Effects of CV-4093, a New Dihydropyridine Calcium Channel Blocker, on Renal Hemodynamics and Function in Stroke-Prone Spontaneously Hypertensive Rats (SHRSP)

Shiro MORIMOTO, Tadashi OHYAMA, Kazuhiro HISAKI and Yasuo MATSUMURA
Department of Pharmacology, Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan
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Abstract—Renal effects of CV-4093, a newly developed dihydropyridine calcium channel blocker, were examined using anesthetized stroke-prone spontaneously hypertensive rats, and the findings were compared with those of nicardipine. An intravenous injection of CV-4093 (2 μg/kg) produced long-lasting hypotension with a slow-onset accompanied by moderate renal vasodilation. There were no appreciable alterations in glomerular filtration rate (GFR) and urine formation, except that urine flow (UF) increased significantly during the first 10 min after injection. When CV-4093 was administered at 10 μg/kg, the hypotensive action was markedly augmented. Eighty minutes after the injection, a decrease in mean arterial pressure of about 45 mmHg was observed. Simultaneously, renal blood flow increased significantly from the control value of 5.76±0.46 ml/g•min to 6.94±0.28 ml/g•min. Renal vascular resistance decreased immediately after the injection, and the response lasted for over 3 hr, thereby indicating the marked and sustained renal vasodilating effect of CV-4093. GFR was constant throughout the experiment, but UF and urinary excretion of sodium were increased significantly. Fractional excretion of sodium was also elevated, thereby suggesting an inhibitory action of CV-4093 on renal tubular reabsorption of sodium. Nicardipine at a dose of 10 μg/kg, a dose producing an effective hypotensive action, caused no significant increases in RBF and urine formation. The renal vasodilating and diuretic actions of CV-4093 may provide a beneficial effect in the treatment of hypertension.

Dihydropyridine calcium channel blockers have been used in the treatment of hypertension (1). The antihypertensive effect of these drugs is due primarily to a decrease in peripheral vascular resistance, resulting from the inhibition of the calcium influx into vascular smooth muscle cells mainly through the voltage-dependent calcium channels (2). CV-4093, 2-(4-diphenylmethyl-1-piperazinyl)ethyl methyl(±)-1,4-dihydro-2,6-dimethyl-4-(m-nitrophenyl)-3,5-pyridinedicarboxylate dihydrochloride, is a new dihydropyridine calcium channel blocker (3). The inhibitory effects of CV-4093 on K+ induced contraction and 45Ca influx in various vascular tissues have already been reported (4). This agent can exert a more potent and long-lasting antihypertensive action in spontaneously hypertensive, renal hypertensive and deoxycorticosterone acetate-salt hypertensive rats than in normotensive rats (5).

It has been shown that the onset of cerebrovascular lesions in stroke-prone spontaneously hypertensive rats (SHRSP) can be delayed by controlling the renal perfusion pressure, suggesting a possible relationship between the pathogenesis of cerebrovascular lesions in SHRSP and renal hemodynamic changes (6). CV-4093 has been reported to be markedly effective in preventing the development of stroke in SHRSP, compared with nicardipine (7). These findings led us to
evaluate the renal actions of CV-4093 in SHRSP. In the present study, the effects of CV-4093 on renal hemodynamics and function were investigated using SHRSP and were compared with those of nicardipine.

