Antihypertensive Effect of CV-4093·2HCl, a New Calcium Antagonist, in Three Rat Models of Hypertension

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Abstract—The hypotensive action of CV-4093·2HCl (CV-4093), a new calcium antagonist, was studied in spontaneously hypertensive, renal hypertensive, DOCA-salt hypertensive and normotensive rats. CV-4093 (3 and 10 mg/kg, p.o.) dose-dependently decreased systolic blood pressure in the three types of hypertensive rats. At the dose of 10 mg/kg, the compound decreased the blood pressure to the normotensive level between 1 and 3 hr after it was administered; the antihypertensive effect lasted for at least 8 hr. The systolic blood pressure in normotensive rats was also decreased at 3 and 10 mg/kg, p.o., but less evidently than in the hypertensive rats. When the antihypertensive effect of CV-4093 was compared with that of seven known calcium antagonists in spontaneously hypertensive rats, it was the most potent and the most long-lasting.

Calcium antagonists interfere with the excitation-contraction coupling mechanism in smooth and cardiac muscle by decreasing the influx of Ca^{2+} (1). They reduce the contractile activity of the heart, and prompt coronary and systemic vasodilation. Since this latter effect reduces elevated blood pressure, calcium antagonists are now being used clinically to treat hypertension (2, 3).

CV-4093·2HCl (CV-4093), methyl 2-(4-diphenylmethyl-1-piperazinyl)ethyl(±)-1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)-3,5-pyridinedicarboxylate dihydrochloride, is a new calcium antagonist (4). CV-4093 has long-lasting inhibitory actions on calcium currents in smooth muscle cells of the rabbit pulmonary artery (5), and it shows an antihypertensive action without a decrease in cardiac output in spontaneously hypertensive rats of stroke-prone strain (SHR) (6). In the present report, the effect of CV-4093 on blood pressure was studied in three types of experimental hypertension: SHR, deoxycorticosterone acetate (DOCA)-salt hypertensive rats and renal hypertensive rats. The hypotensive effect of CV-4093 was compared with that of seven known calcium antagonists in SHR.

Materials and Methods

Animals: Normotensive male Wistar-Kyoto rats, 10 weeks of age, and male stroke-prone SHR, 9–11 weeks of age, were used. The source of the SHR has been reported elsewhere (7). Renal hypertensive rats (two-kidney, one-clip Goldblatt type) were produced by the following method: Under ether anesthesia, the left renal artery of male Wistar-Kyoto rats (6 weeks of age) was clamped by a silver ribbon (slit width, 0.23 mm). Systolic blood pressure was measured once a week. Rats with a systolic blood pressure of 170–200 mmHg were used between 3 and 4 weeks after surgery. DOCA-salt hypertensive rats were produced by the following procedures: The left kidney of male Wistar-Kyoto rats (7 weeks of age) was removed under pentobarbital anesthesia (50 mg/kg, i.p.). The animals were maintained on a 1% NaCl solution as drinking water and subcutaneously injected with 5 mg/kg DOCA suspended in 5% arabic gum once a week. Those with a systolic blood pressure of 170–200 mmHg were used between 3 and 5 weeks after surgery.

Measurement of systolic blood pressure:

Systolic blood pressure in the tail artery was measured using a pulse pick-up method (7). The initial blood pressure was measured in the afternoon of the day before the experiment. The changes in systolic blood pressure were measured 1, 3, 5, 8 and 24 hr after the calcium antagonists were administered.

Drugs: CV-4093, nicardipine·HCl (nicardipine), nifedipine, nimodipine, felodipine, diltiazem·HCl (diltiazem), verapamil·HCl (verapamil) and cinnarizine were synthesized in our Central Research Division. All drugs, except verapamil which was dissolved in saline, were suspended in 5% arabic gum. All were given orally.

Statistical analysis: The ED20 value, which is the dose required to decrease systolic blood pressure of the rats by 20% of the related initial value, was calculated by regression analysis. Statistical differences were evaluated using two-way analysis of variance followed by Scheffe's test.

