Inhibitory Effects of AE0047, a New Dihydropyridine Ca\(^{2+}\) Channel Blocker, on Renal Nerve Stimulation-Induced Renal Actions in Anesthetized Dogs

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ABSTRACT

The effects of AE0047, a newly developed dihydropyridine Ca\(^{2+}\) channel blocker, and nicardipine on changes in the renal function and norepinephrine (NE) overflow induced by renal nerve stimulation (RNS) were examined in anesthetized dogs. RNS at a low frequency (0.5–2.0 Hz) caused significant decreases in the urine flow, urinary excretion of sodium, and fractional excretion of sodium, and also inducing increases in the basal renal blood flow, glomerular filtration rate, and filtrate dynamics. RNS at a high frequency (2.5–5.0 Hz), which diminishes the renal blood flow, glomerular filtration rate, and filtrate fraction, elicited more potent decreases in urine formation and increases in NESR than those seen for the low-frequency RNS. Increases in the basal renal blood flow and urine formation were observed. During AE0047 (50 ng/kg/min) infusion, low-frequency RNS-induced antidiuretic action and increase in NESR were markedly attenuated. Qualitatively similar results were observed for high-frequency RNS. In addition, high-frequency RNS-induced renal vasoconstriction was significantly suppressed by AE0047 infusion at higher doses. Lower doses of AE0047 (10 ng/kg/min) tended to attenuate the low- and high-frequency RNS-induced antidiuretic actions, although neither of the RNS-induced increases in NESR were suppressed by lower doses of AE0047. Nicardipine (50 ng/kg/min) also increased the level of basal urine formation, but the RNS-induced changes in renal function and increases in NESR were not affected by this drug. These results suggest that AE0047 suppresses the RNS-induced NE overflow from renal nerve endings, which is probably involved in the inhibitory effects of the drug on the antidiuretic action elicited by RNS.

In the treatment of hypertension, peripheral vasodilators frequently lead to salt and water retention due to diminished urine formation because of reduced renal perfusion pressure and reflex activation of the renal sympathetic nerves (Lemonetti et al., 1971; Koch-Weser 1974). L-type Ca\(^{2+}\) channel blockers nicardipine (Abe et al., 1983), felodipine (DiBona and Sawin, 1984), nifedipine (Bell and Lindner 1984; Imagawa et al., 1986a), manidipine (Morimoto et al., 1989), and nisoldipine (Kageyama et al., 1990) have been observed to produce diuresis, natriuresis, and renal vasodilation in anesthetized dogs and rats. However, renal nerve stimulation (RNS)-induced renal vasoconstriction and antidiuretic actions are not suppressed by the same type of Ca\(^{2+}\) channel blockers (Ogawa et al., 1984, Imagawa et al., 1986b, Johns and Manitius, 1986), thereby suggesting that the above-mentioned renal responses are independent of Ca\(^{2+}\) influx through the L-type Ca\(^{2+}\) channel. Recently, cilnidipine, which can inhibit both L- and N-type Ca\(^{2+}\) currents (Fujii et al., 1997), has been reported to suppress the \(^{[3H]}\)norepinephrine (NE) overflow induced by periartrial nerve stimulation in the perfused mesenteric vasculatures of spontaneously hypertensive rats (Hosono et al., 1995). In addition, this drug attenuated the RNS-induced renal vascular and tubular responses by inhibiting the NE overflow from renal nerve endings in anesthetized dogs (Takahara et al., 1997).

Vatanidipine hydrochloride (AE0047; Fig. 1) is a newly developed dihydropyridine-type Ca\(^{2+}\) channel blocker that produces not only potent hypotensive effect but also a long duration of action (Ohtaki et al., 1989). This compound also has a long-lasting diuretic and natriuretic effect in anesthetized dogs (Hayashi et al., 1993). In isolated dog mesenteric artery, AE0047 has been reported to inhibit the contractile response and NE release to transmural electrical stimulation by interfering with the inward movement of Ca\(^{2+}\) into nerve terminals.