Materials and Methods
Animal preparation: Male SHRSP (13 weeks old) and age-matched Wistar Kyoto rats (WKY) were used. The source of the animals has been reported elsewhere (8, 9). Each rat was fasted beginning 18 hr before the experiments. Tap water was available ad libitum. Animals were anesthetized with sodium thiobutabarbital (100 mg/kg, i.p.) and placed on a heated surgical tray that maintained a rectal temperature between 37°C and 38°C. After tracheotomy, the right femoral vein was cannulated for infusion of physiological saline containing 1% inulin and for bolus injection of drugs. The left carotid and right femoral arteries were also cannulated for blood sampling and to measure mean arterial pressure (MAP), respectively. After an abdominal midline incision was made, the left kidney was exposed, and the renal artery was carefully stripped of connective tissue, followed by the application of 5% phenol in 70% ethanol to exclude the influence of sympathetic nerve activity. An electromagnetic flow probe (1.0 mm in diameter, Nihon Kohden) connected to a square-wave flowmeter (MFV-2100, Nihon Kohden) was positioned on the renal artery to measure renal blood flow (RBF). A polyethylene cannula was inserted into the left ureter for urine collection. The urinary bladder was cannulated to ensure free drainage of urine from the right kidney. At the end of the surgical operation, about 2 ml of inulin solution was infused slowly to supplement the loss of body fluid and as a priming dose of inulin (about 70 mg/kg); this was followed by a sustained infusion of the same solution at a rate of 0.04 ml/min. MAP and RBF were continuously recorded on a polygraph (RM 6000, Nihon Kohden), throughout all the experiments. A 60- to 90-min period was allowed for stabilization of MAP, RBF and urine flow (UF).

Experimental protocol: After the equilibration period, urine samples were collected during two 20-min control clearance periods. Following the control periods, CV-4093 (2 or 10 µg/kg) or nicardipine (10 µg/kg) was injected intravenously (injection volume, about 0.2 ml). In the control experiments, the vehicle solution (physiological saline containing 2.5% polyethylene glycol-400 and 0.2% ethanol) was administered. During the first 10 min after injection, UF was measured but urine was not collected in order to take into account the dead space in the collection system. Following this, urine samples were collected during four consecutive 20-min periods. Blood samples (0.2 ml each) were obtained at 20 min before drug or vehicle injection and at 30 min and 70 min after the injection, respectively. The blood loss was supplemented by an equal volume of physiological saline. Plasma was immediately separated by centrifugation.

Analytical procedures: Urine and plasma inulin levels were measured by spectrofluorometry (Hitachi 650-60) according to the method of Vurek and Pegram (10). Glomerular filtration rate (GFR) was calculated from the inulin clearance. Urine and plasma sodium concentrations were determined using a flame photometer (Hitachi 205D).

Fractional excretion of sodium (FENS) was estimated as

\[
F_{\text{ENS}}(\%) = \frac{U_{\text{Na}}V}{(P_{\text{Na}} \times \text{GFR})} \times 100
\]

where \(U_{\text{Na}}V\) is urinary excretion of sodium and \(P_{\text{Na}}\) is the plasma sodium concentration.

Drugs: CV-4093 was a kind gift from Takeda Chemical Industries, Ltd. (Osaka, Japan). Nicardipine was purchased from Sigma Chemical Co. (St. Louis, MO). These drugs were dissolved in a mixture of 60% polyethylene glycol-400 and 40% ethanol and were then diluted with physiological saline. Drug solutions were freshly prepared before use.

Statistical analysis: The data in the two control periods were combined, and the mean values calculated. All results are expressed as the mean±S.E. Statistical analysis of the control data and the data after the drug administration was performed using the Kruskal-Wallis nonparametric analysis of variance followed by a Dunnett-type multiple comparison test. In comparing the basal values of renal hemodynamics and function in the
SHRSP and the WKY, Student’s unpaired t-test was used. Differences were considered to be significant at P<0.05.

Results

Table 1 summarizes the body weight, the left kidney weight and the basal renal hemodynamics and function under anesthesia in WKY and SHRSP at 13 weeks old. The body weight and the left kidney weight in SHRSP were slightly, but significantly, lower than those in WKY. The MAP, UF, U\text{Na,V} and FE\text{Na} were significantly higher in SHRSP than in WKY. There were no significant differences in other variables between the two strains of rats.

Table 2 shows the results of the control clearance experiments in anesthetized SHRSP. No significant changes in MAP and in renal hemodynamics and function were observed at any measuring point throughout the experiments.

