

Effects of Benidipine in a Rat Model of Experimental Angina

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To compare the antianginal effects of 1,4-dihydropyridine-type calcium-channel blockers, we evaluated the effects of benidipine, amlodipine, nifedipine, and efonidipine on vasopressin-induced myocardial ischemia in rats, an experimental model of angina. Intravenous administration of benidipine (3 $\mu\text{g}/\text{kg}$), amlodipine (1000 $\mu\text{g}/\text{kg}$), and nifedipine (100 $\mu\text{g}/\text{kg}$) suppressed the vasopressin-induced S-wave depression, an index of myocardial ischemia. Efonidipine (100 $\mu\text{g}/\text{kg}$, *i.v.*) tended to inhibit the S-wave depression. At the antianginal dose of each drug, amlodipine, nifedipine, and efonidipine decreased blood pressure significantly, whereas benidipine had little effect on blood pressure at a dose of 3 $\mu\text{g}/\text{kg}$. These results indicate that benidipine, unlike the other 1,4-dihydropyridine-type calcium-channel blockers examined in this study, inhibits vasopressin-induced coronary vasospasm with fewer undesirable effects such as hypotension in rats, suggesting that benidipine may be useful in the treatment of angina pectoris.

Key words—1,4-dihydropyridine; benidipine; angina

INTRODUCTION

Calcium-channel blockers are prescribed widely for the treatment of cardiovascular disorders such as angina pectoris and hypertension. They are classified into the following three groups on the basis of chemical structure, which exhibit different pharmacologic and therapeutic properties: 1) 1,4-dihydropyridines (DHPs), 2) benzothiazepine, and 3) phenylalkylamines.¹⁾ DHPs exhibit higher selectivity for vasculature than benzothiazepine (diltiazem) and have less effect on nodal tissue of the heart.¹⁾ Benidipine is a DHP-derived calcium-channel blocker with slow-onset and long-lasting vasodilating effects and is clinically useful in the treatment of hypertension and angina pectoris.^{2,3)}

Arginine vasopressin (AVP) is an antidiuretic hormone and a vasoconstrictor, as demonstrated in dogs, where AVP produces vasoconstriction of small coronary arteries and increases total coronary resistance.⁴⁾ AVP-induced angina is a useful model for evaluating the efficacy of compounds in vasospastic angina.^{5–8)} Karasawa et al.⁵⁾ reported that benidipine has beneficial effects in various experimental angina models, including the AVP-induced angina model. We have demonstrated recently that the antianginal efficacy of benidipine 10 $\mu\text{g}/\text{kg}$ was similar to that of diltiazem

1000 $\mu\text{g}/\text{kg}$ in the AVP-induced angina rat model, indicating that benidipine exerts potent antivasospastic anginal effects.⁹⁾ However, there are no reports of studies comparing the antianginal effects of benidipine with those of amlodipine and efonidipine. Efonidipine is an L- and T-type dual calcium-channel blocker, whereas nifedipine and amlodipine are L-type calcium-channel blockers.¹⁰⁾ Although amlodipine was demonstrated to inhibit L- and N-type calcium channels, the effect of amlodipine on the N-type calcium channel is controversial.^{11–13)} It has been reported that benidipine inhibits not only the L-type calcium channel, but also especially inhibits the N- and T-type calcium channels.³⁾ In an effort to evaluate the effectiveness of benidipine among DHP-derived calcium-channel blockers, we investigated the comparative antianginal effects of benidipine, amlodipine, nifedipine, and efonidipine using the AVP-induced angina model.

MATERIALS AND METHODS

Experimental Animals Male Donryu rats (8–9 weeks old; Japan SLC, Shizuoka, Japan) were used in this study. Rats were kept at $23 \pm 1^\circ\text{C}$ under a 12-h light-dark cycle, and had free access to tap water and commercial chow (FR-2, Funabashi Farms, Chiba, Japan). All animals received humane care in compliance with the ethical standards formulated in the guidelines issued by the Science and International

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Affairs Bureau of the Japanese Ministry of Education, Science, Sports and Culture, No. 141, 1987: "Animal Experiments in Universities etc.," and the protocol was approved by the Bioethical Committee of the Pharmaceutical Research Center, Kyowa Hakko Kogyo Co., Ltd.

