HYPOTENSIVE RESPONSE OF SPONTANEOUSLY HYPERTENSIVE RATS TO CENTRALLY ADMINISTERED DILTIAZEM AND ITS METABOLITES: IN RELEVANCE TO THE HYPOTENSIVE ACTION BY ORAL ADMINISTRATION

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In order to study whether a part of the antihypertensive action of orally administered diltiazem is mediated by the central nervous system and whether metabolites of diltiazem contribute to the actions of diltiazem, we compared the effects of diltiazem and its main metabolites on blood pressure by intravenous administration with those by intracerebroventricular administration to spontaneously hypertensive rats (SHR). Contents of diltiazem and its metabolites in the brain of SHR after oral administration were also studied. Hypotensive action of intravenously administered diltiazem was stronger than that of its main metabolites in terms of potency and durability. Diltiazem (30 μ g or more) and its main metabolites (100 μ g) showed weak hypotensive action by intracerebroventricular administration. Oral administration of diltiazem (30 mg/kg) decreased mean blood pressure by about 40 mmHg 30 min after the administration. At this time, contents of unchanged form and metabolites of diltiazem in the brain were 0.78 ± 0.10 and 7.58 ± 0.89 μ g/whole brain, respectively. It was suggested that hypotensive action of diltiazem was mainly attributable to the unchanged form and that the contribution of central action to the acute hypotensive action of diltiazem was unlikely, although centrally administered diltiazem can cause systemic hypotension.

Keywords — spontaneously hypertensive rat; diltiazem; metabolite; plasma level; brain content; intracerebroventricular administration

INTRODUCTION

Recently, clinical attention has been given to Ca antagonists as a new type of antihypertensive agents. The long-term antihypertensive effect of diltiazem, one of the Ca antagonists,^{1–3)} was also demonstrated in animal experiments.^{4–6)} The site of action of diltiazem is considered to be the vascular smooth muscle but other possibilities have not been excluded yet. In this respect, diuretic action⁶⁾ and centrally mediated hypotensive action⁷⁾ were reported for diltiazem.

Diltiazem is a 1,5-benzothiazepine derivative.⁸⁾ Its structure resembles that of some drugs which act on the central nervous system, such as diazepam. It has been reported that diltiazem is distributed into the central nervous system in mice.⁹⁾ From these findings, it is possible to conclude that a part of the antihypertensive action of orally administered diltiazem is mediated by its central action.

To assess the above mentioned possibility, we examined the hypotensive actions of diltiazem and its main metabolites by intracerebroventricular administration. The results were compared with those obtained by intravenous ad-

ministration and by oral administration to spontaneously hypertensive rats (SHR). We also examined the contents of diltiazem and its metabolites in the brain of SHR upon oral administration.

MATERIALS AND METHODS

1. Animals — Fourteen to 18 week-old male SHR (Charles River Japan), weighing 240–350 g, were used in this study. SHR were kept for more than 4 d before use in our animal room in which the temperature was kept constant (23 ± 1 °C). SHR were given standard rat chow (Nihon Clea, CE-2) and drinking water *ad libitum* during the period.

2. Oral Administration — SHR were anesthetized with pentobarbital Na, 50 mg/kg, i.p., to implant an arterial catheter. A polyethylene catheter (PE-60), which had been made fine with heat and filled with heparin Na solution (1000 units/ml), was inserted into the abdominal aorta from the left femoral artery under sterile conditions. The catheter was guided under the skin and exposed from the back of the neck. Incisions were sutured and penicillin G was injected

intramuscularly at the contralateral lower limb.

Hypotensive action of diltiazem was studied after a stabilization period of 2 d or more. Before the experiment, SHR were fasted overnight. In this period, SHR were given 20% sucrose solution as the drinking water.

Blood pressure was measured by a pressure transducer (Nihon Kohden, MPU-0.5), which was connected to the implanted catheter, and heart rate was measured by a cardiometer (Nihon Kohden, RT-5) triggered by the signal of the pulse pressure. Mean blood pressure was obtained by RC circuit. Pulse pressure, mean blood pressure and heart rate were simultaneously recorded on a recticorder (Nihon Kohden, RJG-3006). SHR were placed in a transparent plastic cage and measurements of blood pressure and heart rate were carried out under conscious and unrestrained states.

