Relation between Blood Pressure and Plasma Catecholamine Concentration after Administration of Calcium Antagonists to Rats

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Three types of calcium antagonists, diltiazem, verapamil and nicardipine, were separately infused into Sprague-Dawley (SD) rats (under pentobarbital anesthesia $n=5$) through the left femoral vein at four different flow rates. Mean arterial blood pressure, heart rate and the concentration of plasma catecholamines (CAs), epinephrine (E), norepinephrine (NE) and dopamine (DA), were measured for each calcium antagonist, and the correlations between them were studied. Blood samples were collected within the infusion from common juglar vein. Plasma concentrations of CAs were determined by a HPLC-ethylenediamine condensation reaction-peroxyoxalate chemiluminescence detection system (HPLC-ED-PO-CL).

The plasma concentration of CAs increased corresponding to the blood pressure reduction. The reduction induced by each calcium antagonist correlated with the logarithm of plasma NE concentration. The relation was expressed as $Y = -\alpha \log X + m$ (Y , blood pressure; X , concentration of plasma NE; α , slope; and m , intercept). The correlation coefficients (r_s) were -0.950 (diltiazem), -0.975 (verapamil) and -0.978 (nicardipine) (*versus* -0.734 for control). The α for nicardipine (108.4) was greater than those of diltiazem (85.4) and verapamil (80.8) (*versus* 31.0 for control), meaning that blood pressure reduction was greater in the case of nicardipine than diltiazem and verapamil, with an identical increment of plasma NE concentration. These data indicate that the contribution of the sympathetic nervous system to maintaining blood pressure reduced by nicardipine is less than that observed following the infusion of diltiazem and verapamil.

Similar good inverse correlations between blood pressure and the logarithm of plasma concentration of E were observed with the three drugs infused ($r = -0.928$, -0.966 and -0.948 for diltiazem, verapamil and nicardipine, respectively).

A slight correlation ($r = -0.810$) was obtained between blood pressure and the logarithm of plasma DA concentration following the infusion of nicardipine.

On the infusion of nicardipine, the heart rate remained at the same value as the starting point during the first and second dose infusion (0.50 and 1.01 $\mu\text{g/kg/min}$, respectively) and was then reduced significantly after the additional infusions (2.02 $\mu\text{g/kg/min}$), whereas the heart rates started to decrease from the first point of treatment with diltiazem and verapamil.

Keywords calcium antagonist; rat plasma catecholamine; blood pressure; sympathetic nervous system; HPLC; peroxyoxalate chemiluminescence

Calcium antagonists such as diltiazem, verapamil and dihydropyridines (nifedipine, nitrendipine, nicardipine, *etc.*) have been used as potent vasodilators for anti-hypertensive therapy because of their inhibition of transmembrane Ca^{2+} influx into cardiac and muscle cells and the resultant blood pressure reduction.¹⁾

Plasma norepinephrine (NE) was reported to increase as blood pressure was lowered by a short term treatment using dihydropyridine-type drugs as a result of a baroreceptor-mediated reflex in sympathetic nervous activity,²⁻⁵⁾ whereas other reports revealed that other types of antagonists, diltiazem and verapamil did not have a significant effect on plasma NE level.^{6,7)}

However, the lack of a sensitive, selective methods for the determination of plasma catecholamines (CAs) would have obstructed a detailed analysis of the correlation of blood pressure and heart rate with plasma CAs on an individual basis. Recently, we have developed such a method which requires less than 100 μl rat plasma.⁸⁾ It consists of the separation of CAs by HPLC, a post-column derivatization with ethylenediamine (ED) condensation and sensitive detection by a peroxyoxalate chemiluminescence (PO-CL) reaction.⁹⁾ In the preliminary experiment, determination of plasma CAs after the administra-

tion of diltiazem revealed that the blood pressure reduction was correlated with an increase in the logarithm of plasma NE concentrations. The data indicate that the blood pressure reduction caused by diltiazem may be compensated for by enhancement of the sympathetic nervous system activity.¹⁰⁾

Thus, in this paper, measuring the plasma CAs, we further investigated the relations between blood pressure reduction by three different types of calcium antagonists, diltiazem, verapamil and nicardipine, and the sympathetic nervous system activity in Sprague-Dawley (SD) rats.