Results

Hypotensive effect of CV-4093: The hypotensive effect of CV-4093 (1, 3 and 10 mg/kg, p.o.) on normotensive rats is shown in Table 1. The change in systolic pressure is expressed as a percent change from the related initial value. CV-4093 caused a dose-dependent decrease in blood pressure. Oral doses of 3 and 10 mg/kg reduced the blood pressure by 8.3 and 35.6%, respectively, 1 hr after it was administered. The hypotensive effect of the highest dose lasted for at least 8 hr. The antihypertensive effects of CV-4093 on the three types of hypertensive rats were compared with those of nicardipine (Table 2). Both drugs decreased the systolic pressure

in a dose-dependent manner. The maximum effect of 10 mg/kg of CV-4093 was observed between 1 and 3 hr, and the hypotensive action lasted until at least 8 hr after it was administered in the three types of hypertensive rats. When the antihypertensive effects were compared at the same dose, CV-4093 was more potent than nicardipine in all three models. The ED20 values of CV-4093 were 2.3, 2.4, 3.0 and 4.3 mg/kg in renal hypertensive, DOCA-salt hypertensive, SHR and normotensive rats, respectively.

Comparison with known calcium antagonists: The hypotensive action of CV-4093 was compared with that of seven known calcium antagonists including nicardipine in SHR (Tables 2 and 3). The dose-dependencies of the hypotensive effects of the drugs are summarized in Fig. 1. All compounds, except cinnarizine, dose-dependently decreased systolic blood pressure. Cinnarizine did not reduce blood pressure. The calculated ED20 values were as follows: CV-4093, 3.0; felodipine, 4.4; nifedipine, 5.6; nicardipine, 10.4; nimodipine, 28.0; verapamil, 33.2; diltiazem, 100 mg/kg. CV-4093 and verapamil showed more persistent action than the others when the duration of hypotensive action was compared at the dose which reduced the systolic pressure by 15–19% of the related initial value 1 hr after the drugs were administered (Table 4). The action of CV-4093 (3 mg/kg) and verapamil (30 mg/kg) persisted for at least 8 hr; the actions of nimodipine (30 mg/kg) and diltiazem (100 mg/kg) diminished within 8 hr; and the actions of felodipine (3 mg/kg), nifedipine (5 mg/kg) and nicardipine (10 mg/kg) diminished within 5 hr.

Table 1. Hypotensive effect of CV-4093 in normotensive rats

Dose (mg/kg)	N	Initial B.P. (mmHg)	% Change				
			1 hr	3 hr	5 hr	8 hr	24 hr
Vehicle	5	125±1	1.1±0.6	1.1±0.8	2.4±0.3	2.4±0.5	2.7±0.6
1	5	127±1	-0.6±1.0	0.5±0.4	1.0±0.3	0.5±0.6	1 ±0.4*
3	5	124±1	-8.3±1.1**	-5.5±0.8*	0 ±0.7	1.4±0.2	1.9±0.4
10	5	127±1	-35.6±2.9**	-35.5±0.9**	-30.3±0.7**	-23.4±0.8**	0.3±0.7*

Each value represents the mean±S.E.M. *P<0.05, **P<0.01, significantly different from rats injected with the vehicle at the corresponding time. N=number of rats used.

Table 2. Hypotensive effects of CV-4093 and nicardipine in SHR, DOCA-salt and renal hypertensive rats