Diltiazem was dissolved in deionized water and the volume of oral administration was adjusted to 1 ml/100 g of body weight. Diltiazem (30 mg/kg) or the same volume of deionized water was administered orally after the SHR were accustomed to the new environment and blood pressure and heart rate became stabilized.

3. Intravenous Administration — 3-1. Conscious SHR: A polyethylene catheter for measurement of blood pressure was introduced into the abdominal aorta *via* the tail artery under ether anesthesia. Similarly, a polyethylene catheter for intravenous administration was introduced into the postcava *via* the tail vein. The arterial and the venous catheters were filled with heparin Na solution (1000 units/ml) and 0.9% NaCl solution, respectively. Pulse pressure, mean blood pressure and heart rate were measured as mentioned in section 2.

After the SHR fully recovered from the anesthesia, experiments were started. Diltiazem (30–1000 $\mu\text{g/kg}$), deacetyl-diltiazem (M_1 , 30–1000 $\mu\text{g/kg}$) and *N*-demethyl-diltiazem (M_A , 100–3000 $\mu\text{g/kg}$) were dissolved in 0.9% NaCl solution and were administered through the venous catheter in stepwise elevated doses. The volume of the injection was adjusted to 0.1 ml/100 g of body weight. The same volume of 0.9% NaCl solution was administered in each case to estimate the influence of the administration procedure.

3-2. Anesthetized SHR: Experiments were carried out under pentobarbital Na anesthesia

(50 mg/kg, i.p.). Polyethylene catheters were inserted into the right femoral artery and the left femoral vein for measurement of blood pressure and for intravenous administration, respectively. Pulse pressure, mean blood pressure and heart rate were measured as mentioned in section 2, and administration of drugs was performed as mentioned in section 3-1. Supplemental doses of pentobarbital Na were injected through the venous catheter during the experiment.

4. Intracerebroventricular (i.c.v.) Administration — A catheter for i.c.v. administration had been implanted on the day before the experiment in a manner similar to that previously described by Noble *et al.*¹⁰⁾

Under pentobarbital Na anesthesia (50 mg/kg, i.p.), the skin of the head was incised and the skull was exposed. A hole, 1 mm in diameter, was made on the skull by a dental electric drill at 1.5 mm left of and 1.5 mm behind the bregma. A catheter for i.c.v. administration, which was 9 mm in length and made from a 20 G injection needle, was inserted into the hole to 4 mm depth from the surface of the skull. The catheter was fixed with dental cement (Crown, Bridge & Inlay Cement, G-C Dental Industrial Corporation, Tokyo). The skin was sutured and penicillin G was injected into the muscle of lower extremities.

The effects of diltiazem and its metabolites were studied on the day after the operation under pentobarbital Na anesthesia (50 mg/kg, i.p.). Pulse pressure, mean blood pressure and heart rate were measured as mentioned in section 2. Diltiazem (10–100 μg), M_1 (100 μg) and M_A (100 μg) were dissolved in 10 μl of 0.9% NaCl solution and administered through the catheter. SHR of the control group were administered the same volume of 0.9% NaCl solution. At the end of the experiment, black ink was injected into the catheter to ensure that the tip of the catheter was in the lateral ventricle.

5. Plasma Levels and Brain Contents of Diltiazem and Its Metabolites — Before the experiment, SHR were fasted overnight and given 20% sucrose solution as drinking water. Thirty mg/kg of ^{14}C -diltiazem (85 $\mu\text{Ci/kg}$) were dissolved in deionized water and were administered orally. The volume of the oral administration was adjusted to 1 ml/100 g of body weight. Thirty or 60 min after the administration, about 8 ml of the blood was collected from the abdom-

