Original Article

Cilnidipine, an N+L-Type Dihydropyridine Ca Channel Blocker, Suppresses the Occurrence of Ischemia/Reperfusion Arrhythmia in a Rabbit Model of Myocardial Infarction

Hiroshi NAGAI, Shinya MINATOGUCHI, Xue-Hai CHEN, Ningyuan WANG, Masazumi ARAI, Yoshihiro UNO, Chuanjiang LU, Yu MISAO, Hirohito ONOGI, Hiroyuki KOBAYASHI, Genzou TAKEMURA, Rumi MARUYAMA, Takako FUJIWARA*, and Hisayoshi FUJIWARA

Dihydropyridine Ca channel blockers are widely prescribed for the treatment of hypertension and coronary artery diseases, but it remains unknown whether these agents protect against arrhythmias. We investigated whether cilnidipine, an N+L-type Ca channel blocker, reduces the incidences of ventricular premature beats (VPBs) and, if so, *via* what mechanisms. Japanese white rabbits underwent 30 min of ischemia and 48 h of reperfusion. Cilnidipine (0.5 or 1.0 μg/kg/min, i.v.) or saline (i.v.) was administered from 30 min before ischemia to 30 min after reperfusion. Electrocardiogram and blood pressure were monitored and the incidences of VPBs were measured. At 48 h after reperfusion, myocardial infarct was measured. Myocardial interstitial noradrenaline levels were determined before, during and after 30 min of ischemia with cilnidipine (0.5 and 1.0 μg/kg/min) or saline. The incidences of VPBs during ischemia and reperfusion were significantly attenuated in the cilnidipine 0.5 group (15.6±3.1 and 6.8±1.9 beats/30 min) and in the cilnidipine 1.0 group (10.4±4.9 and 3.5±1.0 beats/30 min) compared to the control group (27.2±4.5 and 24.2±3.1 beats/30 min), respectively. Myocardial interstitial noradrenaline levels were significantly reduced in the cilnidipine 0.5 and 1.0 groups compared to the control group during ischemia and reperfusion. The antiarrhythmic effect of cilnidipine may be related to the attenuation of cardiac sympathetic nerve activity. This finding may provide new insight into therapeutic strategies for hypertensive patients with ventricular arrhythmias. (*Hypertens Res* 2005; 28: 361–368)

***Key Words*: cilnidipine, reperfusion arrhythmia, infarct size, noradrenaline**

Introduction

Calcium (Ca) channel blockers are widely used for the treatment of ischemic heart diseases and hypertension because of their coronary and systemic vasodilating effects (*1*, *2*). One of the many Ca channel blockers, cilnidipine, an N-type and L-type dihydropyridine Ca channel blocker, has been reported to block the N-type Ca^{2+} current in rat sympathetic neurons (*3*) and to inhibit vascular sympathetic neurotransmission through its N-type Ca channel-blocking action (*4*) in addition to exerting a vasodilating action on the L-type Ca channel. Cilnidipine has also been reported to suppress cardiac sympathetic nerve activation in canine blood-perfused papillary

From the Second Department of Internal Medicine, Gifu University School of Medicine, Gifu, Japan; *Kyoto Women's University, Kyoto, Japan.
Address for Reprints: Hisayoshi Fujiwara, M.D., Ph.D., Second Department of Internal Medicine, Gifu University School of Medicine, Yanagido 1–1, Gifu 501–1191, Japan. E-mail: gifuim-gif@umin.ac.jp
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muscle (*5*), to cause less reflex tachycardia (*6*), to improve insulin sensitivity in patients with essential hypertension (*7*), and to decrease arterial stiffness in elderly hypertensive patients (*8*). It has been reported that myocardial interstitial noradrenaline levels are elevated during ischemia and reperfusion (*9*, *10*), and this increased noradrenaline is generally agreed to be involved in the pathophysiology of ischemia and reperfusion injuries such as reperfusion arrhythmia and lethal reperfusion injury. It has also been reported that myocardial catecholamine depletion significantly reduced the incidence of arrhythmias during ischemia and reperfusion (*11*, *12*) and that Ca channel blockers such as diltiazem and verapamil significantly reduced the incidence of ischemia and reperfusion injury (*13*). Therefore, we hypothesized that cilnidipine, which inhibits cardiac sympathetic nerve activity by blocking N-type Ca^{2+} channels, may reduce the incidence of ischemia and reperfusion arrhythmia and the infarct size. Accordingly, we examined 1) whether cilnidipine would reduce the incidence of arrhythmias during ischemia and reperfusion and the infarct size, and 2) whether cilnidipine would attenuate the myocardial interstitial noradrenaline levels during ischemia and reperfusion in a rabbit model of myocardial infarction.