ABBREVIATIONS: RNS, renal nerve stimulation; NE, norepinephrine; AE0047, (–)-2-[4-(4-benzhydrylpiperazin-1-yl) phenyl] ethyl methyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate dihydrochloride; MAP, mean arterial blood pressure; HR, heart rate; RBF, renal blood flow; GFR, glomerular filtration rate; UF, urine flow; UNaV, urinary excretion of sodium; FENa, fractional excretion of sodium; FF, filtration fraction; RVR, renal vascular resistance; NESR, norepinephrine secretion rate.
endings (Okamura et al., 1992.). In this study, we examined the effects of AE0047 on the RNS-induced changes in the renal function and NE overflow in anesthetized dogs by comparing these findings with those seen for nicardipine.

### Materials and Methods

**Animal Preparation.** Adult mongrel dogs of either sexes weighing 11 to 16 kg were used. These dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.), given maintenance doses as needed, and placed on a heated surgical table that maintained the rectal temperature between 37 and 38°C. After tracheal intubation, respiration was supported by artificial ventilation of room air with a Harvard respirator. Polyethylene catheters were placed in the right brachial artery and vein for arterial blood sampling and for the infusion of saline containing 0.45% inulin, respectively. Mean arterial pressure (MAP) and heart rate (HR) were monitored with a pressure transducer (AP601G; Nihon Kohden). Approximately 2 h was allowed for stabilization. HR, and RBF were continuously recorded on a polygraph (RM6000G; Nihon Kohden). Nicardipine was purchased from the Sigma (St. Louis, MO), and all other chemicals were purchased from Nacalai Teque, Inc. (Kyoto, Japan) and Wako Pure Chemical Industries Ltd. (Osaka, Japan). As previously reported (Hayashi et al., 1991). The NERS was calculated by:

$$\text{NERS} (\text{pg/g/min}) = (\text{NEV} - \text{NEA}) \text{RPF}$$

where RPF is the renal plasma flow (microliters per gram per minute), NEV is the renal venous plasma NE concentration (picograms per milliliter), and NEA is the renal arterial plasma NE concentration (picograms per milliliter).

**Drugs.** AE0047 (vatanidipine hydrochloride), synthesized by Yo-shitomi Pharmaceutical Industries Ltd. (Osaka, Japan), was used. Nicardipine was purchased from Sigma (St. Louis, MO), and all other chemicals were purchased from Nacalai Teque, Inc. (Kyoto, Japan) and Wako Pure Chemical Industries Ltd. (Osaka, Japan).

**Statistical Analysis.** All data are expressed as the mean ± S.E. For statistical analyses, we performed Student’s t test for two-sample comparisons and one-way ANOVA followed by Bonferroni’s multiple comparison test for multiple comparisons. For all comparisons, differences were considered significant at $P < .05$ and $P < .01$.