inal aorta under ether anesthesia into a syringe, which contained 100 units of heparin Na solution (0.1 ml in volume). The blood was centrifuged (0 °C, $1000 \times g$, 10 min) and the plasma was obtained. After the blood sampling, the brain was removed from SHR and divided into the cerebrum and the brain stem. Brains were rinsed with 0.9% NaCl solution and adhered water was removed with filter paper. After this procedure, the brains were weighed and homogenized by a polytoron. About 100 mg of the blood and the brain samples were combusted with an automatic combustion apparatus (Aloka, ASC-112). The radioactivity was measured with a liquid scintillation counter (Packard, Tri-Carb 460CD). The remaining blood and brain samples were extracted with 5% NaHCO_3 and ethyl acetate. Then, ethyl acetate was evaporated. The rest was dissolved with a small amount of methyl alcohol and spotted on a thin-layer plate with the authentic sample of diltiazem. After developing the plate, diltiazem was determined by measuring the radioactivity of the area corresponding to the R_f value of this drug.

6. *Statistical Methods* — Data are expressed as means $\pm$ S.E.M. or geometric means (95% confidence limits). Results were compared with

FIG. 1. Time Courses of the Blood Pressure and Heart Rate after Oral Administration of Diltiazem in Conscious and Unrestrained SHR

○, control group, $N=6$; △, diltiazem group, 30 mg/kg, p.o., $N=7$. Each point and vertical bar represent mean $\pm$ S.E.M. of experiments. Some S.E.M. are within the scale of the symbols. Pre-treatment values are shown at 0 min.

their own predosing values. Statistical analysis was performed by the paired t -test and when $p < 0.05$, results were considered to be statistically significant.

RESULTS

1. Oral Administration

Time courses for changes in mean blood pressure and heart rate in the control group and in the diltiazem-treated group are shown in Fig. 1.

The mean blood pressure in the control group increased about 15 mmHg immediately after the administration of deionized water. The increase in the mean blood pressure recovered gradually. On the other hand, the mean blood pressure in the diltiazem group decreased soon after the administration and the peak response was observed 30 min after the administration. At this time, the mean blood pressure was about 40 mmHg lower than that before the administration of diltiazem. The mean blood pressure then gradually rose but remained lower than that prior to the diltiazem treatment. Compared to the value obtained before treatment, significant hypotension (about 10 mmHg, $p < 0.01$) was still observed 2.5 h after the administration.

The heart rate in the control group and in the diltiazem group increased immediately after the administration. However, it decreased more rapidly in the diltiazem group. The heart rate in the diltiazem group decreased below the predosing level after 20 min. Compared to the predosing level, there was a significant decrease in heart rate during a period 60–90 min after the administration.

2. Intravenous Administration

2-1. *Conscious SHR* — Peak responses of mean blood pressure and heart rate which were observed within 1 min to diltiazem, M_1 and M_A in conscious SHR are shown in Fig. 2.

The hypotensive actions of diltiazem and M_1 were equivalent but that of M_A was about 1/3 as potent as the former two drugs. The dose that decreased the mean blood pressure 20 mmHg was 300 $\mu\text{g/kg}$ (220–420 $\mu\text{g/kg}$, 95% confidence limits) for diltiazem, 290 $\mu\text{g/kg}$ (200–400 $\mu\text{g/kg}$) for M_1 and 1170 $\mu\text{g/kg}$ (1100–1250 $\mu\text{g/kg}$) for M_A , respectively. The hypotension lasted 406 ± 93 , 115 ± 28 and 375 ± 47 s with diltiazem at 1 mg/kg, M_1 at 1 mg/kg and M_A at 3 mg/kg, respectively.

In conscious SHR, the heart rates increased

FIG. 2. Changes in Mean Blood Pressure and Heart Rate of Conscious SHR after Intravenous Administration of Diltiazem (○), M₁ (□) or M_A (Δ)

Control values of the mean blood pressure and heart rate were 166.9 ± 8.3 mmHg and 382.9 ± 9.0 beats/min, respectively. Each point and vertical bar represent mean $\pm$ S.E.M. of 7 experiments. Some S.E.M. are within the scale of the symbols. Broken lines show secondary decreasing phases in the heart rate which were observed immediately after the initial transient increasing phases.

38.3 ± 5.1 beats/min with intravenous administration of 0.9% NaCl solution. In the cases of diltiazem and M₁, the heart rates showed biphasic responses; a decrease after a transient increase. M_A caused only an increase in the heart rate.