Methods

In this study, all rabbits received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the U.S. National Institutes of Health (NIH publication 8523, revised 1985). The study protocol was approved by the Ethical Committee of Gifu University School of Medicine, Gifu, Japan.

Animal Selection

Male Japanese white rabbits (Chubu-Kagaku-Shizai Co., Nagoya, Japan) each weighing 2.0–2.5 kg were used. None of the rabbits had any clinically evident infections.

Surgical Preparation

Rabbits were anesthetized with sodium pentobarbital (30–40 mg/kg, i.v.) and additional doses were administered when required throughout the experiment. They were intubated and ventilated with room air supplemented with a low flow of oxygen by a mechanical ventilator (tidal volume, 20–30 ml; respiratory rate, 20–30/min; model SN-480-5; Shinano, Tokyo, Japan). Serial blood gas analysis was performed, and ventilatory parameters were adjusted to keep the arterial blood gas within the physiologic range. Surgery was performed under sterile conditions. The right carotid artery and jugular vein were cannulated to monitor peripheral arterial pressure and to administer drugs or saline and to take blood samples. Then, rabbits were administered heparin (500 U/kg). A thoracotomy was performed in the third intercostal space, and the heart was exposed after excising the pericardium. A 4-0 silk suture on a small curved needle was passed through the myocardium beneath the middle segment of the large arterial branch coursing down the middle of the anterolateral surface of the left ventricle (LV). A small vinyl tube was passed into both ends of the suture, and the coronary branch was occluded by pulling the snare, which was then fixed by clamping the tube with a mosquito hemostat. Myocardial ischemia was confirmed by regional cyanosis and electrocardiographic change. Reperfusion was confirmed by myocardial blush over the risk area after releasing the snare. After the initial preparation but before coronary occlusion, the animals were assigned randomly to one of the three groups. All rabbits were allowed to rest for 20 min after completion of the surgical preparation before the start of the protocol. The rabbits underwent a 30-min occlusion of an anterolateral branch of the coronary artery followed by a 48-h reperfusion (protocol 1) and a 30-min reperfusion (protocol 2).

Protocol 1: Detection of Ischemia and Reperfusion Arrhythmia and Measurement of Infarct Size

Figure 1 summarizes the experimental protocol 1. The animals were subjected to 30 min of ischemia and 48 h of reperfusion. The cilnidipine 0.5 μg/kg group ($n=10$) was intravenously administered 0.5 μg/kg/min of cilnidipine for 90 min starting at 30 min before ischemia and ending at 30 min after reperfusion. The cilnidipine 1.0 μg/kg group ($n=10$) was intravenously administered a 10 μg/kg/min of cilnidipine for 90 min starting at 30 min before ischemia and ending at 30 min after reperfusion. The control group ($n=10$) was administered saline intravenously instead of cilnidipine. Hemodynamic parameters such as mean blood pressure, heart rate and electrocardiogram were monitored by a Power Labo system (AD Instruments Pty Ltd., New South Wales, Australia) throughout the experiment. After the experiment, the chest was closed and the rabbits were allowed to recover from anesthesia for 2 days.