The changes in MAP in SHRSP following intravenous injection of CV-4093 or nicardipine are shown in Fig. 1. At the lower dose (2 μg/kg), CV-4093 produced a long lasting hypotension with a slow-onset. A significant decrease in MAP was observed 60 min after the injection (Fig. 1A, approx. 20 mmHg decrease). When CV-4093 was administered at a higher dose (10 μg/kg), the hypotensive action was markedly augmented. Maximum response (approx. 45 mmHg decrease) was observed between 40-80 min after the injection, and this decrease was sustained for over 3 hr.

Nicardipine (10 μg/kg) produced a decrease of about 70 mmHg in MAP immediately after the administration (Fig. 1B), but thereafter the pressure restored gradually, although it was still lower than that in the control 2–3 hr after the injection.

Renal hemodynamic responses in SHRSP after the administration of CV-4093 or nicardipine are shown in Figs. 2–4. The administration of CV-4093 at 2 μg/kg caused an increase in RBF. One hour after the injection, an increase in RBF of about 20% was observed, but this increase was not statistically significant. Simultaneously, moderate but non-significant decreases in the calculated renal vascular resistance (RVR) were observed. Eighty minutes after an injection of 10 μg/kg of CV-4093, the RBF was 6.94±0.28 ml/g·min (P<0.05 versus the control value), having increased gradually from the control value of 5.76±0.46 ml/g·min (Fig. 2A). RVR decreased immediately after the injection, and this decrease was sustained for over 3 hr (Fig. 3). The administration of nicardipine (10 μg/kg) caused a transient decrease in RBF (Fig. 2B), but the decreased level gradually returned to the control level. The change in RVR following nicardipine administration was similar to that seen following administration of the lower dose of CV-4093 (Fig. 3). As shown in Fig. 4, there were no significant alterations in GFR after the injection of CV-4093 or nicardipine.

Table 3 summarizes the renal excretory responses in SHRSP following administration of CV-4093 or nicardipine. The lower dose of CV-4093 produced a significant increase in

| Table 1. Basal renal hemodynamics and function in anesthetized WKY and SHRSP |
|---------------------------------|-----------|-----------|
|                                 | WKY       | SHRSP     |
|                                 | (n=8)     | (n=9)     |
| MAP (mmHg)                      | 127±1     | 171±4***  |
| RBF (ml/g·min)                  | 6.22±0.84 | 5.71±0.70 |
| RVR (mmHg/ml·g·min)             | 25.6±5.7  | 35.6±7.1  |
| GFR (ml/g·min)                  | 1.01±0.09 | 1.28±0.12 |
| UF (ml/g·min)                   | 3.46±0.41 | 6.32±0.67**|
| U\text{Na,V} (μEq/g·min)        | 0.31±0.06 | 1.28±0.24**|
| FE\text{Na} (%)                 | 0.21±0.05 | 0.68±0.12**|
| Body wt. (g)                    | 295±5     | 270±3***  |
| Left kidney wt. (g)             | 1.35±0.04 | 1.23±0.03*|

Each value represents the mean±S.E. All values except body wt. and left kidney wt. are average values of data in two control clearance periods. *P<0.05, **P<0.01, ***P<0.001, compared with WKY.
Table 2. Renal hemodynamics and function in control experiments with SHRSP