2-2. Anesthetized SHR — Changes in the mean blood pressure and heart rate to diltiazem, M₁ and M_A in anesthetized SHR are shown in Fig. 3. Peak responses were also observed within 1 min in this experiment as in the cases of conscious SHR.

The drugs exerted hypotensive actions in a dose dependent manner. The doses decreased mean blood pressure by 20 mmHg were $115.6 \mu\text{g/kg}$ (76.9 – $173.7 \mu\text{g/kg}$) for diltiazem, $168.5 \mu\text{g/kg}$ (130.0 – $218.5 \mu\text{g/kg}$) for M₁ and $727.1 \mu\text{g/kg}$ (446.3 – $1184.3 \mu\text{g/kg}$) for M_A, respectively. The hypotension lasted 745 ± 49 , 227 ± 22 and 700 ± 52 s with diltiazem at 1 mg/kg, M₁ at 1 mg/kg and M_A at 3 mg/kg, respectively.

FIG. 3. Changes in Mean Blood Pressure and Heart Rate of Anesthetized SHR after Intravenous Administration of Diltiazem (○), M₁ (□) and M_A (Δ)

Control values of the mean blood pressure and heart rate were 142.3 ± 2.7 mmHg and 330.3 ± 9.8 beats/min, respectively. Each point and vertical bar represent mean $\pm$ S.E.M. of 7 experiments. Some S.E.M. are within the scale of symbols.

In contrast to conscious SHR, the drugs decreased the heart rate in a dose dependent manner in anesthetized SHR. Negative chronotropic action of M_A seemed to be slightly weaker than those of diltiazem and M₁ in comparison with their hypotensive actions.

3. i.c.v. Administration

Figure 4 shows the time courses of mean blood pressure and heart rate for the control group and diltiazem groups (10, 30 and 100 μg), and Fig. 5 shows those for the M₁ group (100 μg) and for the M_A group (100 μg).

No changes in mean blood pressure and heart rate were observed by 0.9% NaCl solution or by 10 μg of diltiazem. However, 30 μg of diltiazem decreased mean blood pressure by 5 mmHg ($p < 0.05$) and decreased the heart rate slightly ($p < 0.05$). One hundred μg of diltiazem decreased mean blood pressure about 20 mmHg and decreased heart rate about 20 beats/min. Peak responses in hypotension were observed 3 to 5 min after the administration and in heart rate, 7 to 10 min after the administration. Significant changes in mean blood pressure and heart rate caused by 100 μg of diltiazem lasted for 20 and 30 min, respectively.

FIG. 4. Time Courses of Changes in Mean Blood Pressure and Heart Rate in Anesthetized SHR after Intracerebroventricular Administration of Diltiazem

●, control group, $N=5$; ○, diltiazem $10\text{ }\mu\text{g}$, $N=5$; Δ, diltiazem $30\text{ }\mu\text{g}$, $N=6$; □, diltiazem $100\text{ }\mu\text{g}$, $N=6$. Each point and vertical bar represent mean $\pm$ S.E.M. of the experiment. Some S.E.M. are within the scale of symbols. Pretreatment values were $121.0 \pm 3.5\text{ mmHg}$ for mean blood pressure and $301.8 \pm 7.9\text{ beats/min}$ for the heart rate ($N=22$).

One hundred μg of M_A decreased mean blood pressure about 10 mmHg and decreased the heart rate 18 beats/min (Fig. 5). Significant changes in mean blood pressure and heart rate lasted for 20 and 30 min, respectively. M_1 , at a dose of $100\text{ }\mu\text{g}$, decreased mean blood pressure 8 mmHg ($p<0.05$), but it caused no changes in the heart rate. A decrease in mean blood pressure after administration of M_1 was recovered within 10 min.