### Results

**Effects of Intrarenal Arterial Infusion of AE0047 at a Low Dose (10 ng/kg/min) on RNS-Induced Renal Actions.** RNS at a low frequency decreased urine flow (UF), urinary excretion of sodium (UnaV), and fractional excretion of sodium (FENa) by 41% to 67%, respectively, without affecting the systemic and renal hemodynamics (Fig. 2; Table 1). RNS at a high frequency produced a more potent reduction in urine formation (UF, UnaV, and FENa decreased by 76, 73, and 45% from control values of 19.0 ± 2.9, 3.64 ± 0.75 μEq/g/min, and 2.9 ± 0.77%, respectively, without affecting the systemic and renal hemodynamics (Fig. 2; Table 1). RNS at a high frequency produced a more potent reduction in urine formation (UF, UnaV, and FENa decreased by 76, 73, and 45% from control values of 19.0 ± 2.9, 3.64 ± 0.75 μEq/g/min, and 2.9 ± 0.77%, respectively, without affecting the systemic and renal hemodynamics (Fig. 2; Table 1). RNS at a high frequency produced a more potent reduction in urine formation (UF, UnaV, and FENa decreased by 76, 73, and 45% from control values of 19.0 ± 2.9, 3.64 ± 0.75 μEq/g/min, and 2.9 ± 0.77%, respectively, without affecting the systemic and renal hemodynamics (Fig. 2; Table 1). RNS at a high frequency produced a more potent reduction in urine formation (UF, UnaV, and FENa decreased by 76, 73, and 45% from control values of 19.0 ± 2.9, 3.64 ± 0.75 μEq/g/min, and 2.9 ± 0.77%, respectively, without affecting the systemic and renal hemodynamics (Fig. 2; Table 1).
and renal vascular resistance (RVR) increased by ~34%; Table 1). When AE0047 (10 ng/kg/min) was administered intrarenally, the basal level of RBF was elevated by ~27% and that of RVR decreased by ~20% (Table 1), without affecting MAP and HR. The GFR remained unchanged during the intrarenal arterial infusion of AE0047, which thus resulted in a significant decrease in FF. In the presence of 10 ng/kg/min AE0047, the low- and high-frequency RNS-induced decreases in urine formation tended to be attenuated (Fig. 2). High-frequency RNS-induced renal vasoconstriction also was slightly attenuated by this dose of AE0047 (Table 1).

**Effects of Intrarenal Arterial Infusion of AE0047 at a High Dose (50 ng/kg/min) on RNS-Induced Renal Actions.** When high doses (50 ng/kg/min) of AE0047 were administered intrarenally, slight and nonsignificant increases in RBF, GFR, and FF were observed. However, a significant decrease in MAP and an increase in HR also were seen (Table 2). A qualitatively similar decrease in RVR and increases in

### Table 1

Effects of AE0047 (10 ng/kg/min) on the RNS-induced changes in systemic and renal hemodynamics in anesthetized dogs

<table>
<thead>
<tr>
<th>Control</th>
<th>Low RNS</th>
<th>High RNS</th>
<th>AE0047 Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>HR (beats/min)</td>
<td>RBF (ml/g/min)</td>
<td>RVR (mm Hg/ml/g/min)</td>
</tr>
<tr>
<td>153.7 ± 1.8</td>
<td>154.5 ± 1.8</td>
<td>3.7 ± 1.2</td>
<td>41.2 ± 2.8</td>
</tr>
<tr>
<td>155.3 ± 7.4</td>
<td>156.5 ± 7.4</td>
<td>3.7 ± 0.9</td>
<td>42.0 ± 2.9</td>
</tr>
<tr>
<td>153.2 ± 8.7</td>
<td>152.3 ± 8.7</td>
<td>3.9 ± 0.8</td>
<td>40.0 ± 2.7</td>
</tr>
<tr>
<td>157.5 ± 8.3</td>
<td>156.2 ± 7.9</td>
<td>3.0 ± 0.4</td>
<td>53.9 ± 6.8</td>
</tr>
</tbody>
</table>

* P < .05; † P < .01 versus each control value (Student's t test).

* P < .01; † P < .05 versus low-frequency RNS-induced percentage of change (Bonferroni's multiple comparison test).

* P < .05, ‡ P < .01 versus control value during saline infusion (Student's t test).

* P < .05 versus high-frequency RNS-induced percentage of change during saline infusion (Bonferroni's multiple comparison test).
the basal levels of UF, UnaV, and FENa were observed with the higher dose as seen with the lower dose (Fig. 3). In the presence of AE0047 at higher doses, low- and high-frequency RNS-induced decreases in urine formation and high-frequency RNS-induced renal vasoconstriction were markedly attenuated. The observed changes in RBF, UF, UnaV, and FENa were 2.4, 14, 10, and 8% by low-frequency RNS and 4.7, 19, 19, and 16% by high-frequency RNS, respectively (Fig. 3; Table 2).

