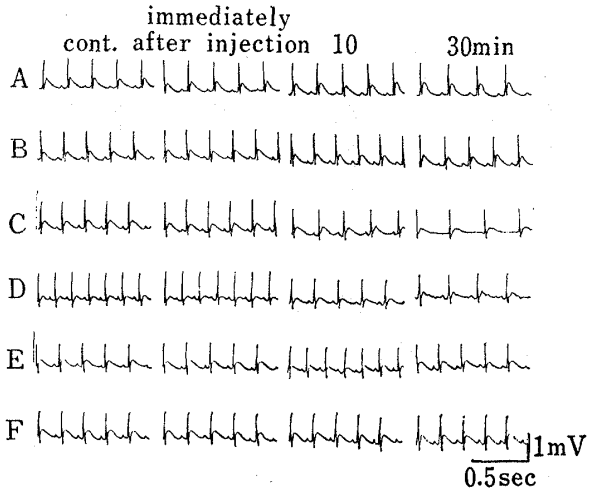

Fig. 4. Lead II Electrocardiograms recorded from Anesthetized Rats, before and after Intravenous Injections of 9-Substituted Berberines

A: I, 2.5×10^{-6} mole/kg; B: II, 2.5×10^{-6} mole/kg;
C: III, 2.5×10^{-6} mole/kg; D: IV, 2.5×10^{-6} mole/kg;
E: V, 6.2×10^{-7} mole/kg; F: VI, 3.1×10^{-7} mole/kg

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I or II was given to rats pretreated with atropine sulfate (2.0 and 6.0 mg/kg, *i.v.*) or hexamethonium bromide (5.0 mg/kg, *i.v.*). The response to I or II was not influenced by the prior administration of these drugs.

To examine whether or not the effect of the test compounds on blood pressure was dependent upon the drug action at brain, experiments were done on spinal rats. As shown in Fig. 3, hypotensive response was not observed.

b) Effects on Blood Pressure and Electrocardiogram——Results are shown in Fig. 4. Intravenous injection of 2.5×10^{-6} mole/kg of I, a sufficient dose to cause a slight rise of blood pressure, produced no or only a slight change in lead II electrocardiographic pattern. Namely, in some experiments, the dose of I increased the height of QRS complex. II (2.5×10^{-6} mole/kg, hypotensive dose) produced no change in ECG pattern. After a dose of III or IV (2.5×10^{-6} mole/kg, *i.v.*), which caused a sustained fall of blood pressure, amplitude of QRS complex and Q–Q interval decreased (Fig. 4), and in course of time disappearance of P wave and deformation of T wave were sometimes observed. At the early stage when the fall of blood pressure was caused by III or IV, however, any change was not observed in ECG.

Cardiovascullar Experiments in Toads

a) Effects on Isolated, Perfused Heart——In control experiments, infusion of isotonic glucose solution did not change heart rate and amplitude of contraction. Effects of the test compounds are shown in Fig. 5. Perfusion of I (final concentration of 1.6×10^{-4}M) resulted in an increase in rate and amplitude. A concentration of 6.4×10^{-4}M caused an arrhythmia,

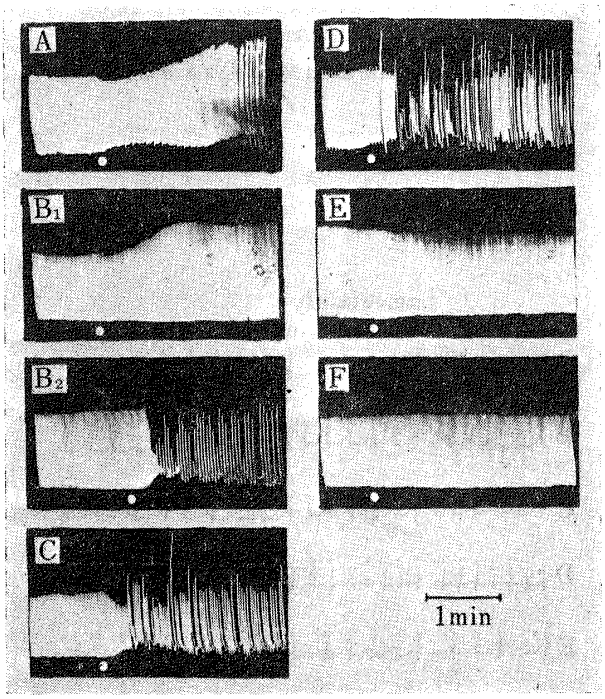

Fig. 5. Contractions recorded from Isolated Perfused Heart of *Bufo vulgaris*, before and after Administration of 9-Substituted Berberines