4. Plasma Levels and Contents in the Brain

The plasma level of diltiazem 30 min after the administration (diltiazem, 30 mg/kg , *p.o.*) was about $3 \times 10^{-7}\text{ g/ml}$, which was larger than that 60 min after the administration. On the other hand, the plasma level of the metabolites was about $3 \times 10^{-5}\text{ g/ml}$ 60 min after the admin-

FIG. 5. Time Courses of Changes in Mean Blood Pressure and Heart Rate in Anesthetized SHR after Intracerebroventricular Administration of the Metabolites of Diltiazem

Δ, $M_1\text{ }100\text{ }\mu\text{g}$, $N=7$; ○, $M_A\text{ }100\text{ }\mu\text{g}$, $N=5$. Each point and vertical bar represent mean $\pm$ S.E.M. of the experiment. Some S.E.M. are within the scale of symbols. Pretreatment values were $127.5 \pm 5.0\text{ mmHg}$ for mean blood pressure and $289.5 \pm 13.6\text{ beats/min}$ for the heart rate ($N=12$).

istration, which was larger than that was 30 min after the administration (Table I).

Calculated from concentrations, the contents of diltiazem and the metabolites in the whole brain 30 min after the administration, when the hypotensive action of orally administered diltiazem was maximum (*cf.* Fig. 1), were 0.78 ± 0.10 and $7.58 \pm 0.89\text{ }\mu\text{g/whole brain}$, respectively (Table I).

DISCUSSION

Hypotensive actions of intravenously administered diltiazem and its main metabolites in anesthetized SHR were stronger than those in conscious SHR. This was probably due to the participation of baroreflex. Therefore, we studied the actions of i.c.v. administered diltiazem

TABLE I. Plasma Levels and concentrations in the Brain of Diltiazem and Metabolites in SHR after Oral Administration (30 mg/kg , *p.o.*, $N=5$)

	Sampling time (min)	Plasma level ($\mu\text{g/ml}$)	Cerebrum ($\mu\text{g/g tissue}$)	Brain stem and cerebellum ($\mu\text{g/g tissue}$)
Diltiazem	30	0.33 ± 0.04	0.45 ± 0.06	0.41 ± 0.04
	60	0.20 ± 0.01	0.22 ± 0.03	0.21 ± 0.02
Metabolites	30	28.42 ± 0.97	4.12 ± 0.45	4.31 ± 0.45
	60	34.50 ± 0.69	3.35 ± 0.35	3.63 ± 0.36

and its main metabolites on blood pressure and heart rate in a more sensitive system, *i.e.* anesthetized SHR. We found that diltiazem, at a dose of 10 μg , caused no effect on systemic blood pressure, but larger doses of diltiazem (30 μg or more) and a large dose (100 μg) of the metabolites decreased systemic blood pressure significantly by i.c.v. administration.

The dose, 30 μg , corresponds to about 100 $\mu\text{g}/\text{kg}$ of the body weight of SHR (240–350 g). When this dose was administered intravenously to anesthetized SHR, the systemic blood pressure decreased about 20 mmHg. It was possible that the hypotensive action of diltiazem by i.c.v. administration was caused by leakage from the cerebral ventricle to the systemic circulation. However, the order of hypotensive potencies of diltiazem and its metabolites was diltiazem = $M_1 > M_A$ by intravenous administration and the order was diltiazem $> M_A > M_1$ by i.c.v. administration. When 100 μg of diltiazem was administered intracisternally to rabbits,¹¹⁾ the peak plasma level of the radioactivity reached about 3×10^{-8} g eq/ml. Although the investigators did not determine the proportion of the unchanged form and the metabolites, the plasma level appeared to be too low to cause systemic hypotension. Furthermore, the time course of the plasma level appears to be different from that of the hypotension obtained by i.c.v. administration in our experiment. Therefore, the hypotensive actions of diltiazem and its metabolites by i.c.v. administration are not caused by leakage but probably caused by their central action. In fact, Higuchi *et al.* reported that microinjected diltiazem directly into the nucleus tractus solitarius decreased blood pressure and heart rate in anesthetized Wistar Kyoto rats.⁷⁾

On the other hand, the content of diltiazem in the brain of SHR 30 min after oral administration of diltiazem at a dose of 30 mg/kg was about 0.8 $\mu\text{g}/\text{whole brain}$. This dose of diltiazem decreased systemic blood pressure about 40 mmHg 30 min after the administration, compared to the i.c.v. doses, the content of diltiazem in the brain was too small to cause systemic hypotension. This conclusion is also applicable to the cases of M_1 and M_A .