Postmortem Study

At the end of the study, the rabbits were intravenously administered heparin (500 U/kg) and sacrificed by an overdose of pentobarbital. The heart was excised and mounted on a Langendorff apparatus. The coronary branch was reoccluded and Evans blue dye (4%; Sigma Chemical Co., St. Louis, USA) was injected from the aorta at 80 mmHg to determine the area at risk. The LV was sectioned into 7 slices parallel to the atrio-ventricular ring. Each slice was weighed, incubated in a 1% solution of triphenyl tetrazolium chloride (TTC) at 37°C to visualize the infarct area, and photographed. The areas of the ischemic region and the infarcted myocardium were traced on each LV slice and multiplied by the slice's weight, then expressed as a fraction of the risk region or LV for each heart. The slice with the snare occluder was excluded from analysis, because direct damage by the occluder usually

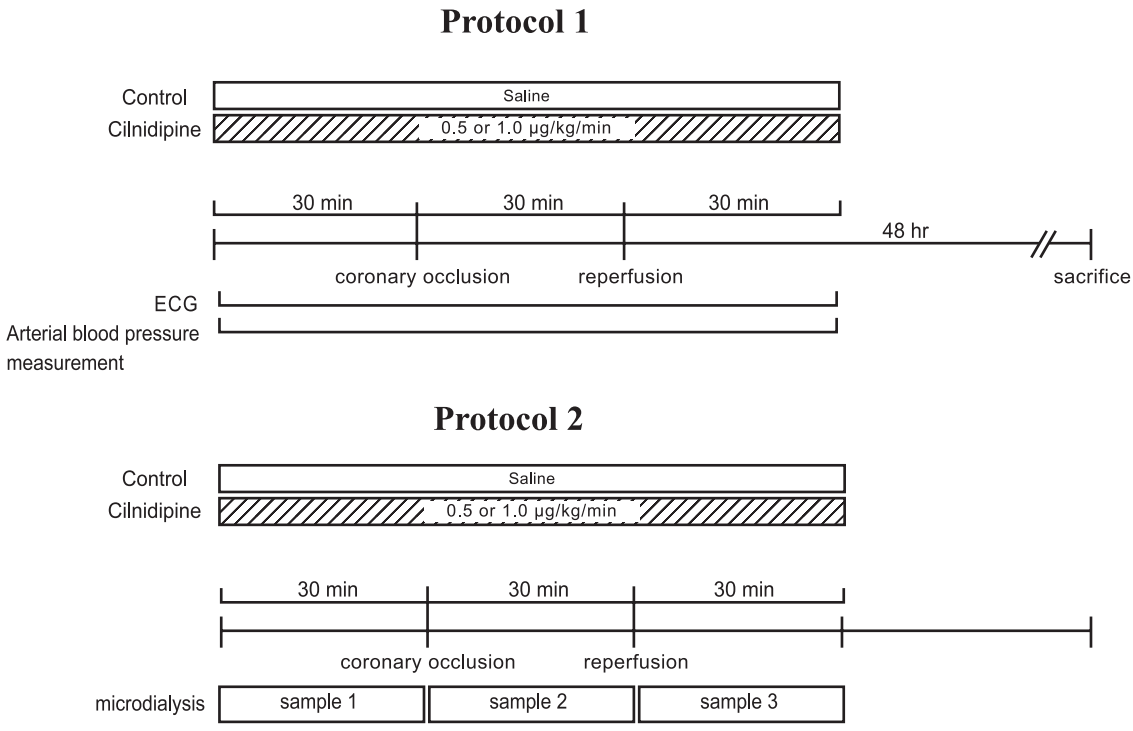

Fig. 1. *Experimental protocols 1 and 2.*

caused small artificial necrotic foci.

