SUMMARY

Cilnidipine is a dihydropyridine calcium channel blocker that acts on both L-type and N-type calcium channels.

The effects of cilnidipine given intravenously at doses of 2.5, 5.0, and 10 µg/kg were studied using an ex vivo hydronephrosis model in spontaneously hypertensive rats. The effects of nifedipine at a dose of 10 µg/kg were also studied using the same model as a reference.

Cilnidipine caused dose-dependent blood pressure reduction and dilatation of the glomerular afferent arterioles; the arteriolar diameter after cilnidipine infusion at 2.5, 5.0, and 10 µg/kg was 101% ± 3%, 112% ± 4%, and 123% ± 6% relative to baseline, respectively. With cilnidipine, dilatation of the efferent arterioles was also observed; it was maximal after 5 to 10 minutes. Five minutes after administration of 2.5, 5.0, and 10 µg/kg of cilnidipine, the efferent arteriolar diameter was 103% ± 2%, 109% ± 4%, and 119% ± 4% of baseline, respectively. This efferent arteriolar dilating action of cilnidipine was abolished after pretreatment with ω-conotoxin, a selective N-type calcium channel blocker. A dose-dependent increase of glomerular blood flow volume was also observed after cilnidipine infusion. Nifedipine, an L-type calcium channel blocker, at a dose of 10 µg/kg reduced systolic blood pressure to a similar extent as cilnidipine at a dose of 10 µg/kg, but only dilated the afferent arterioles and had no significant effect on efferent arterioles.

Cilnidipine dilated both the afferent and efferent glomerular arterioles. The efferent arteriolar dilating effect of cilnidipine may be attributed to its inhibition of the N-type calcium channel. (Int Heart J 2008; 49: 723-732)

Key words: N-type calcium channel, Efferent arterioles, Cilnidipine, Omega-conotoxin, Hydronephrotic kidney model

Since the kidney is liable to be affected by hypertension, renal protection is an important aspect of antihypertensive treatment. Therefore, one of the essential characteristics of antihypertensive drugs should be to suppress the occurrence and progression of hypertensive renal damage. Hypertensive renal dysfunction is
thought to be caused by glomerular damage due to glomerular hypertension,\(^2,3\) which has been observed in salt-sensitive rats and in spontaneously hypertensive rats (SHRs) with persistent hypertension.\(^4\) Intraglomerular pressure is mainly regulated by the afferent and efferent arterioles. Antihypertensive drugs that dilate the efferent arteriole along with the afferent arteriole may have an advantage in improving glomerular hypertension. Angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers,\(^5-7\) and some long-acting dihydropyridine (DHP) calcium blockers\(^7-9\) have been shown to dilate both the afferent and efferent arterioles.

Cilnidipine is a dihydropyridine (DHP) calcium channel blocker that inhibits both L-type and N-type calcium channels.\(^10-12\) The L-type calcium channel is the usual target of DHP calcium blockers, thus it is DHP-sensitive,\(^13\) and the N-type calcium channel is DHP-insensitive. Several studies have shown that when norepinephrine (NE) is released from sympathetic nerve terminals there is an influx of extracellular calcium through calcium channels.\(^14-16\) Norepinephrine constricts both the afferent and efferent arterioles.\(^17\) It has been shown that cilnidipine suppresses NE release from the perfused vascular bed of SHR mesentry.\(^18\) It has also been histologically confirmed that all parts of the renal vascular system receive sympathetic innervation.\(^19,20\)

Therefore, we hypothesized that the inhibitory action of cilnidipine on N-type calcium channels could dilate both afferent and efferent arterioles. In the present study using a rat hydronephrotic model, the effect of cilnidipine on the renal arterioles was studied and compared with that of nifedipine, which inhibits the L-type calcium channel but not the N-type calcium channel.

**Methods**

**Animals:** Sixty-three, 8-week-old, female spontaneous hypertensive rats (SHRs, Charles River Japan) were used. The average body weight of the SHRs at 16 weeks-old was 211 ± 13.4 (SD) g.

**Drugs:** Cilnidipine (Fujirebio Co. Ltd., Tokyo) and nifedipine (Sigma-Aldrich, St. Louis, Missouri, USA) were dissolved in dimethyl sulfoxide (DMSO); these solutions were then diluted with physiological saline before use.