MATERIALS AND METHODS

Reagents CAs (norepinephrine, NE; epinephrine, E; and dopamine, DA), dihydroxybenzylamine (DHBA, the internal standard for CAs), and alumina (WA-4) were purchased from Sigma (St. Louis, MO, U.S.A.). Trifluoroacetic acid (TFA) was obtained from Pierce (Rockford, IL, U.S.A.). Acetonitrile, ethanol, dioxane, ethyl acetate and distilled water, all of HPLC grade, were purchased from Wako (Osaka, Japan). Hydrogen peroxide and bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl]oxalate (TDPO) were also from Wako. Sodium hexane-

sulfonate and imidazole (zone-refined) were obtained from Tokyo Kasei (Tokyo, Japan). The purified ED prepared for washing semi-conductors was a gift from Wako. Nembutal sodium solution (50 mg/ml) was purchased from Dabbott Laboratories (IL, U.S.A.) and pentobarbital sodium salt was from Tokyo Kasei. Diltiazem hydrochloride was kindly donated by Tanabe Seiyaku Co., Ltd. (Biological Research Laboratory, Saitama, Japan). Nicardipine hydrochloride was purchased from Sigma and verapamil hydrochloride was from Nacalai. Male SD rats weighing from 240 to 340 g were obtained from Nihon Seibutu Zairyo Center (Tokyo, Japan).

Alumina was purified with hydrochloric acid as described previously.¹¹⁾

Infusion of the Drugs and Rat Plasma Treatment A similar procedure to that described in the previous paper was adopted.¹⁰⁾ SD rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally). Diltiazem, verapamil and nicardipine were separately dissolved in saline at concentrations of 3.27, 1.96 and 9.81×10^{-2} mg/kg/ml, respectively. Pentobarbital was dissolved in saline at a concentration of 41.7 mg/kg/ml. A solution of diltiazem was infused through the left femoral vein with a Model 901 infusion/withdrawal pump (Harvard Apparatus, MA, U.S.A.) by changing the flow rate stepwise from 5.1 to 51 μ l/min. These flow rates corresponded to infusions at 16.7, 33.7, 67.5 and 167 μ g/kg/min. The period for each infusion was 25 min. Solutions of verapamil and nicardipine were separately infused at the same flow rate used for diltiazem; these flow rates corresponded to 10.0, 20.2, 40.4 and 100 μ g/kg/min for verapamil, and 0.50, 1.01, 2.02 and 5.00 μ g/kg/min for nicardipine. Saline was infused into the control rats at the same flow rate used for diltiazem. A syringe containing a solution of nicardipine was covered with aluminium film during the infusion in order to prevent decomposition by light. The pentobarbital solution was infused to keep the rats anesthetized through the right femoral vein with a Model Familic-100N syringe pump (Jasco, Tokyo, Japan) at a flow rate of 4 μ l/min. Arterial blood pressure and heart rate were measured through the left femoral artery with a Model DTX Disposable Transducer (Spectramed Inc., Oxnard, CA, U.S.A.), which was connected to a Model RP-5 carrier amplifier for arterial blood pressure (Nihon Kohden, Tokyo, Japan) and a Model RT-5 pulse rate tachometer for heart rate (Nihon Kohden), and were recorded on a Model RJG-3004 rectifier (Nihon Kohden).