Drugs Benidipine (hydrochloride), amlodipine (besilate), and efonidipine (hydrochloride) were synthesized at Kyowa Hakko Kogyo (Tokyo, Japan). Nifedipine (hydrochloride) and AVP were purchased from Sigma (St. Louis, MO, USA). Benidipine, amlodipine, efonidipine, and nifedipine were dissolved in physiologic saline containing 0.1 vol% Tween 80 (Wako Pure Chemical, Osaka, Japan). Drug concentrations were adjusted to yield an injection volume of 0.5 ml/kg of body weight. The doses of benidipine and nifedipine were selected on the basis of a study conducted by Karasawa et al.⁵⁾ The dose of amlodipine was selected in reference to a previous report.¹⁴⁾ The doses of efonidipine were selected according to the study by Sato et al.¹⁵⁾ AVP was diluted with physiologic saline to a concentration of 1 IU/ml solution.

Effects on Vasopressin-Induced Angina Model in Rats The antianginal effects of the drugs were assessed according to the method of Hirata et al.⁶⁾ Rats were anesthetized with sodium pentobarbital (60 mg/kg, *i.p.*). The standard limb lead II electrocardiogram (ECG) was recorded to measure S-wave depression. The rats were allocated to the following 11 groups ($n=8$ /group): vehicle; benidipine 1 $\mu\text{g}/\text{kg}$; benidipine 3 $\mu\text{g}/\text{kg}$; benidipine 10 $\mu\text{g}/\text{kg}$; amlodipine 100 $\mu\text{g}/\text{kg}$; amlodipine 300 $\mu\text{g}/\text{kg}$; amlodipine 1000 $\mu\text{g}/\text{kg}$; nifedipine 30 $\mu\text{g}/\text{kg}$; nifedipine 100 $\mu\text{g}/\text{kg}$; efonidipine 10 $\mu\text{g}/\text{kg}$; and efonidipine 100 $\mu\text{g}/\text{kg}$. The test drugs were injected intravenously 5 min prior to the injection of AVP. AVP (1 IU/kg) was injected into the jugular vein, and ECG changes were recorded for the first 10 min following AVP injection.

Effects on Blood Pressure and Heart Rate in Rats In a separate experiment, the effects of vehicle, benidipine (1, 3, and 10 $\mu\text{g}/\text{kg}$), amlodipine (100, 300, and 1000 $\mu\text{g}/\text{kg}$), nifedipine (30 and 100 $\mu\text{g}/\text{kg}$), and efonidipine (100 $\mu\text{g}/\text{kg}$) on blood pressure and heart rate were measured in normal rats anesthetized with sodium pentobarbital (60 mg/kg, *i.p.*; each group consisted of 5 animals). Blood pressure was recorded on a polygraph through a blood pressure transducer connected to a cannula inserted into the carotid artery, and heart rate was measured based

on the blood pressure pulse wave. Since AVP was injected 5 min after the intravenous administration of each drug, the changes in blood pressure and heart rate at that time were summarized as the cardiovascular effects of the tested drugs.

Statistical Analysis All values are expressed as mean \pm S.E. All statistical calculations were performed using a computer and statistical analysis software (SAS, version 9.1.3, SAS Institute, Inc., Cary, NC, USA). Statistical analysis of the S-wave was performed using the Kruskal-Wallis test followed by Steel's test for multiple comparisons. Statistical analysis of blood pressure or heart rate was performed using the Wilcoxon rank-sum test for comparison between two groups, or using the Kruskal-Wallis test followed by Steel's test for multiple comparisons. A difference was considered statistically significant at $p < 0.05$.