**Preparation of the hydronephrotic model:** After the rats were anesthetized with ether, hydronephrotic kidney was induced by permanent ligation of the left ureter. The rats were maintained on standard rat chow with free access to tap water for 2 months after surgery.

The hydronephrotic kidneys were prepared for direct in vivo observation of the renal microvasculature under light microscopy according to a previously described method.\(^7,21,22\) After the rats were anesthetized with pentobarbital (50
mg/kg, intraperitoneally), the left jugular vein was cannulated to permit continuous infusion of isotonic saline (60 µL/minute), and the left carotid artery was cannulated to allow continuous measurement of arterial blood pressure with an electric manometer (Nihon-Kohden Inc., Tokyo). The hydronephrotic left kidney was split longitudinally with an electrocautery knife, spread out as a thin sheet, and then placed in a water bath filled with saline, which was kept at 37°C. Microscopic examination of the renal microcirculation was performed by transillumination. The microscopic images were recorded on videotape. The velocity of the red blood cells (RBC) in the arterioles was measured continuously using a red cell velocity tracker (model 102 B-C, IPM, Ann Arbor, Michigan, USA).

Each kidney was allowed to equilibrate in the water bath filled with saline before cilnidipine or nifedipine was injected. Only one rat was used for each dose of cilnidipine or nifedipine, since the blood pressure and the arteriolar diameters became unstable 60 minutes after the start of the experiment in the control rats, and the effects of a bolus injection of cilnidipine last more than 60 minutes because cilnidipine is a long-acting calcium channel blocker. In each rat, only one glomerulus in which both the afferent and efferent arterioles could be identified was observed during the entire experiment, because if the high power microscopic field was changed, it was difficult to properly position the microscope to again find the same glomerulus. When no glomeruli could be found in which both the afferent and efferent arterioles could be identified, these rats were excluded from the analysis of the arteriolar diameters; only their blood pressure and/or glomerular blood flow data were used for analysis.

**Drug administration and observation:** Cilnidipine or nifedipine was injected as a bolus via the cannula placed in the left external jugular vein. Cilnidipine was administered at doses of 2.5 µg/kg (n = 9), 5 µg/kg (n = 9), or 10 µg/kg (n = 8), while nifedipine was administered at a dose of 10 µg/kg (n = 9). During preliminary experiments in SHRs, it was found that nifedipine 10 µg/kg and cilnidipine 10 µg/kg had equivalent hypotensive effects (data not shown). In control rats, the vehicle alone (DMSO at a final concentration of 1%) was injected (n = 8 for cilnidipine, n = 8 for nifedipine). The afferent and efferent arteriolar diameters were recorded on videotape at 2, 5, 10, 15, 20, and 30 minutes after drug injection.

**Effect of closure of the N-type calcium channel:** To assess the effect of N-type channel inhibition, cilnidipine (10 µg/kg) was injected 30 minutes after the hydronephrotic kidney was pretreated with ω-conotoxin, a selective inhibitor of this channel (Sigma-Aldrich); ω-conotoxin was added at 1 µM to the physiological saline in the chamber (n = 6). As a control, the same dose of cilnidipine was injected into rats (n = 6) with hydronephrotic kidneys that were not pretreated with ω-conotoxin. The arteriolar diameters were measured 30 minutes after the cilnidipine injection.
Analysis of data: During videotape playback, the arteriolar images were captured on computer (Power Macintosh 7500, Apple Computer, Inc., Cupertino, California, USA) using a video capture board. The arteriolar diameters were then measured and analyzed (NIH image 1.61, State View 4.02, Abacus Concepts, Inc., Berkeley, California, USA). The diameters of the distal afferent arterioles (from 20 to 50 µm from the glomerulus) and the proximal efferent arterioles (from 20 to 50 µm from the glomerulus) were determined.

The relative value of the glomerular blood flow volume was calculated as the RBC velocity multiplied by the square of the arteriolar diameter.7,21,23) The changes in the arteriolar diameter and the changes in the glomerular blood flow volume are expressed as the mean ± SE, taking the values prior to drug administration as 100%.