A: I, 6.4×10^{-4}M; B-1: II, 4.0×10^{-5}M;
B-2: II, 1.28×10^{-3}M; C: III, 1.6×10^{-4}M;
D: IV, 1.6×10^{-4}M; E: V, 8.0×10^{-5}M;
F: VI, 4.0×10^{-5}M

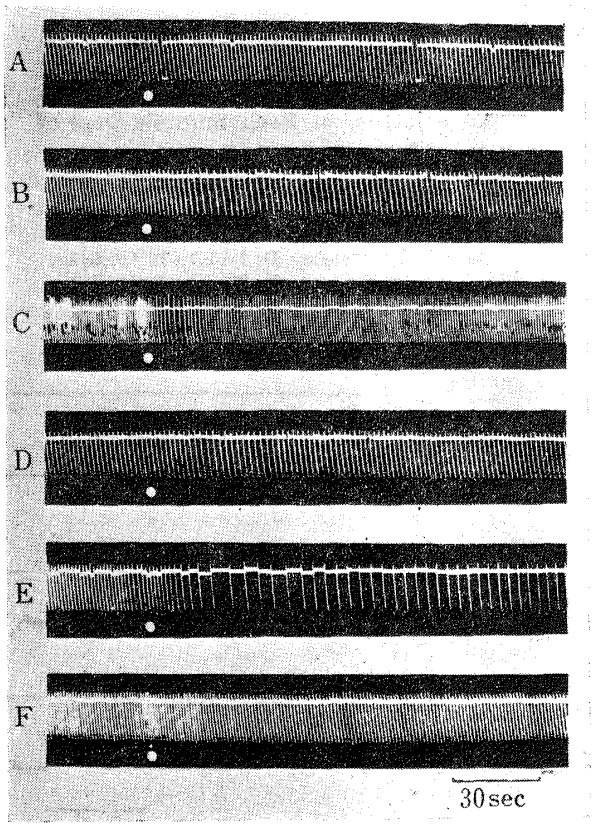

Fig. 6. Peripheral Resistance in Hind Limb Preparations of *Bufo vulgaris* (Laewen–Trendelenburg's Method)

Each deflection indicates the drop flowed from v. abdominalis.

A: I, 1.25×10^{-7} mole; B: II, 4.0×10^{-6} mole;
C: III, 5.0×10^{-7} mole; D: IV, 5.0×10^{-7} mole;
E: V, 1.25×10^{-7} mole; F: VI, 1.25×10^{-7} mole

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which returned to normal state after the solution was exchanged for drug-free Ringer's solution. II (4.0×10^{-5}M) caused an increase in amplitude of contraction. Higher doses decreased the contractility, and 1.28×10^{-3}M caused a perfect inhibition. Perfusion of either III or IV (2.0×10^{-5}M or more) resulted in a decrease in rate and amplitude. In a concentration of 1.6×10^{-4}M of III or IV, there occurred an arrhythmic contraction, which no more returned to normal state even after the solution was exchanged for drug-free Ringer's solution. V or VI (4.0×10^{-5}M) did not have any effect on either amplitude or rate of contraction.

b) Effects on Perfused Hind limb Preparation——Effects of the test compounds on peripheral vascular resistance in hind limb of *Bufo vulgaris* are shown in Fig. 6. I (1.25×10^{-7} mole), injected into a. abdominalis, had no effect on the number of drops flowing out from v. abdominalis. Injection of II (2.0×10^{-6} mole) had no effect on the drop number, but 4.0×10^{-6} mole caused a temporary decrease. Effect of III or IV (5.0×10^{-7} mole) was not uniform. Number of drops did not change or slightly decreased after administration of III or IV. V (3.1×10^{-8}—1.25×10^{-7} mole) markedly decreased the outflow, whereas VI (1.25×10^{-7} mole) had no effect.

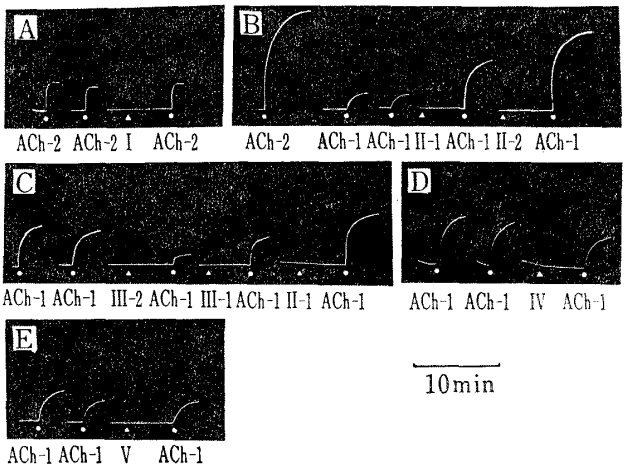

Fig. 7. Effects of 9-Substituted Berberines on Strips of M. rectus abdominis of Frogs contracted by Acetylcholine