RESULTS

When AVP was injected in the rats, a depression of the S-wave in the lead II ECG appeared (Fig. 1), suggesting subendocardial ischemia.¹⁶⁾ Changes in the ST-segment were used as an index of ischemic severity. According to a previous report,⁵⁾ the fall of the S-wave was regarded as the fall of ST. Maximal S-wave depression was observed about 4 min after AVP injection (data not shown), and is consistent with the results of previous reports.⁷⁻⁹⁾ Benidipine significantly inhibited the depression at 3 and 10 $\mu\text{g}/\text{kg}$ in a dose-dependent manner (Fig. 2). Amlodipine 1000 $\mu\text{g}/\text{kg}$ and nifedipine 100 $\mu\text{g}/\text{kg}$ also significantly inhibited the S-wave depression. Efonidipine 100 $\mu\text{g}/\text{kg}$ tended to inhibit the S-wave depression.

Benidipine 10 $\mu\text{g}/\text{kg}$, amlodipine 1000 $\mu\text{g}/\text{kg}$, nifedipine 100 $\mu\text{g}/\text{kg}$, and efonidipine 100 $\mu\text{g}/\text{kg}$

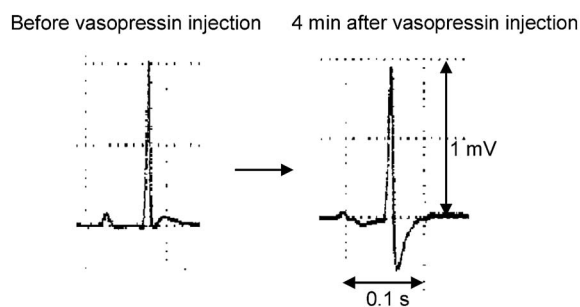


Fig. 1. Typical Tracings of Electrocardiogram (Lead II) of Anesthetized Rats Following Intravenous Injection of Vasopressin