ANOVA and Dunnett’s post-hoc test were used to compare the cilnidipine groups with the control group. When the nifedipine group was compared with the control group, the unpaired t-test was employed. For all analyses, statistical significance was set at P < 0.05.

The experiment was performed in accordance with the institutional guideline for animal experiments of the University of Tokyo. All experiments were performed at the University of Tokyo from 1999 to 2000.

RESULTS

In the control group, no significant changes in systolic blood pressure, afferent arteriolar diameter, efferent arteriolar diameter, or glomerular blood flow were found throughout the experiments.

A dose-dependent decrease of the systolic blood pressure was observed after the intravenous administration of cilnidipine at doses of 2.5, 5, and 10 µg/kg; the maximum effect occurred 2-5 minutes after drug administration, followed by gradual recovery (Figure 1A). The decrease in blood pressure at 5 minutes after drug administration at doses of 2.5, 5, and 10 µg/kg was 13 ± 3 mmHg, 10 ± 3 mmHg, and 23 ± 3 mmHg, respectively. As shown in Figure 1B, though 2.5 µg/kg of cilnidipine did not dilate the afferent arterioles, dilatation of these arterioles was observed at higher doses; it reached a maximum at 5 minutes and then had disappeared almost 30 minutes after administration. At cilnidipine doses of 2.5, 5, and 10 µg/kg, the afferent arteriolar diameter at 5 minutes was 101% ± 3%, 112% ± 4%, and 123% ± 6% compared to the baseline, respectively (P < 0.001 versus control for 10 µg/kg). Dilatation of the efferent arterioles was also observed; it was maximal 5-10 minutes after drug administration (Figure 1C). At cilnidipine doses of 2.5, 5, and 10 µg/kg, the efferent arteriolar diameter at 5 minutes was 103% ± 2%, 109% ± 4%, and 119% ± 4% compared to the baseline,
respectively ($P < 0.05$ versus control for $5 \mu g/kg$, and $P < 0.001$ versus control for $10 \mu g/kg$). These changes became less prominent with time; however, with a cilnidipine dose of $10 \mu g/kg$, arteriolar dilation persisted even 30 minutes after administration ($112 \pm 6\%$, not significant). With cilnidipine, a dose-dependent increase in the glomerular blood flow was also observed (Figure 1D); a significant increase was observed when $10 \mu g/kg$ of cilnidipine was injected. While both afferent and efferent arteriolar dilatation reached a maximum within a short period and then decreased, the glomerular blood flow increase persisted throughout the observation period.

As shown in Figure 2A, the systolic blood pressure decreased by $26 \pm 4$ mmHg after intravenous administration of $10 \mu g/kg$ of nifedipine; the maximal
decrease was reached at 5 minutes, and then the blood pressure recovered gradually. The afferent arteriolar diameter reached a maximum of $113\% \pm 6\%$ at 5 minutes after nifedipine injection ($P < 0.001$ versus control), and then decreased and returned almost to baseline by 20 minutes (Figure 2B). On the other hand, there were no significant changes in the efferent arteriolar diameter after nifedipine administration (Figure 2C). Although the glomerular blood flow increased after nifedipine injection, the increase was not significant (Figure 2D).

Compared to the untreated hydronephrotic kidney, glomerular afferent arteriolar dilatation by cilnidipine was attenuated when the hydronephrotic kidney was pretreated with $\omega$-conotoxin; however, the decrease in the diameter was not significant. Conversely, dilation of the efferent arterioles by cilnidipine was completely abolished after $\omega$-conotoxin pretreatment ($P < 0.05$, Figure 3).
DISCUSSION

The present study using an *ex vivo* SHR hydronephrotic model found that cilnidipine dilated both the afferent and efferent arterioles. In contrast, nifedipine dilated only the afferent arteriole. The blocking experiment using ω-conotoxin showed that cilnidipine dilated the efferent arteriole by blocking the N-type calcium channel and dilated the afferent arteriole by blocking the L-type calcium channel.