Drugs	Dose (mg/kg)	N	Initial B.P. (mmHg)	1 hr	3 hr	% Change 5 hr	8 hr	24 hr
SHR								
Vehicle		15	185±1	1.7±0.8	1.0±1.0	0.3±0.9	1.4±0.7	4.1±0.8
CV-4093	1	5	184±1	-1.8±0.6	1.1±0.5	1.6±0.5	1.1±0.8	4.0±0.8
	3	5	183±2	-15.4±1.3**	-13.1±1.7**	-7.8±0.7**	-5.5±0.8**	4.0±0.6
	10	5	181±1	-43.9±0.6**	-42.3±0.8**	-34.1±0.4**	-28.8±0.5**	4.3±0.4
Nicardipine	3	5	181±3	-3.6±0.9**	0.1±0.9	0.4±0.4	1.4±1.4	2.3±1.0
	10	5	184±3	-19.2±1.1**	-6.1±1.8**	1.4±1.4	0.7±1.1	2.6±1.5
	30	5	189±1	-34.4±1.0**	-34.6±1.1**	-23.1±0.7**	-17.5±0.6**	3.6±0.4
DOCA-salt hypertensive rats								
Vehicle		10	181±4	2.1±0.7	1.0±1.0	0.4±0.8	-5.1±1.3	1.1±1.0
CV-4093	1	8	182±3	-6.7±1.4	-5.8±2.1	-2.7±1.6	-4.9±1.2	0.4±1.3
	3	9	187±3	-20.1±1.9**	-19.1±1.5**	-12.3±1.3**	-13.8±1.6**	0.4±0.9
	10	8	182±3	-47.1±1.9**	-46.6±1.9**	-37.7±3.2**	-31.6±2.6**	-2.9±1.8
Nicardipine	3	8	180±3	-3.4±1.8	-2.5±1.5	-1.2±1.4	-4.1±2.0	3.9±1.8
	10	9	184±3	-29.4±2.8**	-11.7±1.4**	-0.4±1.1	-5.0±1.6	1.2±1.3
Renal hypertensive rats								
Vehicle		8	185±3	1.2±1.3	0.7±1.0	-0.3±1.6	-2.4±1.4	4.8±1.1
CV-4093	1	8	182±2	-5.0±2.0	-5.5±1.9	-2.9±1.5	-0.5±0.9	4.4±1.4
	3	8	184±3	-24.5±2.4**	-19.9±2.4**	-7.4±0.8	-5.1±1.5	2.6±1.9
	10	8	186±3	-48.5±1.0**	-49.5±1.6**	-42.2±2.2**	-30.8±3.2**	-6.4±3.9*
Nicardipine	3	8	185±4	-2.1±1.7	1.5±1.3	3.2±1.6	-1.6±2.5	5.9±1.4
	10	8	183±3	-23.5±4.0**	-12.4±1.9**	-2.5±1.0	-2.1±1.1	3.9±1.3

Each value represents the mean±S.E.M. *P<0.05, **P<0.01, significantly different from rats injected with vehicle at the corresponding time. N=number of rats used.