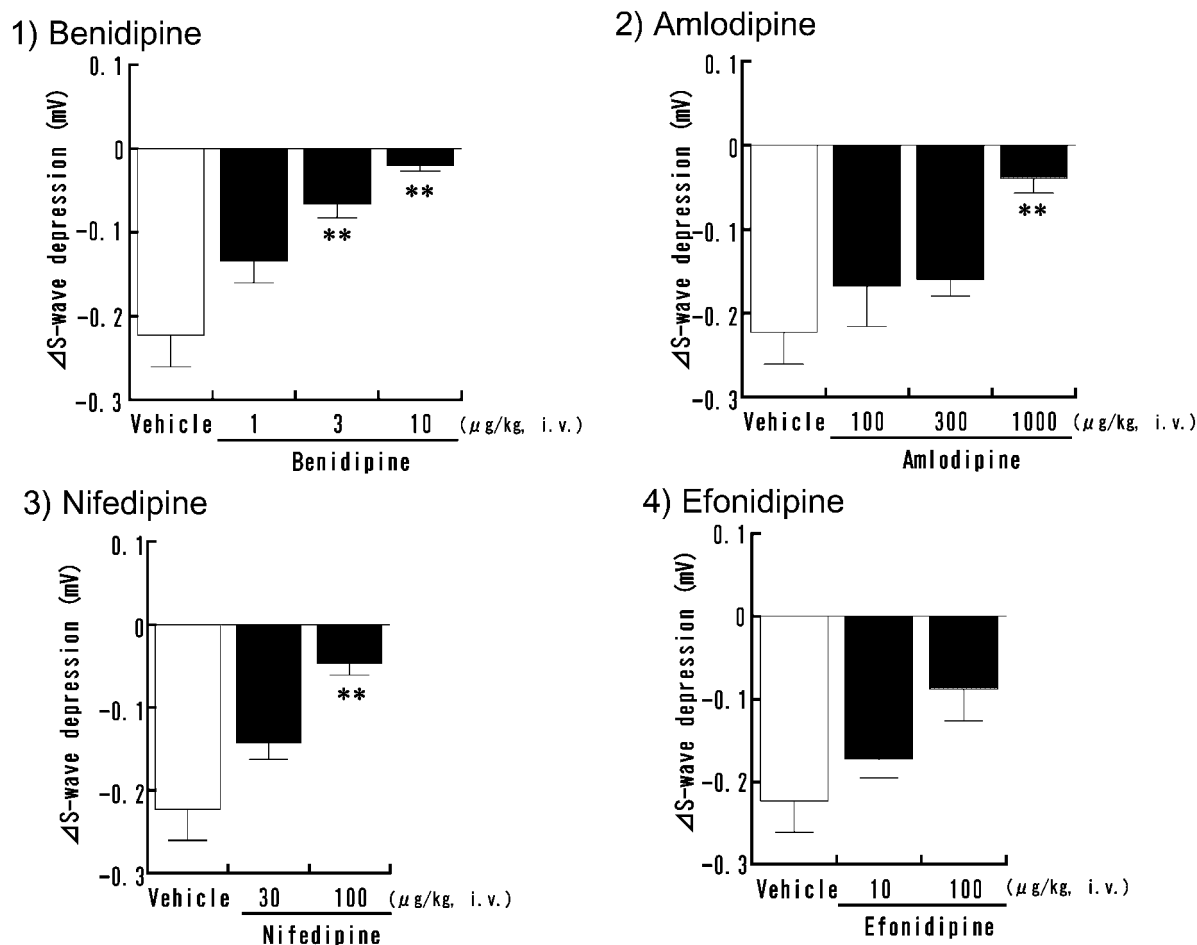


Fig. 2. Effects of Benidipine (1), Amlodipine (2), Nifedipine (3), and Efonidipine (4) on the Maximum Changes in Vasopressin-induced S-wave Depression in the Electrocardiogram (Lead II) in Anesthetized Rats

Drugs were administered intravenously 5 min prior to vasopressin injection. Columns are means \pm S.E. of 8 rats. ** $p < 0.01$ vs. vehicle.

decreased blood pressure significantly (Table 1). None of the drug treatments significantly affected heart rate 5 min after each drug administration.

DISCUSSION

In the present study, we compared the effectiveness of the DHP-derived calcium-channel blockers benidipine, amlodipine, nifedipine, and efonidipine. Benidipine had the most potent antianginal effect among the test drugs. Benidipine inhibited AVP-induced myocardial ischemia at a dose that did not affect blood pressure or heart rate. On the other hand, amlodipine, nifedipine, and efonidipine decreased blood pressure at antianginal doses. These results demonstrate that benidipine may be useful as an antianginal agent with fewer side effects such as hypotension and tachycardia.

In the present study, the effect of benidipine $10 \mu\text{g}/\text{kg}$ was comparable to that of nifedipine $100 \mu\text{g}/\text{kg}$

and amlodipine $1000 \mu\text{g}/\text{kg}$. On the basis of these antianginal values, benidipine is 10-fold more potent than nifedipine and 100-fold more potent than amlodipine. The K_i value of benidipine for the DHP binding site is $0.13 \text{ nmol}/\text{l}$, indicating that benidipine has a higher affinity for the DHP binding site than nifedipine ($K_i = 1.2 \text{ nmol}/\text{l}$).¹⁷⁾ Moriyama and Karasawa¹⁴⁾ reported that the EC_{50} value of benidipine for vasorelaxation was about 40-fold lower than the value of amlodipine in canine coronary arteries precontracted with KCl. Moreover, the duration of the hypotensive action of benidipine $10 \mu\text{g}/\text{kg}$ (i.v.) was almost the same as that of amlodipine $1500 \mu\text{g}/\text{kg}$ (i.v.) in anesthetized dogs.¹⁴⁾ These observations indicate that benidipine has about 150-fold more potent vasodilating effect than amlodipine. Thus the differences in calcium-blocking potency could explain the difference in the antispasmodic effects of these DHPs.

Table 1. Effects of Intravenous Injection of Benidipine, Amlodipine, Nifedipine, and Efonidipine on Mean Blood Pressure and Heart Rate

	Time after drug injection	Vehicle	Benidipine ($\mu\text{g}/\text{kg}$)		
			1	3	10
Mean blood pressure (mmHg)	0 min	93.0 \pm 4.7	101.3 \pm 3.1	90.5 \pm 3.9	95.1 \pm 2.0
	5 min	92.3 \pm 4.2	94.9 \pm 2.4	82.5 \pm 5.1	69.3 \pm 4.5*
Heart rate (beats/min)	0 min	340.6 \pm 16.8	337.0 \pm 8.8	326.2 \pm 6.3	343.8 \pm 14.1
	5 min	334.8 \pm 20.1	336.6 \pm 11.8	321.8 \pm 13.8	351.2 \pm 11.8