Many studies have examined the effects of calcium blockers on renal arterioles. It has been shown that the short-acting drugs of this drug class, such as nifedipine and diltiazem, selectively dilate the afferent arterioles without affecting the efferent arterioles. However, among the more recently developed DHP calcium blockers, there are several drugs that dilate the efferent arterioles in addition to dilating the afferent arterioles. A glomerular vascular relaxing factor other than nitric oxide was shown to be involved in the efferent arteriolar dilating effect of manidipine. However, the precise mechanisms by which calcium channel blockers dilate the efferent arteriole have not yet been elucidated.

Cilnidipine inhibits the N-type calcium channel, which is DHP-insensitive, and also inhibits the L-type calcium channel at similar concentrations. The N-type calcium channel is involved in sympathetic neurotransmission, and it was shown that cilnidipine suppresses the release of norepinephrine from sympathetic nerve terminals in perfused SHR mesentery specimens.
It was shown that cilnidipine suppresses sympathetic nerve activity of SHRs as well as normotensive rats.\textsuperscript{27,28} It was reported that the efferent arterioles of normotensive WKY rats were regulated by sympathetic nerves in the same way as SHRs.\textsuperscript{29} Thus, we assumed that cilnidipine has a vasodilatory effect on the efferent arterioles of SHRs and normotensive rats.

It has been demonstrated histologically that sympathetic nerves are distributed to all parts of the renal vascular system,\textsuperscript{19,20} including the afferent and efferent arterioles of the glomeruli, though the density of these nerves is higher in the afferent arterioles than in the efferent arterioles.\textsuperscript{20} However, low-frequency (0.5-1.5 Hz) electrical stimulation of the renal sympathetic nerves causes a similar increase in the resistance of the afferent and efferent arterioles.\textsuperscript{26} In addition, an investigation of perfused rat kidneys revealed that \(\omega\)-conotoxin, which specifically inhibits N-type calcium channels, significantly suppresses norepinephrine release after sympathetic nerve stimulation.\textsuperscript{30} Therefore, N-type calcium channels should be present in renal sympathetic nerves and should have a role in the release of neurotransmitters. Steinhausen, \textit{et al}\textsuperscript{17} and Yuan, \textit{et al}\textsuperscript{31} reported that the afferent and efferent arterioles were both constricted by sympathetic activity that involved norepinephrine.

In anesthetized dogs, Takahara, \textit{et al} showed that cilnidipine attenuated renal nerve stimulation-induced renal vasoconstriction, as well as antinatriuresis; they concluded that suppression of renal sympathetic neurotransmission by antagonism of N-type calcium channels contributed to these effects.\textsuperscript{32} In the present study, the dilatation of the efferent arterioles by cilnidipine was abolished after pretreatment with \(\omega\)-conotoxin, which is a specific blocker of N-type calcium channels. This suggests that dilation of the efferent arterioles was dependent on the inhibition of norepinephrine release from the renal sympathetic nerve terminals in the glomeruli. Uchino, \textit{et al} performed a micropuncture study and showed that the \(\alpha_1\) antagonist terazosin can decrease the resistance of both afferent and efferent arterioles of SHRs; they concluded that the efferent arterioles have a stronger response than the afferent arterioles to \(\alpha_1\) receptor stimulation.\textsuperscript{33} Although cilnidipine does not bind to the \(\alpha_1\) receptor,\textsuperscript{18} it may have caused efferent arteriolar dilatation by indirectly attenuating \(\alpha_1\) receptor stimulation by decreasing free norepinephrine levels.

Since cilnidipine markedly dilated the efferent arterioles, cilnidipine was assumed to lower intraglomerular pressure. Glomerular hypertension is considered to be a risk factor for glomerular damage,\textsuperscript{29} and the ACE inhibitor imidapril, which has an efferent arteriolar dilating action, is thought to be useful in that they provide a kidney protective effect.\textsuperscript{34} Recently, in Japan, it has been shown that cilnidipine decreased urinary protein in CKD patients.\textsuperscript{35} Although the precise mechanism by which cilnidipine decreases urinary protein needs to be clarified,
it is possible that the efferent arteriolar dilating effect of cilnidipine may play some part in this mechanism.

**Conclusions:** Cilnidipine dilated the afferent arteriole mainly by blocking L-type calcium channels and dilated the efferent arteriole mainly by blocking N-type calcium channels. Therefore, cilnidipine may lower glomerular pressure and have a renal protective effect.

**REFERENCES**