Protocol 2: Measurement of Myocardial Interstitial Noradrenaline Levels

Twenty-four rabbits were assigned to investigate the effect of cilnidipine on the level of myocardial interstitial noradrenaline during ischemia and reperfusion. A microdialysis probe (PNF 1700; 20-mm length, 0.31-mm OD, 0.2-mm ID; transverse type, MW 50 kDa cut-off; Asahi Medical, Tokyo, Japan) for dialysate sampling was implanted in the risk region of the myocardium, which was served by the anterolateral coronary artery along the axis of the ventricular fibers and reached from the epicardial outer layer to the endocardial inner layer of the myocardium. Probe placement was confirmed at autopsy. The microdialysis probe was perfused with Ringer's solution at a rate of 10 μl/min. After a 60-min rest following the completion of instrumentation, the dialysate was sampled during 30-min pre-ischemia, during 30-min ischemia and during 30-min reperfusion in the presence of saline ($n=10$) or cilnidipine (0.5 and 1.0 μg/kg/min, $n=7$ each, i.v., starting at 30 min before ischemia). Dialysate samples were frozen at −83°C until further analysis. The dialysate noradrenaline concentrations were measured by directly injecting the samples into a high performance liquid chromatography (HPLC) system (MCM column; MC Medical, Tokyo, Japan) coupled with an electrochemical detection device (coulochem II; ESA, Chemsford, USA).

Statistical Analysis

Values are expressed as the group means±SEM. An χ^2 test was used to test differences in the survival rate and the incidence of ventricular fibrillation and ventricular tachycardia. To compare the group means of the area at risk, infarct size, number of VPBs and hemodynamic parameters, one-way analysis of variance (ANOVA) was performed, and if the ANOVA was significant, a Scheffe's F test was performed to assess the significance of the differences among groups. The effects of treatments on hemodynamics were analyzed with ANOVA with repeated measures. Differences with a value of $p<0.05$ were considered significant.

Chemicals and Materials Used

Cilnidipine was provided by Mochida Pharmaceutical Co., Ltd. (Tokyo, Japan).

Results

Mortality, Ventricular Fibrillation, Ventricular Tachycardia and Animal Exclusion

Fifty-two rabbits were initially enrolled in the protocol 1 study. There were no significant differences in the number of animals assigned to each group or in the incidence of ventricular fibrillation (VF), ventricular tachycardia (VT) or mortal-

Table 1. Hemodynamic Parameters

	Group	Basal	Before occlusion	Occlusion		Reperfusion	
				20 min	30 min	20 min	30 min
SBP (mmHg)	Control	97±5	92±5	78±7	77±6	79±4	80±4
	Cil 0.5	96±3	78±3*	70±2	70±2	70±3	70±4
	Cil 1.0	95±7	78±5	62±5	62±4*	63±4*	63±4*
MBP (mmHg)	Control	82±4	78±5	66±5	65±4	65±3	65±4
	Cil 0.5	84±3	61±3*	54±2*	53±3*	52±3*	52±3*
	Cil 1.0	84±7	59±3**	50±3*	50±2*	51±2**	51±2**
DBP (mmHg)	Control	74±4	70±5	60±4	59±3	58±3	58±4
	Cil 0.5	78±4	52±3**	46±3*	45±4*	44±4**	44±4**
	Cil 1.0	78±7	50±4**	43±4*	42±3*	45±3*	45±4*
HR (beats/min)	Control	267±11	264±9	264±11	264±10	263±10	264±9
	Cil 0.5	270±7	261±7	253±7	252±7	252±8	252±8
	Cil 1.0	275±5	254±11	244±11	243±10	243±8	243±8
Double product	Control	258±12	241±8	202±15	201±14	208±12	208±11
(× 100)	Cil 0.5	260±10	203±12*	177±10	177±9	177±10	177±9
	Cil 1.0	261±20	197±16*	152±16*	152±15*	153±11**	153±10**

SBP, systolic blood pressure; MBP, mean blood pressure; DBP, diastolic blood pressure; HR, heart rate; Cil 0.5, cilnidipine 0.5 μg/kg/min; Cil 1.0, cilnidipine 1.0 μg/kg/min. *$p<0.05$, **$p<0.01$ *vs.* control.

ity among the three groups. Among these animals, 6 were excluded because of technical problems: these were 3 from the control group, 2 from the cilnidipine 0.5 group and 1 from the cilnidipine 1.0 group. Of the remaining 46 rabbits, 11 had VF and VT during coronary occlusion and reperfusion: 4 (VF:3, VT:1) from the control group, 4 (VF:3, VT:1) from the cilnidipine 0.5 group and 3 (VF:2, VT:1) from the cilnidipine 1.0 group. Five rabbits died after the first day of the experiment: 2 from the control group, 1 from the cilnidipine 0.5 group, and 2 from the cilnidipine 1.0 group. Thus, the experiments were completed in the remaining 30 rabbits, and the data from these animals were used for the analysis of ventricular premature beats and infarct size.