| Time min | MAP mmHg | RBF ml/g·min | RVR mmHg/ml·g·min | GFR ml/g·min | UF μl/g·min | UNaV μEq/g·min | FENa % |
|----------|----------|--------------|-------------------|--------------|-------------|----------------|--------|
| -40-0    | 171±4    | 5.71±0.70    | 35.6±7.1          | 1.28±0.12    | 6.32±0.67   | 1.28±0.24      | 0.68±0.12    |
|          |          | Intravenous injection of the vehicle |                  |              |             |                |        |
| 10-30    | 170±5    | 5.62±0.66    | 36.3±7.2          | 1.16±0.11    | 5.81±0.87   | 1.23±0.23      | 0.73±0.15    |
| 30-50    | 167±4    | 5.67±0.62    | 34.4±6.1          | 1.16±0.12    | 5.30±0.57   | 1.06±0.16      | 0.64±0.11    |
| 50-70    | 163±3    | 5.76±0.58    | 32.1±5.1          | 1.19±0.12    | 5.03±0.47   | 1.14±0.17      | 0.72±0.19    |
| 70-90    | 164±4    | 5.78±0.55    | 32.1±5.1          | 1.24±0.10    | 5.22±0.49   | 1.16±0.18      | 0.68±0.17    |

Each value represents the mean±S.E. (n=9).

Table 3. Renal excretory responses in SHRSP to intravenous injection of CV-4093 or nicardipine

| Time min | CV-4093 (2 μg/kg, i.v.) | CV-4093 (10 μg/kg, i.v.) | Nicardipine (10 μg/kg, i.v.) |
|----------|-------------------------|---------------------------|-------------------------------|
|          | UF μl/g·min | UNaV μEq/g·min | FENa % | UF μl/g·min | UNaV μEq/g·min | FENa % | UF μl/g·min | UNaV μEq/g·min | FENa % |
| -40-0    | 6.63±1.60    | 1.70±0.51    | 0.84±0.30 | 6.29±0.94    | 1.37±0.29    | 0.63±0.09 | 7.95±1.67    | 1.67±0.47    | 0.82±0.22 |
| 0-10     | 17.20±5.62*  | ---          | ---      | 26.41±3.75** | ---          | ---      | 13.95±4.81   | ---          | ---      |
| 10-30    | 10.81±3.56   | 2.25±0.56    | 1.16±0.29 | 17.83±3.89** | 4.29±1.01*   | 1.99±0.40* | 10.70±1.92   | 2.54±0.52    | 1.26±0.29 |
| 30-50    | 7.72±2.21    | 1.51±0.38    | 0.82±0.18 | 11.24±2.54   | 2.99±0.73    | 1.45±0.32 | 10.56±2.07   | 2.44±0.45    | 1.25±0.26 |
| 50-70    | 5.52±1.35    | 1.08±0.29    | 0.54±0.11 | 8.60±1.81    | 2.35±0.58    | 1.08±0.23 | 8.97±1.96    | 1.98±0.38    | 0.99±0.18 |
| 70-90    | 4.92±1.22    | 1.17±0.25    | 0.57±0.12 | 6.31±1.10    | 1.78±0.45    | 0.77±0.15 | 6.85±1.47    | 1.45±0.33    | 0.76±0.18 |

Each value represents the mean±S.E. (n=9). *P<0.05, **P<0.01, compared with the values observed before the administration of CV-4093 or nicardipine.
Renal Action of CV-4093

UF during the first 10 min after the injection, but after this, the UF gradually returned to the control level. No significant changes in U\textsubscript{Na}V and FE\textsubscript{Na} were noted in any animal in the lower dose group throughout the experiments. The administration of the higher dose of CV-4093 caused a significant diuretic and natriuretic action. In the first clearance period (10–30 min after the injection), UF, U\textsubscript{Na}V and FE\textsubscript{Na} increased to 2.8, 3.1 and 3.2 times their control values, respectively, and then these values returned to the control levels gradually. No significant effects on urine formation were observed after the administration of nicardipine, although UF was increased slightly during the first 10 min after the injection.

Fig. 1. Changes in mean arterial pressure (MAP) in SHRSP after intravenous injection of CV-4093 or nicardipine. CV-4093 (○, 2 μg/kg; ●, 10 μg/kg) or nicardipine (△, 10 μg/kg) was injected at the time noted by the arrow. The lower panel indicates the data obtained during the first 10 min after the injection. Each point and bar represents the mean±S.E. (n=9). *P<0.05, **P<0.01, compared with the values observed before the administration of CV-4093 or nicardipine.