Amlodipine ($\mu\text{g}/\text{kg}$)			Nifedipine ($\mu\text{g}/\text{kg}$)		Efonidipine ($\mu\text{g}/\text{kg}$)
100	300	1000	30	100	100
104.4 \pm 1.9	88.5 \pm 3.3	91.5 \pm 1.3	89.9 \pm 2.2	86.3 \pm 4.7	91.3 \pm 2.6
104.5 \pm 3.5	83.5 \pm 2.0	68.9 \pm 4.2*	79.1 \pm 3.3	71.2 \pm 4.3*	61.7 \pm 3.7*
343.6 \pm 13.0	310.8 \pm 8.8	329.2 \pm 14.3	346.4 \pm 5.4	339.6 \pm 26.3	328.4 \pm 6.7
347.6 \pm 14.8	320.6 \pm 11.4	331.4 \pm 16.7	350.4 \pm 5.1	332.2 \pm 26.3	310.4 \pm 5.9

Data are mean \pm S.E. of 5 rats. * $p < 0.05$ vs. the vehicle group.

The DHP benidipine has a higher selectivity for coronary vessels than cardiac tissues.^{14,18} DHPs differ in their vascular vs. myocardial selectivity, with benidipine representing a highly vascular-selective DHP, while nifedipine and amlodipine are fairly unselective.^{19,20} When the vascular selectivity of DHPs was evaluated using isolated coronary arteries and the right ventricular papillary muscles of dogs, the coronary artery selectivity of benidipine was 14.4-fold higher than that of nifedipine and 19-fold higher than that of amlodipine.^{14,18} The high coronary selectivity of benidipine may contribute to its potent antianginal effects.

Efonidipine is reported to relax the coronary artery at a concentration that does not induce any cardio-depressive effect.¹⁵ Intravenously injected efonidipine 100 $\mu\text{g}/\text{kg}$ significantly inhibited vasopressin-induced depression of the ST-wave in rats.¹⁵ In the present study, efonidipine 100 $\mu\text{g}/\text{kg}$ tended to inhibit the S-wave depression, but the effect was not significant. Furthermore, efonidipine decreased blood pressure at this dose. A precise explanation for the lack of efficacy is uncertain. The discrepancy may be due to differences in models and experimental protocols. Efonidipine is an L- and T-type dual calcium-channel blocker.¹⁰ Benidipine was found to have triple calcium-channel (L, N, and T) blocking action.³ In the present study, the L-type calcium channel blocker nifedipine but not efonidipine significantly inhibited S-wave depression, indicating that the blocking effect on T-type calcium channels might not con-

tribute markedly to the antianginal efficacy of benidipine in the AVP-induced angina model. Further studies will be needed to clarify the roles of L-, T-, and N-type calcium-channel blockade in antianginal effects.

Benidipine is clinically useful in the treatment of angina pectoris.^{2,3,21} The cohort study reported recently by the Department of Cardiology, Kyushu University, endorsed the results obtained in premarketing clinical studies.¹⁸ The effects on prognosis were evaluated for nitrates, beta-blockers, and calcium antagonists, including nifedipine, benidipine, and amlodipine, in 726 patients receiving vasospastic angina treatment. Of these drugs, only benidipine was identified as determining the prognosis of vasospastic angina. Those results indicated that benidipine exerted a better prognostic effect than other calcium-channel blockers in relation to therapy for patients with vasospastic angina. In this study, benidipine exerted a greater antispastic effect at lower doses than nifedipine or amlodipine. The highly selective coronary vasodilator effect of benidipine might contribute to its better prognostic effects in patients with vasospastic angina.

In conclusion, the present results indicate that benidipine exerts potent antianginal effects in the vasopressin-induced angina rat model and exhibits fewer undesirable effects such as hypotension and tachycardia, suggesting that benidipine may be useful in the treatment of angina pectoris.

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