Hemodynamic Parameters

Table 1 shows the hemodynamic parameters: systolic blood pressure (SBP), mean blood pressure, diastolic blood pressure (DBP), heart rate, and the double product (SBP × heart rate). Although there was no significant difference in blood pressure before ischemia among the 3 groups, blood pressure was decreased during ischemia and reperfusion in the cilnidipine 0.5 and 1.0 groups compared to the control group. There was no significant difference in heart rate at each time point throughout the experiment. The double product was significantly decreased in the cilnidipine 1.0 group but not in the cilnidipine 0.5 group as compared to the control group.

Effect on Infarct Size

As shown in Fig. 2A, the mean percentages of the area at risk (percentage of the LV) were 44.5±5.0%, 45.2±2.0%, and 40.5±2.1% in the control group, cilnidipine 0.5 group, and cilnidipine 1.0 group, respectively. No significant difference was noted among the 3 groups.

As shown in Fig. 2B, the infarct size as a percentage of the area at risk was reduced significantly in the cilnidipine 1.0 group (35.2±1.5%) but not in the cilnidipine 0.5 group (39.9±3.2%) compared with the control group (44.5±3.6%).

Effect on the Incidence of Ventricular Premature Beats (VPBs) during Ischemia and Reperfusion

As shown in Fig. 3, VPBs were observed during 30-min ischemia and 30-min reperfusion in the control group. The incidence of VPBs during ischemia was significantly reduced in the cilnidipine 0.5 group (15.6±3.1 beats/30 min) and cilnidipine 1.0 group (10.4±4.9 beats/30 min) in a dose-dependent manner as compared to the control group (27.2±4.5 beats/30 min). The incidence of VPBs during reperfusion was also significantly reduced in the cilnidipine 0.5 group (6.8±2.0 beats/30 min) and cilnidipine 1.0 group (3.5±1.0 beats/30 min) in a dose-dependent manner as compared to the control group (24.2±3.1 beats/30 min).

Effect on Myocardial Interstitial Noradrenaline Levels

As shown in Fig. 4, myocardial interstitial noradrenaline levels were significantly increased in the control group during 30 min ischemia and during 30 min reperfusion compared with the pre-ischemic period. Pre-ischemic treatment with cilnidipine 0.5 and 1.0 μg/kg/min dose-dependently and significantly decreased the myocardial interstitial noradrenaline