Table 3. Hypotensive effects of six calcium antagonists in SHR

Drugs	Dose (mg/kg)	N	Initial B.P. (mmHg)	1 hr	3 hr	% Change 5 hr	8 hr	24 hr
Vehicle		5	187±3	3.2±0.7	1.7±1.5	0.6±1.0	0.5±0.7	3.8±1.8
Nifedipine	1	5	199±1	1.0±0.5	1.6±0.3	1.7±0.4	2.1±0.4	2.1±0.2
	3	5	185±2	-8.9±1.5**	-0.4±1.0	1.6±1.2	2.9±1.4	2.3±1.7
	5	5	198±2	-14.9±0.6**	-9.1±0.7**	-1.9±0.6	0.9±0.2	2.6±0.2
	10	5	187±3	-31.4±2.7**	-18.2±2.0**	-3.7±3.5	1.8±0.5	2.1±1.7
Vehicle		5	189±1	1.6±0.5	0.6±0.2	0.6±0.7	1.5±0.5	4.1±0.5
Felodipine	1	5	187±1	0.7±0.5	2.2±0.3	1.1±0.7	1.2±0.5	4.1±0.3
	3	5	185±2	-17.1±0.6**	-8.5±0.7**	-1.2±0.7	1.5±0.4	2.0±0.5
	10	5	186±2	-29.4±0.7**	-26.9±0.6**	-18.4±0.9**	-10.8±1.2**	1.5±0.8*
Vehicle		5	195±2	1.0±1.0	1.2±0.6	2.2±0.5	2.6±0.2	3.3±0.9
Nimodipine	3	5	179±1	0.9±0.5	1.8±0.5	0.9±0.4	2.7±0.2	2.7±0.3
	10	5	196±2	-11.3±0.5**	-12.0±0.8**	-0.1±0.7	1.8±0.4	2.6±0.5
	30	5	181±1	-16.7±1.5**	-16.9±0.6**	-7.4±0.7**	-0.4±0.6	1.9±0.3
	100	5	191±1	-27.6±0.9**	-34.2±1.1**	-33.8±0.9**	-31.8±1.2**	3.5±0.3
Vehicle		10	189±2	0.5±0.4	0 ±0.4	0.3±0.5	1.2±0.4	4.1±0.6
Diltiazem	30	5	184±2	0.5±0.6	1.3±1.1	2.1±1.1	1.9±1.2	4.3±1.2
	100	5	194±2	-15.7±0.9**	-9.9±0.5**	-1.1±0.6*	0.7±0.6	2.3±0.5
Vehicle		5	172±1	-0.5±0.6	0.4±0.5	1.7±0.4	1.2±0.5	3.4±0.3
Verapamil	10	5	182±1	0.2±0.9	1.5±0.8	0.7±0.5	0.6±0.7	3.0±0.6
	30	5	183±1	-16.0±0.3**	-16.0±0.7**	-9.5±0.5**	-5.3±0.8**	2.2±0.6
	100	5	170±1	-36.5±0.8**	-41.6±0.6**	-41.8±0.5**	-37.2±0.5**	2.6±0.5
Cinnarizine	30	5	180±2	1.6±0.6	2.0±0.5	3.2±0.6	2.1±0.6	N.D.
	100	5	181±1	0.4±0.7	2.1±0.4	1.8±0.7	1.4±0.2	N.D.

N.D.=not determined. Each value represents the mean±S.E.M. *P<0.05, **P<0.01, significantly different from rats injected with vehicle at the corresponding time. N=number of rats used.

Table 4. Duration of hypotensive action of CV-4093 and other calcium antagonists in SHR

Drugs	Dose (mg/kg)	% Change at a peak period	Duration of action (hr)
CV-4093	3	-15	8
Verapamil	30	-16	8
Nimodipine	30	-17	5
Diltiazem	100	-16	5
Felodipine	3	-17	3
Nifedipine	5	-15	3
Nicardipine	10	-19	3

Fig. 1. Antihypertensive effect of CV-4093 and other calcium antagonists in SHR. (1) CV-4093, (2) felodipine, (3) nifedipine, (4) nicardipine, (5) nimodipine, (6) verapamil, (7) diltiazem, (8) cinarizine.

Discussion

The contractility of smooth muscle depends on the intracellular Ca^{2+} ions to activate myofibrillar ATPase which generates the contractile energy (8–10). Not only a greater responsiveness to added Ca^{2+} and higher membrane permeability to Ca^{2+} in the arteries (11, 12) but also an increase in calcium contents of cardiovascular tissues (13, 14) have been reported in experimental hypertensive animals. These findings suggest that intracellular Ca^{2+} is greatly involved in the development of hypertension and that a new calcium antagonist that selectively acts on resistance vessels will be useful for treating hypertension. Recently, Nakaya et al. (15) reported that CV-4093 is a calcium antagonist with little cardiodepressant action, but with a highly selective vascular effect.

In the present study, CV-4093 administered p.o. caused a dose-dependent decrease in systolic blood pressure in normotensive and three types of hypertensive rats. The hypotensive effect of CV-4093, however, was greater in hypertensive rats than in normotensive rats. This may be due to a correction of the abnormal calcium availability in the vasculature of these hypertensive rats. The antihypertensive effect of CV-4093 was compared with that of seven known calcium antagonists. The calculated ED₂₀ value of CV-4093 was the smallest, and CV-4093 was one of the compounds that exerted the most persistent effect. These results indicate that among the calcium antagonists examined, CV-4093 is the most potent and long-lasting antihypertensive agent.

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