Blood (0.15 ml) was collected 13 times through the common jugular vein, 1 min before the infusion of each calcium antagonist or saline, and 5, 15 and 24 min after the beginning of each step of the infusion. Each calcium antagonist was infused into five SD rats (15 rats total), and three rats were infused with saline as a control. To the plasma (80 μ l) was added alumina (5 mg), 10 μ M 3,4-dihydroxybenzylamine (internal standard solution, 10 μ l) and 1.5 M tris-hydrochloric acid buffer (pH 8.7, 100 μ l).⁸⁾ CAs were extracted from the alumina with 0.1 M perchloric acid (100 μ l). A 50 μ l aliquot of the supernatant, which corresponds to 40 μ l plasma, was subjected to the HPLC system. The plasma CA were determined by HPLC-PO-CL detection.⁸⁾

Means and their standard errors of mean arterial blood pressure, heart rate and plasma CA concentrations and correlations between these parameters were calculated using the Student's *t*-test.

Calibration Curve The same pretreatment was performed for 100 μ l of rat plasma to which a 10 μ l standard mixture containing 0.25, 0.50, 0.75 or 1 pmol each of NE, E and DA, and 100 pmol DHBA was added.

HPLC-PO-CL Detection System The HPLC detection system consisted of a Model 880-PU pump (JASCO) for delivering the eluent, a Model 885-PU pump (JASCO) for the fluorogenic reagent solution, a Model 885-PU pump (JASCO) for the chemiluminogenic reagent solution, a Model TC-100 heating bath (JASCO), a Model 825-CL chemiluminescence detector (JASCO) equipped with a Model Y-46 cut-off filter (Kenko, Tokyo, Japan), a Model 807-IT data processor (JASCO), a Model 851-AS auto-sampler (JASCO), two Model KZU-1 mixing devices (25 μ l, Kyowa Seimitsu, Tokyo, Japan),¹²⁾ and PTFE tubing (1.6 mm o.d. $\times$ 0.5 mm i.d. $\times$ 15 m) wound in a knitted fashion.

HPLC Conditions The eluent consisted of 50 mM potassium acetate (pH 3.20)–50 mM potassium phosphate (pH 3.20)–acetonitrile (92.15:4.85:3, v/v/v) containing 1 mM sodium 1-hexanesulfonate. The flow rate was adjusted to 0.5 ml/min. A catechopack (150 mm $\times$ 4.6 mm, JASCO) used as a separation column was kept at 40 °C. The fluorogenic reagent solution was prepared by dissolving 105 mM ED and 175 mM imidazole in acetonitrile–ethanol (90:10, v/v), the flow rate of which was 0.25 ml/min; the reaction coil length for the fluorogenic reaction was 15 m $\times$ 0.5 mm i.d. The reaction temperature was 80 °C. The chemiluminogenic reactions were made as follows: 0.25 mM TDPO, 150 mM hydrogen peroxide and 110 mM TFA in dioxane–ethyl acetate (50:50, v/v); reagent flow rate, 1.4 ml/min.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram obtained from a standard mixture of NE, E, DA and DHBA (the internal standard), and a chromatogram obtained from plasma (corresponding to 40 μ l) of an SD rat, which was collected at 15 min after the fourth infusion of verapamil (100 μ g/kg/min, 51 μ l/min).

As reported previously,¹⁰⁾ the continuous infusion of diltiazem resulted in a gradual decrease both in mean arterial blood pressure (from 117 ± 7.3 to 65 ± 4.5 mmHg) and in heart rate (from 403 ± 10.5 to 281 ± 14.2 beats/min) and a gradual increase in concentration of plasma NE (from 1.0 ± 0.1 to 4.2 ± 1.0 pmol/ml) ($n=5$). A similar result was also obtained following the infusion of the other calcium antagonists: for verapamil, blood pressure, from 114 ± 3.5 to 59 ± 1.8 mmHg; heart rate, from 400 ± 27.8 to 266 ± 9.9 beats/min; concentration of plasma NE, from 0.8 ± 0.0 to 4.4 ± 0.2 pmol/ml ($n=5$); and for nicardipine, blood pressure, from 128 ± 4.7 to 62 ± 1.7 mmHg; heart rate, from 403 ± 12.0 to 319 ± 17.8 beats/min; concentration of plasma NE, from 1.0 ± 0.2 to 5.1 ± 1.0 pmol/ml ($n=5$); and for the control, blood pressure, from 116 ± 7.2 to 105 ± 5.6 mmHg; heart rate, from 395 ± 3.7 to 368 ± 32