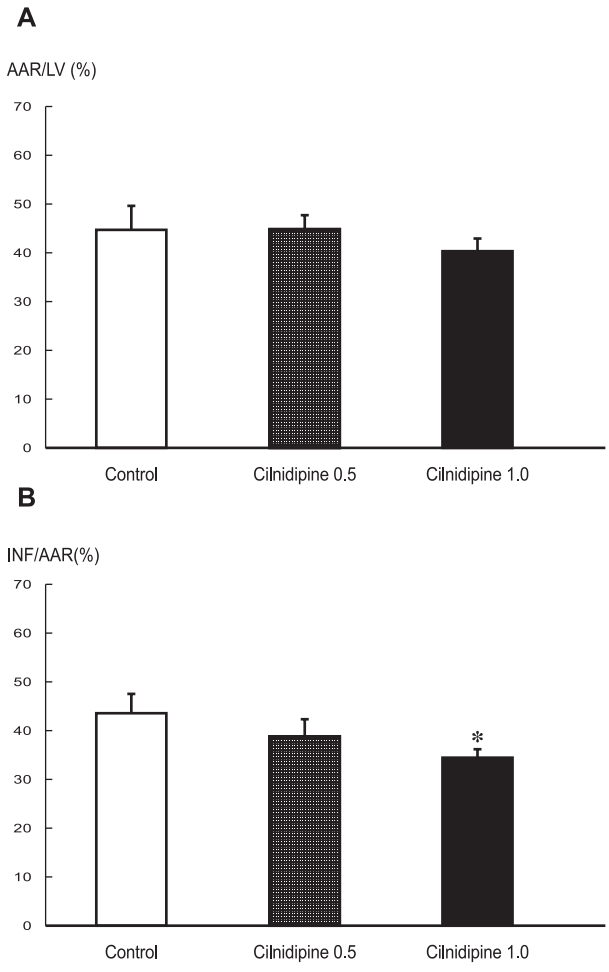

Fig. 2. *A: Area at risk (AAR) expressed as a percentage of the left ventricle (LV). No significant difference was noted among the groups. B: Infarct size expressed as a percentage of the area at risk. The infarct size was modestly but significantly reduced in the cilnidipine 1.0 group but not in the cilnidipine 0.5 group.* *$p < 0.05$ vs. *the controls.*

levels during ischemia and reperfusion periods, and cilnidipine 1.0 μg/kg/min but not 0.5 μg/kg/min decreased the basal level of noradrenaline, as compared to those in the control group.

Discussion

Effects on Heart Rate and Blood Pressure

Cilnidipine at doses of 0.5 μg/kg/min and 1.0 μg/kg/min significantly decreased the mean blood pressure. However, cilnidipine at doses of 0.5 μg/kg/min and 1.0 μg/kg/min did not affect the heart rate throughout the experiment, although 1.0 μg/kg/min of cilnidipine tended to decrease the heart rate during ischemia and reperfusion compared to the control group. The double product (SBP × heart rate) was significantly decreased in the cilnidipine 1.0 group but not in the cilnidipine 0.5 group as compared to the control group. In the present study, despite the fact that cilnidipine reduced the mean blood pressure, the heart rate did not increase and in fact tended to decrease in the cilnidipine 1.0 group, suggesting that the baroreceptor reflex was blunted by the treatment with cilnidipine. This may be explained by the fact that cilnidipine decreased the levels of myocardial interstitial noradrenaline, which may have attenuated cardiac sympathetic nerve activity, resulting in the reduced baroreceptor reflex.

Effects on Infarct Size

Cilnidipine at a dose of 1.0 μg/kg/min but not 0.5 μg/kg/min modestly but significantly reduced the infarct size. It is generally accepted that the infarct size is determined over the several hours after infarction (*14*, *15*) and is affected by oxygen consumption (*16*). The double product, an indicator of oxygen consumption, was significantly decreased in the cilnidipine 1.0 group but not in the cilnidipine 0.5 group as compared to the control group. In the present study, the infarct size-reducing effect of cilnidipine at a dose of 1.0 μg/kg/min may have been at least partly mediated through the reduction in double product. Furthermore, the fact that cilnidipine 0.5 μg/kg/min tended to reduce the infarct size and cilnidipine 1.0 μg/kg/min significantly reduced the infarct size may be related to the dose-dependent decrease in myocardial interstitial noradrenaline levels due to cilnidipine treatment. Therefore, the infarct size-reducing effect of cilnidipine may be mediated through the attenuation of cardiac sympathetic nerve activity during ischemia and reperfusion, since β-blockers are reported to reduce the infarct size (*17*). However, the precise mechanism by which cilnidipine reduces the infarct size remains to be investigated.