If high concentrations of diltiazem and/or its metabolites are distributed in the site of action in the central nervous system in a case of oral administration but not in a case of i.c.v. administra-

tion, it could be possible that diltiazem decreased systemic blood pressure through the actions on the central nervous system. However, the distribution of diltiazem in the brain was reported to be uniform by whole body autoradiography in mice.⁹⁾ Therefore, these assumptions are not likely, and further studies are required for rats. As a result, it is suggested that the central action is not involved in acute hypotensive action of diltiazem by oral administration.

A dose of 10 mg/kg was reported to be the lowest dose of diltiazem to decrease blood pressure by oral administration to rats.^{4,6)} Ninety to 180 mg/d of diltiazem is used in Japan for the treatment of patients with hypertension and sufficient effect was observed.¹²⁾ Recently, a difference in the metabolism of diltiazem between humans and rats was reported.¹³⁾ Maximum plasma level of unchanged form in humans after oral administration of 120 mg of diltiazem was $1 - 3 \times 10^{-7}$ g/ml.¹⁴⁾ On the other hand, we found that the plasma level of unchanged form in SHR 30 min after the administration of diltiazem, at a dose of 30 mg/kg, *p.o.*, was about 3×10^{-7} g/ml. This finding indicates that the dose of diltiazem, 30 mg/kg, *p.o.* in rats is comparable to the clinical dose in terms of plasma level.

We found that the plasma level of unchanged form of diltiazem was only one tenth of that of total metabolites in SHR. It has been reported that the main metabolites of diltiazem are N-demethyl form (M_A) in humans¹³⁾ and deacetyl form (M_1) in rats.¹⁵⁾ Diltiazem, M_1 and M_A decreased blood pressure by intravenous administration to conscious and anesthetized SHR. The order of hypotensive potency of these three compounds was diltiazem = $M_1 > M_A$ and the order of durability of the hypotensive actions was diltiazem = $M_A > M_1$. These orders are comparable to the results obtained in anesthetized dogs.¹⁶⁾

All compounds increased heart rate in conscious SHR. This increase in heart rate should be due to administration procedure and to baroreflex.^{5,17)} Diltiazem and M_1 decreased heart rate after a transient increase, while M_A caused no decrease in the heart rate. Negative chronotropic action of M_A was slightly weaker than those of diltiazem and M_1 in anesthetized SHR. Compared to hypotensive actions, M_A may possess a weaker negative chronotropic action than diltiazem and M_1 in SHR.

As mentioned above, hypotensive potency of M_1 and M_A are similar to or less potent than that of the parent compound, diltiazem. Therefore, it cannot be ruled out that M_1 and/or M_A contribute to the hypotensive action of diltiazem. However, plasma levels of M_1 and M_A in humans after oral administration of diltiazem were reported to be $1/5-1/8^{14)}$ and $1/3^{13)}$ of diltiazem, respectively. Thus, hypotensive action of diltiazem should be caused mainly by diltiazem itself.

In conclusion, the hypotensive action of diltiazem is considered to be caused mainly by the unchanged form and participation of the central action to produce the acute hypotensive effect of diltiazem by oral administration is unlikely, although centrally administered diltiazem can cause systemic hypotension. Participation of the central action to long-term antihypertensive effect of diltiazem should be studied further.