Fig. 1. Chromatogram of a Standard Mixture of CAs (A) and Chromatogram Obtained from SD Rat Plasma (B)

Peaks: NE, norepinephrine, 0.5 pmol; E, epinephrine, 0.5 pmol; IS, internal standard, 3,4-dihydroxybenzylamine, 50 pmol; DA, dopamine, 0.5 pmol (A). The plasma was collected from a rat 15 min after the fourth infusion of verapamil (100 $\mu\text{g/kg/min}$, 51 $\mu\text{l/min}$) (B). The sample treatment procedure and the conditions for the HPLC-PO-CL detection system are described in the text.

Fig. 2. Relation between Mean Arterial Blood Pressure and the Logarithm of Plasma NE Concentration Obtained with the Infusion of Diltiazem (O), Verapamil (□) and Nicardipine (●) into SD Rats

The experimental conditions are described in the text.

beats/min; concentration of plasma NE, from 1.6 ± 0.1 to 2.8 ± 0.4 pmol/ml ($n=3$).

The mean arterial blood pressure reduction induced by the infusion of diltiazem correlated well with the logarithm of the plasma NE concentration with a coefficient of correlation, r , of -0.950 ($n=5$) (Fig. 2). The relation was expressed as $Y = -\alpha \log X + m$, where Y is mean arterial blood pressure, X the concentration of plasma NE, α a slope, and m an intercept. The values for α and m were 85.4 and 119.8, respectively. A similar good inverse correlation was also observed after the infusion of either verapamil or nicardipine (Fig. 2). As summarized in Table I, the good correlation (-0.950 , -0.975 and -0.978 for diltiazem, verapamil and nicardipine, respectively) for the three calcium antagonists indicate that the sympathetic nervous system, responding to the blood pressure re-

TABLE I. Relation between Mean Arterial Blood Pressure and the Logarithm of Plasma NE Concentration

	Diltiazem	Verapamil	Nicardipine	Control
α^a	85.4 ± 8.5	80.8 ± 5.5	108.4 ± 7.1	31.0 ± 8.7
m	119.8 ± 5.8	112.2 ± 4.5	135.3 ± 5.2	124.0 ± 3.6
r	-0.950	-0.975	-0.978	-0.734

Values are mean $\pm$ S.E. a) Comparison of the α s using the Student's t -test resulted in the following: diltiazem > control ($p < 0.01$); verapamil > control ($p < 0.02$); nicardipine > control ($p < 0.01$); nicardipine > diltiazem ($p < 0.05$); nicardipine > verapamil ($p < 0.02$). No significant difference was observed between the α s for others.

TABLE II. Relation between Mean Arterial Blood Pressure and the Logarithm of Plasma E Concentration

	Diltiazem	Verapamil	Nicardipine	Control
α^a	68.0 ± 8.3	73.5 ± 7.9	70.7 ± 7.6	30.0 ± 7.0
m	44.8 ± 6.9	32.0 ± 4.0	52.8 ± 7.2	94.3 ± 3.3
r	-0.928	-0.966	-0.948	-0.786

Values are mean $\pm$ S.E. a) Comparison of the α s using the Student's t -test resulted in the following: diltiazem > control ($p < 0.01$); verapamil > control ($p < 0.01$); nicardipine > control ($p < 0.01$). No significant difference was observed between the α s for others.

duction induced by each calcium antagonist, acted to release NE, as suggested in the previous paper.¹⁰⁾ Thus the signal to the sympathetic nervous system may be originated through a baroreceptor reflex.¹³⁾