Effects of Intrarenal Arterial Infusion of Nicardipine (50 ng/kg/min) on RNS-Induced Renal Actions. When nicardipine (50 ng/kg/min) was infused intrarenally, no significant changes were seen in the systemic and renal hemodynamics (Table 3). However, the basal level of urine
formation tended to increase with nicardipine infusion (Fig.
4). In contrast to cases with AE0047 administration, nicar-
dipine had no effect on low- and high-frequency RNS-induced
antidiuretic and antinatriuretic actions (Fig. 4). In addition,
high-frequency RNS-induced renal vasoconstriction was
slightly accelerated by nicardipine infusion (Table 3).

**TABLE 3**

Effects of nicardipine (50 ng/kg/min) on the RNS-induced changes in systemic and renal hemodynamics in anesthetized dogs

<table>
<thead>
<tr>
<th></th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>RBF (ml/g/min)</th>
<th>RVR (mm Hg/ml/g/min)</th>
<th>GFR (ml/g/min)</th>
<th>FF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saline infusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>136.6 ± 8.1</td>
<td>149.8 ± 7.7</td>
<td>3.7 ± 0.3</td>
<td>38.9 ± 6.2</td>
<td>0.93 ± 0.05</td>
<td>44.5 ± 6.8</td>
</tr>
<tr>
<td>Low RNS</td>
<td>137.8 ± 8.5</td>
<td>153.0 ± 6.4</td>
<td>3.6 ± 0.4</td>
<td>40.4 ± 6.7</td>
<td>0.92 ± 0.08</td>
<td>46.4 ± 10.0</td>
</tr>
<tr>
<td>High RNS</td>
<td>138.2 ± 9.5</td>
<td>147.2 ± 5.6</td>
<td>3.2 ± 0.4</td>
<td>46.7 ± 8.6</td>
<td>0.58 ± 0.11b</td>
<td>29.8 ± 4.1b</td>
</tr>
<tr>
<td><strong>Nicardipine infusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>125.0 ± 8.0'</td>
<td>147.6 ± 6.4'</td>
<td>4.1 ± 0.6</td>
<td>34.4 ± 7.1</td>
<td>0.98 ± 0.09</td>
<td>46.2 ± 8.0</td>
</tr>
<tr>
<td>Low RNS</td>
<td>125.2 ± 7.6</td>
<td>152.2 ± 8.4</td>
<td>4.0 ± 0.5</td>
<td>34.5 ± 6.7</td>
<td>0.94 ± 0.09</td>
<td>44.6 ± 7.7</td>
</tr>
<tr>
<td>High RNS</td>
<td>120.4 ± 7.2</td>
<td>145.0 ± 9.4</td>
<td>3.1 ± 0.6</td>
<td>44.9 ± 8.8</td>
<td>0.58 ± 0.18b</td>
<td>32.6 ± 6.7b</td>
</tr>
</tbody>
</table>

- *P < .05; **P < .01 versus each control value (Student's t test).
- *P < .01 versus control value (Student’s t test).
- %P < .01 versus control value during saline infusion (Student’s t test).
- %P < .01 versus high-frequency RNS-induced percentage of change during saline infusion (Bonferroni’s multiple comparison test).

**Fig. 4.** Effects of nicardipine (50 ng/kg/min) on the RNS-induced changes in urine formation. Each value represents the mean ± S.E. of six dogs. *P < .05, **P < .01 versus each control value. †P < .05 versus control value during saline infusion. ‡P < .05, ‡‡P < .01 versus low-frequency RNS-induced percentage of change during saline infusion.
markedly from a control value of $-156.1 \pm 43.0$ pg/g/min to $1024.6 \pm 81.8$ and $860.2 \pm 83.5$ pg/g/min at 1 and 9 min after the start of the high-frequency RNS, respectively ($n = 18$). According to the following results, the RNS-induced increases in NESR from control are indicated as $\Delta$NESR to clarify the changes in NESR induced by the RNS. The intra-renal arterial infusion of AE0047 at a higher dose (50 ng/kg/min) significantly attenuated the increases in $\Delta$NESR during low-frequency RNS (from $369.8 \pm 95.4$ and $454.7 \pm 118.4$ pg/g/min to $81.0 \pm 82.7$ and $66.0 \pm 43.2$ pg/g/min at 1 and 9 min after the start of low-frequency RNS, respectively). A lower dose of AE0047 (10 ng/kg/min) and nicardipine (50 ng/kg/min) had no significant effect on the low-frequency RNS-induced increases in $\Delta$NESR (Fig. 5). The high-frequency RNS-induced increases in $\Delta$NESR also were attenuated by the higher dose of AE0047. In contrast to the cases demonstrating low-frequency RNS, the high-frequency RNS-induced increases in $\Delta$NESR at 1 min were significantly enhanced by the lower dose of AE0047. However, nicardipine had no effect on the high-frequency RNS-induced changes in $\Delta$NESR (Fig. 6).