Note that the responses to acetylcholine are increased by II and reduced by III and IV. ACh-1: ACh, 1×10^{-6}M; ACh-2: ACh, 1×10^{-5}M; I: I, 1×10^{-6}M; II-1: II, 1×10^{-6}M; II-2: II, 5×10^{-6}M; III-1: III, 1×10^{-7}M; III-2: III, 1×10^{-6}M; IV: IV, 1×10^{-6}M; V: V, 1×10^{-6}M

Skeletal and smooth muscle Preparations

a) Effects on Skeletal Muscle——As shown in Fig. 7, pretreatment with I (5×10^{-5}M) or V (1×10^{-6}M) had no effect on the contraction caused by acetylcholine. II (1×10^{-6}M, 5×10^{-6}M) obviously increased the acetylcholine-induced contraction, as reported by Uchizumi.[4] However, III and IV inhibited the contraction.

b) Effects on Smooth Muscle——Each of the test compounds was given in a concentration of 1×10^{-6}M in the bath.

I and II had no or a slight increasing effect on the contraction caused by acetylcholine (1×10^{-8}M—1×10^{-7}M), whereas III, IV and V showed depressive effects. VI had no effect on the contraction, but inhibited the spontaneous movements.

I and II had weak depressant effects on the preparation contracted by barium chloride (2×10^{-5} g/ml). III, IV, and V were stronger than I and II in depressing the contraction. VI had no effect on it.

Discussion

In the present study, berberine (II, R=OCH_3 in Chart 1) caused a marked but transient hypotension in rats, as has been generally accepted in rabbits, cats and dogs (*e.g.* Jang,[5] Minesita, *et al.*,[6] Uchizumi[4]). It was also found that III (R=OC_4H_9) and IV (R=OC_5H_{11})

4) S. Uchizumi, *Nippon Yakurigaku Zasshi*, **53**, 63 (1957).
5) C. Jang, *J. Pharmacol. Exptl. Therap.*, **71**, 178 (1941).
6) T. Minesita, K. Yamamoto, T. Kogeichi, R. Kido, and T. Miyake, *Shionogi Kenkyusho Nempo*, **5**, 704 (1955).

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caused prolonged hypotension, whereas I (R=OH) had a weak hypertensive action. Therefore, it is evident that 9-substitution of berberine molecule seriously influences the cardiovascular effects.

Uchizumi[4] reported that the acetylcholine-potentiating action was responsible to the mechanism of hypotension of II. Also in the present study, II was found to have an acetylcholine-potentiating action in skeletal muscle of frogs as well as in small intestine of guinea-pigs *in vitro*. The test compounds except II, however, did not show any acetylcholine-potentiating effect on either preparation, III and IV, in contrast with II, being found to inhibit acetylcholine action. Therefore, even though it could be considered that the acetylcholine-potentiating action was responsible to the transient hypotension caused by II, the action could not be the common cause of the hypotesive action of 9-substituted berberines tested in this study. Furthermore, it should be noted that the response of II was not influenced by atropine and hexamethonium.

The dose of III or IV (2.5×10^{-6} mole/kg), which caused hypotension, was found to inhibit the cardiac function in the electrocardiographic study in rats, whereas II (2.5×10^{-6} mole/kg) had no effect on either wave form in ECG or heart rate in rats. In isolated frog heart, which was used to give some complementary result to the evidence for the experiment in rats, II (4.0×10^{-5}M) caused an increase in amplitude of contraction, but, in higher concentrations, II decreased the amplitude. III or IV (2.0×10^{-5}M) caused a decrease in the rate and amplitude of contraction. Jang[5] reported that II stimulated the cat heart and augumented the coronary flow in small and moderate doses, and that in large doses it depressed the heart.

Therefore, it may be reasonable to consider that the depression of heart was one of the probable causes of the prolonged hypotensive responses to II, III, and IV.

Minesita, *et al.*[6] reported that in spinal animals, a dose of some berberine homologue 10 times as large as hypotensive dose in intact animals did not show any hypotensive effect, concluding that the hypotensive action of the compound was centrally mediated. In the present study, any response in blood pressure was not observed after the injection of each test compound in spinal rats, although control level of blood pressure before the injection of the compounds was too low to analyze the drug effect precisely. Then, probability remains that the effect of the test compounds on blood pressure is partly due to their central action.

Acknowledgement We are grateful to Dr. M. Ohta, Pharmaceutical Division, Kowa Co., Ltd., for providing the compounds used in this study.

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