Effects on Myocardial Interstitial Noradrenaline Levels

Myocardial interstitial noradrenaline levels increased during ischemia and reperfusion, suggesting that cardiac sympathetic nerve activity is augmented during ischemia and reperfusion. Although nifedipine did not affect the myocardial interstitial noradrenaline levels, cilnidipine decreased the myocardial interstitial noradrenaline levels during the preischemic phase and during ischemia and reperfusion in an *in vivo* beating rabbit heart. This suggests that cilnidipine attenuates cardiac sympathetic nerve activity at baseline and during ischemia and reperfusion. This is consistent with a previous report that cilnidipine attenuates sympathetic nerve activity (*5*). Previous reports demonstrated that administration of cilnidipine did not affect the basal plasma levels of noradrenaline (*18–20*) but that cardiac sympathetic nerve activity assessed by ^{123}I metaiodobenzylguanidine (MIBG) was decreased (*20*).

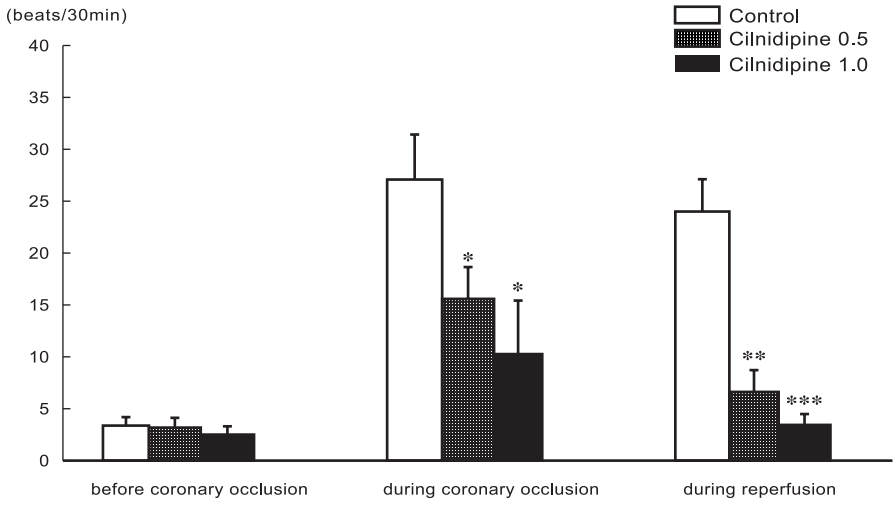

Fig. 3. *Effect of cilnidipine on the incidence of ventricular premature beats (VPBs) during the ischemia and reperfusion periods.* *p < *0.05,* **p < *0.01,* ***p < *0.001* vs. *the controls.*

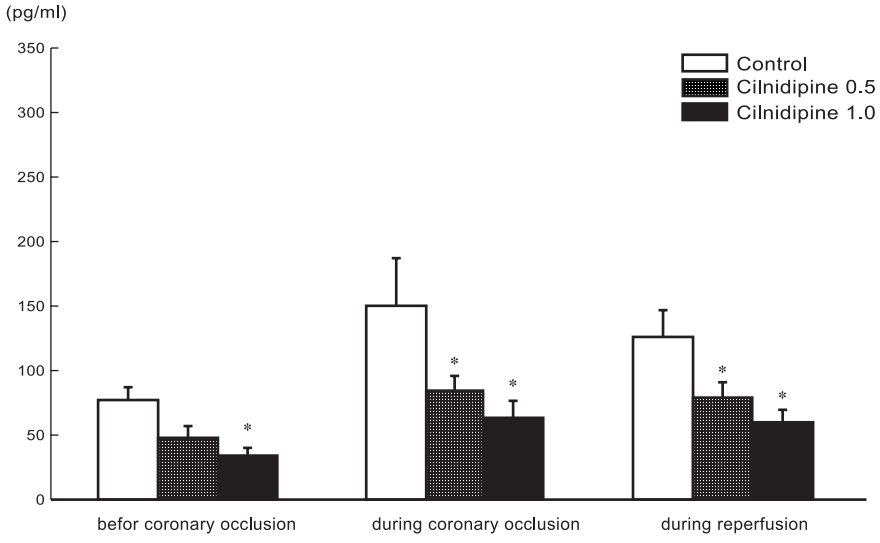

Fig. 4. *Effect of cilnidipine on myocardial interstitial noradrenaline levels.* *p < *0.05,* **p < *0.01* vs. *the controls.*