Discussion

Hypotension induced by peripheral vasodilators causes a baroreceptor-mediated reflex increase in the sympathetic nerve activities and neurohormonal systems. In the kidney, the activation of the renal sympathetic nerve induces NE overflow, renal vasoconstriction, and salt (and water) retention, which thereby lead to diminished urine formation (Leonetti et al., 1971; Koch-Weser 1974; Zambraski et al., 1976; DiBona, 1977). In this study, we demonstrated that AE0047, a novel dihydropyridine-type Ca$^{2+}$ channel blocker, efficiently suppresses the antidiuresis, antinatriuresis, and NE overflow induced by the stimulation of the renal sympathetic nerve.

Hayashi et al. (1993) reported that the intrarenal arterial infusion of AE0047 (50 ng/kg/min) for 25 min produces diuretic and natriuretic effects without elevating the level of RBF. In addition, there were marked increases in urine formation after the termination of drug infusion. In this study, we noted an extremely slow onset and long-lasting renal actions of AE0047, i.e., significant increasing actions of 10 and 50 ng/kg/min of this agent on UF and RBF were observed 20 to 30 min after the start of drug infusion, and these responses reached a plateau at 80 to 90 min. Because the RNS experiment during AE0047 infusion was performed 90 min after the start of drug infusion, there was an increased basal level of RBF. Such a slow onset and long-lasting pharmacological actions of AE0047 have been demonstrated in both in vivo and in vitro studies with anesthetized rats and dogs, and rat aortic strips, respectively (Ohtaki et al., 1989; Nishikawa et al., 1998). Other Ca$^{2+}$ channel blockers administered intrarenally also have been indicated to produce diuretic and natriuretic actions in anesthetized rats and dogs (Abe et al., 1983; Brown and Churchill, 1983; Dietz et al., 1983; Johns, 1985; Imagawa et al., 1986a; Johns and Manitius, 1986; Fukui et al., 1987; Kageyama et al., 1989; Kageyama et al., 1990). Clearly, these renal vasodilatory
effects are considered to contribute, at least in part, to such actions. However, direct inhibitory actions on tubular electrolyte and water reabsorption also are involved, although the precise mechanisms underlying such tubular actions are unknown (Abe et al., 1983; Brown and Churchill, 1983; Dietz et al., 1983; Johns, 1985; Imagawa et al., 1986a; Johns and Manitius, 1986; Fukui et al., 1987; Kageyama et al., 1989; Kageyama et al., 1990). Irrespective of the mechanisms of AE0047-induced diuresis and natriuresis, these effects seem to be beneficial to the treatment of hypertensive diseases.