REFERENCES

- 1) N. Akaike, A. M. Brown, K. Nishi and Y. Tsuda: Actions of verapamil, diltiazem and other divalent cations on the calcium-current of helix neurones, *Br. J. Pharmacol.*, **74**, 87–95 (1981).
- 2) T. Nagao, T. Ikeo and M. Sato: Influence of calcium ions on responses to diltiazem in coronary arteries, *Jpn. J. Pharmacol.*, **27**, 330–332 (1977).
- 3) H. Nakajima, M. Hoshiyama, K. Yamashita and A. Kiyomoto: Electrical and mechanical responses to diltiazem in potassium depolarized myocardium of the guinea pig, *Jpn. J. Pharmacol.*, **26**, 571–580 (1976).
- 4) I. Yamaguchi, K. Ikezawa, S. Murata, H. Narita, T. Ikeo and M. Sato: Hypotensive effects of diltiazem in DOCA/saline hypertensive rats, *Folia Pharmacol. Jpn.*, **75**, 191–199 (1979).
- 5) M. Sato, S. Murata, H. Narita, M. Tomita, K. Yamashita and I. Yamaguchi: Hypotensive effects of diltiazem hydrochloride in the normotensive, spontaneously hypertensive and renal hypertensive rats, *Folia Pharmacol. Jpn.*, **75**, 99–106 (1979).
- 6) H. Narita, T. Nagao, H. Yabana and I. Yamaguchi: Hypotensive and diuretic actions of diltiazem in spontaneously hypertensive and Wistar Kyoto rats, *J. Pharmacol. Exp. Ther.*, **227**, 472–477 (1983).
- 7) S. Higuchi, A. Takeshita, N. Ito, T. Imaizumi, H. Matsuguchi and M. Nakamura: Arterial pressure and heart rate responses to calcium channel blockers administered in the brainstem in rats, *Circ. Res.*, **57**, 244–251 (1985).
- 8) M. Sato, T. Nagao, I. Yamaguchi, H. Nakajima and A. Kiyomoto: Pharmacological studies on a new 1,5-benzothiazepine derivative (CRD-401). I. Cardiovascular actions, *Arzneim.-Forsch.*, **21**, 1338–1343 (1971).
- 9) M. Sakuma, M. Yoshikawa and Y. Sato: The whole body autoradiographic studies on the distribution of ^{14}C -labeled new 1,5-benzothiazepine derivative (^{14}C -CRD-401) in mice, *Chem. Pharm. Bull.*, **19**, 995–1005 (1971).
- 10) E. P. Noble, R. J. Wurtman and J. Axelrod: A simple and rapid method for injecting ^3H -norepinephrine into the lateral ventricle of the rat brain, *Life Sci.*, **6**, 281–291 (1967).
- 11) K. Naito, T. Nagao, M. Otsuka, S. Harigaya and H. Nakajima: Penetration into and elimination from the cerebrospinal fluid of diltiazem, a calcium antagonist, in anesthetized rabbits, *Arzneim.-Forsch.*, **36**, 25–28 (1986).
- 12) S. Yorifuji, Y. Kawai, S. Arami, S. Ikoma, K. Kawamori, H. Kunishige, Y. Kira, K. Kurose, A. Matsuoka, A. Sakai, H. Iwasaka, T. Takamiya, K. Nakao, H. Hirouchi, S. Yamada and H. Itoh: Hypotensive effect of diltiazem hydrochloride (Herbesser tablets) in patients with essential hypertension—pracebo controlled double blind crossover study, *Rinsho Seijinbyo*, **9**, 893–907 (1979).
- 13) J. Sugihara, Y. Sugawara, H. Ando, S. Harigaya, A. Etoh and K. Kohno: Studies on the metabolism of diltiazem in man, *J. Pharmacobio-Dyn.*, **7**, 24–32 (1984).
- 14) P. L. Morselli, M. Rovei, M. Mitchard, A. Durand, R. Gomeri and J. Larribaud: Pharmacokinetics and metabolism of diltiazem in man (observations on healthy volunteers and angina pectoris patients), "New Drug Therapy with a Calcium Antagonist," ed by R. J. Bing, Excerpta Medica, Amsterdam, 1979, pp. 152–168.
- 15) T. Meshi, J. Sugihara and Y. Sato: Metabolic fate of *d-cis*-3-acetoxy-5-2-(dimethylamino) ethyl-2,3-dihydro-2-(*p*-methoxyphenyl) 1,5-benzothiazepine-4(5*H*)-one hydrochloride (CRD-401), *Chem. Pharm. Bull.*, **19**, 1546–1556 (1971).
- 16) H. Yabana, T. Nagao and M. Sato: Cardiovascular effects of the metabolites of diltiazem in dogs, *Cardiovasc. Pharmacol.*, **7**, 152–157 (1985).
- 17) H. Nakaya, A. Schwartz and R. W. Millard: Reflex chronotropic and inotropic effects of calcium channel-blocking agents in conscious dogs. Diltiazem, verapamil and nifedipine compared, *Circ. Res.*, **52**, 302–311 (1983).