Effect on VPBs

In the present study, cilnidipine at doses of 0.5 and 1.0 μg/kg/min significantly reduced the incidence of VPBs both during ischemia and during reperfusion. It has been reported that myocardial catecholamine depletion by pretreatment with 6-hydroxydopamine significantly blunted the ischemia-induced reduction in action potential amplitude and V_{max}, prevented any spontaneous recovery and abolished reperfusion-induced shortening of action potential duration, and finally, significantly reduced the incidence of arrhythmia during ischemia and reperfusion in Langendorff perfused guinea pig hearts subjected to low flow 30 min ischemia and reperfusion (*11*). It has also been reported that myocardial depletion of catecholamines by pretreatment with reserpine significantly attenuated, but did not abolish, cardiac arrhythmias induced by ischemia and reperfusion in an isolated rat heart preparation (*12*). These findings indicate that myocardial catecholamines, especially noradrenaline, are a contributing factor to arrhythmogenesis during ischemia and reperfusion. On the other hand, there are some reports indicating that myocardial catecholamines are a contributing factor to arrhythmia during ischemia (*21*, *22*) but not during reperfusion (*23*, *24*). Furthermore, there is a report demonstrating that the amount of noradrenaline washed out upon reperfusion may contribute to the genesis of reperfusion ventricular arrhythmias but not to that

of reperfusion ventricular fibrillation (*25*). Indeed, this is consistent with the result in the present study that cilnidipine suppressed the incidence of VPBs during ischemia and reperfusion, but there was no significant difference in the mortality rate due to ventricular fibrillation among the control and cilnidipine groups.

It has been postulated that reperfusion-induced arrhythmias are associated with alterations in $[Ca^{2+}]_i$ levels (*26*), and recent studies have shown that an increase in $[Ca^{2+}]_i$ levels was associated with the induction of ventricular arrhythmia (*27*, *28*). Therefore, Ca channel blockers such as verapamil can attenuate electrophysiological alterations (*29*), and ryanodine, an inhibitor of calcium release from the sarcoplasmic reticulum, can decrease the occurrence of reperfusion arrhythmias (*26*). In addition to these mechanisms, it has been reported that oxygen free radicals, which also increase the Ca^{2+} influx, play an important role in the occurrence of reperfusion arrhythmias (*30*, *31*). It is well known that catecholamines also increase Ca^{2+} influx through the L-type Ca channels (*32*). In the present study, cilnidipine significantly decreased the myocardial interstitial noradrenaline levels at baseline levels and during ischemia and reperfusion. This effect may be mediated through the blockade of the N-type Ca^{2+} channels by cilnidipine, which in turn would attenuate the cardiac sympathetic nerve activity (*20*).

In the present study, the attenuation of the incidence of VPBs during ischemia may have been due to the decrease in myocardial interstitial noradrenaline release, and the attenuated incidence of VPBs during reperfusion may have been mediated through a combination of the blockade of L-type Ca channels, the blockade of which decreases Ca^{2+} influx, and the decrease in myocardial interstitial noradrenaline release. However, since cilnidipine did not attenuate the incidence of ventricular fibrillation and ventricular tachycardia, the extent of decrease in myocardial interstitial noradrenaline may have been insufficient to prevent these arrhythmias. In addition, there is still a possibility that the inhibiting effect of cilnidipine on VPBs may have been due to actions other than the N-type Ca channel-blocking action. These mechanisms remain to be investigated.

In conclusion, cilnidipine modestly reduced the myocardial infarct size, and attenuated the incidence of ventricular premature beats during ischemia and reperfusion in a rabbit model of myocardial infarction. These effects may have been mediated through a combination of the blockade of the L-type Ca channels and the reduction in myocardial interstitial noradrenaline levels during ischemia and reperfusion. This finding may provide new insight into therapeutic strategies for hypertensive patients with ventricular arrhythmias.

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