It is well established that RNS enhances renal tubular sodium reabsorption and increases renal vascular tone, both effects being mediated by α₁-adrenoceptors, while also diminishing urine formation and renal hemodynamics (Osborn et al., 1983; Hesse and Johns, 1984; Chiba et al., 1990). In this study, low-frequency RNS elicited a decreased urine formation without affecting the systemic and renal hemodynamics, although high-frequency RNS produced a more potent anti-diuretic and antinatriuretic actions, in addition to reductions in RBF, GFR, and FF. L-type Ca²⁺ channel blockers nifedipine (Imagawa et al., 1986b; Ogasawara et al., 1993), verapamil (Ogawa et al., 1984), diltiazem, and nicardipine (Johns and Manitius, 1986) have been shown to be ineffective on the RNS-induced renal actions, thereby suggesting that the influx of extracellular Ca²⁺ through L-type Ca²⁺ channel has no modulatory effects on α₁-adrenoceptor-mediated antidiuresis and renal vasoconstriction. In this study, we demonstrated that AE0047 markedly suppressed the RNS-induced renal vasoconstriction and antidiuresis, in contrast to the effects observed by nicardipine. To clarify the mechanisms underlying these effects, we determined the NESR during the RNS with or without drug infusion. Our results clearly indicated that the intrarenal administration of AE0047 but not nicardipine, at the same doses, markedly attenuated the NE overflow induced by both low- and high-frequency RNS.

The influx of extracellular Ca²⁺ into nerve endings through the N-type Ca²⁺ channel plays an important role in transmitter release (Hirning et al., 1988). Recently, dihydropyridine derivative cilnidipine, which attenuates both L- and N-type Ca²⁺ currents, was reported to reduce the [³H]NE overflow evoked by periarterial nerve stimulation in the isolated mesenteric artery of spontaneously hypertensive rats (Hosono et al., 1995). The RNS-induced renal actions and NE overflow also were suppressed by this agent, in anesthetized dogs (Takahara et al., 1997). However, Okamura et al. (1992) found that AE0047 inhibits transmural electrical stimulation-induced vasoconstriction and [³H]NE overflow in the dog mesenteric artery, thus suggesting that the drug decreases transmembrane influx of Ca²⁺ into adrenergic nerve endings. In this study, the higher dose of AE0047 suppressed the RNS-induced NE overflow from the renal sympathetic nerves. Collectively, it seems likely that the inhibitory effect of AE0047 on the RNS-induced NE release is due to the decrease of inward Ca²⁺ movement due to the blocking of the N-type Ca²⁺ channel located on the nerve endings, although more direct evidence is needed to support this view.

The lower dose of AE0047 showed a slight but significant increase in NESR during high-frequency RNS. However, this dose of the drug tended to attenuate the low- and high-frequency RNS-induced changes in urine formation and re-
nal hemodynamics. These results suggest that AE0047 can inhibit RNS-induced changes in the renal functions, independent of the NE overflow. Most recently, we found that the lower dose of AE0047 tended to attenuate the antidiuretic action induced by exogenously applied NE (data not shown). At present, we cannot explain why the lower dose of AE0047 only slightly increases the NRESR in high-frequency RNS. One possibility is that the increase in RBF during AE0047 infusion may somewhat overestimate the values of NRESR because the calculation of NRESR includes RBF changes. However, an increased RBF may have a “washout” effect on the NE overflow. A previous study indicated that renal NE spillover is partly affected by a change in the RBF (Garty et al., 1990). It is also possible that AE0047 may increase NRESR via blocked of presynaptic α1-adrenoceptors. Dihydropyridines having some structural similarity to AE0047 have been found to have antagonist activity at various α-adrenoceptor subtypes (Marzabadi et al., 1999). Furthermore, AE0047 contains the arylpiperazine element found in many α-adrenoceptor antagonists. Further studies are needed to evaluate this and other possible mechanisms for the actions produced by the lower dose of AE0047.

In summary, the intrarenal arterial infusion of AE0047 exerted renal vasodilatory, diuretic and natriuretic actions. AE0047 at the lower dose attenuated low- and high-frequency RNS-induced antidiuretic and antinatriuretic responses, independently of NE release. A higher dose of this drug effectively inhibited RNS-induced NE release and changes in renal functions. However, the same dose of nifedipine, which has a similar hypotensive effect to AE0047, had no effect on the renal responses induced by RNS. The inhibitory effect of AE0047 on the RNS-induced renal actions therefore seems to be due, at least in part, to the inhibition of the inward movement of Ca2+ into nerve endings. Thus, AE0047 may be more useful in treatment of hypertensive patients with diminished renal function than other dihydropyridine-type Ca2+ channel blockers.

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References