![Graph showing changes in MAP](image-url)
Discussion

The antihypertensive action of CV-4093 in spontaneously hypertensive rats have been reported to be much more potent and long-lasting than those seen with well-known calcium channel blockers such as nifedipine, nicardipine, verapamil and diltiazem (5). In our study, CV-4093 elicited a long-lasting hypotensive action in anesthetized SHRSP and was more potent than nicardipine in this respect. Similar results have been obtained with conscious SHRSP (6). In vitro studies, CV-4093 has been shown to inhibit high potassium-induced vasoconstriction in various vascular tissues (4, 11). Okabe et al. (12) demonstrated that CV-4093 caused highly selective and long-lasting inhibition of the Ca inward current in single smooth muscle cells of rabbit main pulmonary artery. Since this inhibitory action of CV-4093 was not easily reversed by wash-out, CV-4093 may bind
Fig. 3. Changes in renal vascular resistance (RVR) in SHRSP after intravenous injection of CV-4093 or nicardipine. Other details and symbols are the same as in Fig. 1.

Fig. 4. Changes in glomerular filtration rate (GFR) in SHRSP after intravenous injection of CV-4093 or nicardipine. CV-4093 (□, 2 μg/kg; ■, 10 μg/kg) or nicardipine (△, 10 μg/kg) was injected at the time noted by the arrow. Each column and bar represents the mean±S.E. (n=9).
firmly to the site of action, which is considered to be responsible for the long-lasting hypotensive action of the compound (4, 12).

In the present study, when CV-4093 (10 μg/kg) was injected intravenously into anesthetized SHRSP, a potent renal vasodilating effect lasting for more than 3 hr was observed. On the other hand, the same dose of nicardipine did not show any significant renal vasodilating effect. Nagaoka et al. (6) demonstrated that the onset rate and incidence of cerebrovascular lesions in salt-loaded SHRSP were lowered by controlling the renal perfusion pressure with mild aortic clamping and proposed that the alteration of renal hemodynamics and function induced by the increased renal perfusion pressure, and the resultant renal vascular changes may play an important role in the development of cerebrovascular lesions in SHRSP. The difference between the effects of CV-4093 and nicardipine on renal vasculature observed in our study may partially explain the finding that the former drug was much more effective than the latter in preventing the development of stroke in SHRSP (7).

There is accumulating evidence with respect to the diuretic and natriuretic properties of calcium channel blockers, regardless of whether this effect is mediated by a direct tubular action or is secondary to changes in renal hemodynamics (13, 14). The results of several in vivo studies, using anesthetized rats (15–17) and dogs (18–21), have indicated that the diuretic and natriuretic actions induced by calcium channel blockers are due to a direct inhibition of tubular sodium and water reabsorption. Micropuncture studies using felodipine (22) and nitrendipine (23) strongly support the above findings. In our study, CV-4093 caused increases in UF, UNaV, and FENa after the higher dose of CV-4093 disappeared between 1–1.5 hr after the injection (Table 3), after which the three variables remained at their respective control levels, in contrast to the compound’s long-lasting hypotensive and renal vasodilating effects. A previous study (24) using anesthetized SHRSP demonstrated that the reduction of MAP in SHRSP to a level similar to that in WKY, by aortic constriction, produces marked decreases in sodium and water excretion. In the present experiments, the higher dose of CV-4093 reduced MAP in SHRSP to a level similar to that seen in WKY, and this decreased level was sustained for over 3 hr. However, decreased urine formation was not observed. It is therefore conceivable that the diuretic and natriuretic properties of CV-4093 are responsible for the maintenance of urine formation during antihypertensive treatment.

In conclusion, CV-4093, a new dihydropyridine calcium channel blocker, exerted a marked and long-lasting antihypertensive action in SHRSP. Renal vasodilating and diuretic properties of CV-4093 may contribute to the beneficial effects of this agent in the treatment of hypertension.

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