The slopes (α s) of the three calcium antagonists (85.4, 80.8 and 108.4 for diltiazem, verapamil and nicardipine, respectively) were greater than that of the control (31.0, $p < 0.01$ –0.02). The α for nicardipine was greater than that of diltiazem or verapamil ($p < 0.02$ –0.05), whereas no significant difference was observed between the α s for diltiazem and verapamil ($p > 0.10$). This means that the blood pressure reduction with an identical increment of plasma NE concentration was greater in the case of nicardipine than either diltiazem or verapamil. This may indicate that the contribution of the sympathetic nervous system in maintaining the blood pressure reduced by nicardipine was less than that by diltiazem and verapamil. In the nicardipine group, however, the heart rate was maintained at a normal level until the second infusion, and the maintained heart rate might help the sympathetic nervous system to compensate for the blood pressure reduction.

An increase in the concentration of plasma E was also observed by the infusion of the three calcium antagonists: for diltiazem, from 0.11 ± 0.05 to 0.55 ± 0.25 pmol/ml; for verapamil, from 0.15 ± 0.04 to 0.41 ± 0.12 pmol/ml; and for nicardipine, from 0.14 ± 0.03 to 0.78 ± 0.30 pmol/ml. Similar relations to NE were also observed between the blood pressure and plasma E concentration ($r = -0.928$, -0.966 and -0.948 for diltiazem, verapamil and nicardipine, respectively as $Y = -\alpha \log X + m$, where Y is mean arterial blood pressure, X the concentration of plasma E, α the slope, and m the intercept) as shown in Table II. The α values were 68.0, 73.5 and 70.7 for diltiazem, verapamil and nicardipine, respectively. The data suggest that the sympathetic nervous system also stimulates the adrenal gland to release E to maintain the

TABLE III. Relation between Mean Arterial Blood Pressure and the Logarithm of Plasma DA Concentration

	Diltiazem	Verapamil	Nicardipine	Control
$\alpha^a)$	59.5 ± 23.9	-2.8 ± 34.7	97.0 ± 21.1	11.6 ± 6.7
m	55.5 ± 14.8	90.2 ± 20.5	26.6 ± 14.3	105.0 ± 4.7
r	-0.600	+0.032	-0.810	-0.463

Values are mean ± S.E. a) Comparison of the α s using the Student's *t*-test resulted in the following: diltiazem > control ($p < 0.01$); verapamil > control ($p < 0.02$); nicardipine > control ($p < 0.01$); nicardipine > verapamil ($p < 0.05$). No significant difference was observed between the α s for others.

TABLE IV. Relation between Heart Rate and the Logarithm of Plasma NE Concentration

	Diltiazem	Verapamil	Nicardipine	Control
$\alpha^a)$	182.0 ± 19.9	174.8 ± 11.3	113.4 ± 27.3	63.9 ± 16.0
m	415.0 ± 13.6	392.2 ± 9.2	419.9 ± 20.0	400.1 ± 6.7
r	-0.940	-0.978	-0.781	-0.768

Values are mean ± S.E. a) Comparison of the α s using the Student's *t*-test resulted in the following: diltiazem > control ($p < 0.01$); verapamil > control ($p < 0.02$); nicardipine > control ($p < 0.01$). No significant difference was observed between the α s for others.

blood pressure reduced by the calcium antagonists to a similar degree. It is interesting to note that the sympathetic nervous system and the adrenal gland act differently against the blood pressure reduction caused by different calcium antagonists, as indicated by the α values. In this sense, more data on α brought about by the two types of calcium antagonists, nicardipine and diltiazem (or verapamil), should be accumulated for understanding the role of the sympathetic nervous system on blood pressure regulation.

An increase in concentration of plasma DA, from 0.14 ± 0.04 to 0.44 ± 0.15 pmol/ml, was observed for nicardipine; however, infusion of diltiazem and verapamil did not afford such a gradual increase. Also only in the case of nicardipine was a good correlation ($r = -0.810$) observed (Table III).

The degree of heart rate reduction induced by nicardipine was different from that by diltiazem and verapamil. For easy understanding, the relations between heart rate and the plasma NE concentrations obtained by diltiazem, verapamil and nicardipine are depicted in Fig. 3. The values for α , m , and r are summarized in Table IV. Although the increase in plasma NE concentration, in other words, the onset of the mobilization of sympathetic nervous activity against the effect of the drugs occurred, the heart rate was maintained during the first and second dose infusions (0.50 and 1.01 $\mu\text{g/kg/min}$) at the same value as the starting point of the treatment with nicardipine, whereas the heart rates started to decrease from the first point of treatment with diltiazem and verapamil (Fig. 3). This phenomena might be related to the fact that nicardipine raises the heart rate but diltiazem and verapamil do not at low doses of injection (0.01 $\mu\text{g/kg}$,

Fig. 3. Relation between Heart Rate and the Logarithm of Plasma NE Concentration Obtained with the Infusion of Diltiazem (○), Verapamil (□) and Nicardipine (●) into SD Rats

The experimental conditions are described in the text.

i.v.) into dogs anesthetized with pentobarbital, while the three of them lower the heart rate at high doses of injection (more than 0.03 mg/kg).¹⁴ The present data are also consistent with reports on the direct effects of the three antagonists on the sinus node.¹⁵

In conclusion, plasma concentrations of CAs increased corresponding to blood pressure reduction after the administration of calcium antagonists. By investigating the correlation between plasma concentrations of CAs and blood pressure after the administration of drugs to affect the blood pressure in rats, the degree of contribution of the sympathetic nervous system to the regulation of blood pressure is thus evaluated.

REFERENCES

- 1) W. G. Nayler, *Eur. Heart J.*, **1**, 225 (1980).
- 2) O. L. Pedersen, E. N. Mikkelsen, J. Christensen, H. J. Kornerup, E. B. Pedersen, *Eur. J. Clin. Pharmacol.*, **15**, 235 (1979).
- 3) R. Pedrinelli, F. M. Fouad, R. C. Tarazi, E. L. Bravo, S. C. Textor, *Arch. Intern. Med.*, **146**, 62 (1986).
- 4) M. Bellet, P. Sassano, T. Guyenne, P. Corvol, J. Menard, *Br. J. Clin. Pharmacol.*, **24**, 465 (1987).
- 5) G. Mancina, G. Leonetti, G. B. Picotti, A. Ferrari, M. D. Galva, L. Gregorini, G. Parati, G. Pomidossi, C. Ravazzani, C. Sala, A. Zanchetti, *Clin. Sci.*, **57**, 165s (1979).
- 6) G. Leonetti, C. Cuspidi, L. Sampieri, L. Terzoli, A. Zanchetti, *J. Cardiovasc. Pharmacol.*, **4**, S319 (1982).
- 7) R. R. Evans, W. R. Davis, J. M. Wallace, D. J. DiPette, O. B. Holland, *Am. J. Hypertens.*, **3**, 605 (1990).
- 8) S. Higashidate, K. Imai, *Analyst*, **117**, 1863 (1992).
- 9) S. Kobayashi, K. Imai, *Anal. Chem.*, **52**, 424 (1980).
- 10) S. Higashidate, K. Imai, P. R. Prados, S. Adachi-Akahane, T. Nagao, *Biomed. Chromatogr.*, **8**, 19 (1994).
- 11) K. Imai, M. Sugiura, Z. Tamura, *Chem. Pharm. Bull.*, **19**, 409 (1971).
- 12) S. Kobayashi, K. Imai, *Anal. Chem.*, **52**, 1548 (1980).
- 13) H. Nakaya, A. Schwartz, W. Millard, *Circ. Res.*, **52**, 302 (1983).
- 14) T. Takenaka, M. Asano, I. Miyazaki, K. Shiono, *Kiso To Rinsho*, **14**, 4477 (1980).
- 15) H. Sato, K. Tsuchiya, *J. Cardiovasc. Pharmacol.*, **21**, 685 (1993).