Preferential Inhibitory Effect of Nifedipine on Angiotensin II–Induced Renal Vasoconstriction

JUN-ICHI IMAGAWA, MIZUE KUSABA-SUZUKI, AND SUSUMU SATOH

SUMMARY The inhibitory effects of nifedipine on renal vasoconstrictor response to angiotensin II, norepinephrine, or renal nerve stimulation were tested in anesthetized dogs. Intrarenal infusions of nifedipine (0.3, 1, and 3 μg/min) dose-dependently suppressed the renal vasoconstriction induced by intrarenal injections of angiotensin II (0.03, 0.05, and 0.1 μg) or norepinephrine (0.3–1 μg) but not that by renal nerve stimulation (4–7 Hz). However, the inhibitory effect of nifedipine on angiotensin II–induced vasoconstriction was greater than its effect on norepinephrine-induced or renal nerve stimulation–induced vasoconstriction (i.e., 50% reduction in renal blood flow). Furthermore, a greater renal vasodilation induced by intrarenal bolus injections of nifedipine (1, 3, and 10 μg) but not by acetylcholine (0.1 and 0.3 μg) was observed during the reduction in the perfusion pressure of the contralateral kidney to approximately 50 mm Hg, which resulted in an increase in plasma renin activity and plasma angiotensin II concentration but no change in plasma norepinephrine concentration. There was a significant positive correlation between plasma renin activity and plasma angiotensin II concentration before nifedipine injections and the subsequent increase in renal blood flow produced by each dose of nifedipine. These results indicate that nifedipine has a relatively preferential inhibitory effect on the renal vasoconstriction produced by both exogenous and endogenous angiotensin II in canine renal vasculature. (Hypertension 8: 897-903, 1986)

KEY WORDS • nifedipine • angiotensin II • renal vasoconstriction

CALCIUM entry blockers, including nifedipine, have been reported to inhibit Ca⁺⁺ entry into cardiac muscle and coronary vascular smooth muscle cells by direct inhibition of the slow Ca⁺⁺ channel, thereby producing a negative inotropic effect and coronary vasodilation. A peripheral vasodilative effect of calcium entry blockers has been shown, and their antihypertensive effect has been studied extensively in animals and hypertensive patients. The antihypertensive property of calcium entry blockers is based on the decrease in total peripheral vascular resistance induced by these agents. On the other hand, the kidney is thought to be one of the key organs affecting the systemic blood pressure in normal and pathological states. It has been proposed that increased renal vascular resistance is necessary to sustain elevated pressure. Therefore, the renal vascular effect of calcium entry blockers is an important aspect in an evaluation of their value as antihypertensive drugs. In this context, we previously examined the effect of verapamil on the stimulation produced by the endogenous vasoconstrictor substances angiotensin II (ANG II) and norepinephrine (NE) and by renal nerve stimulation (RNS) and observed that verapamil exerted a relatively greater inhibitory effect on ANG II–induced renal vasoconstriction. Similarly, Yamaguchi et al. who also used autoperfused dog renal vasculature, reported that dilazem, another calcium entry blocker, exhibits an antagonistic effect on the ANG II–induced vasoconstriction, whereas it has no effect on epinephrine-induced vasoconstriction.

In our present experiments, we obtained evidence that nifedipine also exerts an inhibitory effect on ANG II–induced renal vasoconstriction. Thus, a relatively selective antagonistic effect against exogenous ANG II seems to be a common property shared by calcium entry blockers. This finding prompted us to extend our study to assess the renal vasodilative effect of nifedipine on increased endogenous ANG II formation, which was induced by a reduction in the perfusion pressure of the contralateral kidney.
Materials and Methods

Twenty-two mongrel dogs of either sex, weighing 12 to 16 kg, were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and artificially ventilated with room air at 13 breaths/min and a tidal volume of 20 to 25 ml/kg after administration of decamethonium bromide (0.25 mg/kg i.v.) to induce paralysis of the skeletal muscle. Anesthesia was maintained by intravenous infusion of sodium pentobarbital (5 mg/kg/hr). Decamethonium bromide was employed under pentobarbital anesthesia that was of suitable depth and duration. Animals were cared for in accordance with guidelines published by the Prime Minister's Office of Japan on Standards relating to the care and management of experimental animals (Notification No. 6, 1980). The right brachial artery was catheterized to monitor mean systemic blood pressure (MBP) with a pressure transducer (MPU-0.5; Nihon Kohden, Tokyo, Japan), and this transducer was also used for collection of arterial blood samples. The cephalic vein was cannulated for intravenous administration of drugs.

In Groups 1 and 2, the left kidney was exposed by a flank incision using the retroperitoneal approach. A noncannulating electromagnetic flow probe (2.5–3.5 mm in diameter) was placed around the left renal artery for measurement of renal blood flow (RBF) with a square-wave electromagnetic flowmeter (MF-27; Nihon Kohden). For the infusion of nifedipine (Group 1) or vehicle (Group 2), a 26-gauge needle connected to a polyethylene tube (PE-20) was inserted into the renal artery proximal to the flow probe. In addition, another needle, also connected to a polyethylene tube, was inserted into the renal artery for injection of ANG II or NE.

The renal nerves were carefully dissected away from the renal vessels and cut after ligation. Platinum electrodes were placed on the renal nerve plexus at a point where there could be no contact with the renal artery. In Group 3, a catheter was introduced into the abdominal aorta through the left femoral artery to monitor the renal arterial pressure using a second pressure transducer. Left and right kidneys were exposed by bilateral flank incisions using the retroperitoneal approach. Noncannulating electromagnetic flow probes were placed around both renal arteries for measurement of RBF with square-wave electromagnetic flowmeters. An arterial constrictor was placed around the aorta between the origins of the left and right renal arteries to reduce the perfusion pressure of the left kidney. For injections of nifedipine and acetylcholine, two 26-gauge needles connected to polyethylene tubes were inserted into the right renal artery. The MBP, renal arterial pressure, and RBF were recorded on a polygraph with linear-writing pens (PM-85; Nihon Kohden). A 60- to 90-minute equilibration period was allowed after the operation.

Experimental Protocol

Group 1

The experimental protocol for Group 1 (n = 8) consisted of four consecutive 40-minute periods. The first period was the control phase during which vehicle, 0.1 ml/min, was infused into the renal artery. Subsequently, nifedipine was infused into the renal artery at 0.3 μg/min during the second period, 1 μg/min during the third period, and 3 μg/min during the fourth period. All nifedipine infusions were delivered at 0.1 ml/min. Intrarenal injections of ANG II and NE and RNS were applied during all four 30-minute periods, starting 10 minutes after the commencement of the vehicle (control period) or nifedipine infusions. Animals received three graded bolus injections of ANG II (0.03, 0.05, and 0.1 μg), one dose of NE, and one level of frequency of RBF for 30 seconds (supramaximal voltage, 10–20 V; duration, 1 msec). On application of the vasoconstrictor stimuli, RBF was immediately reduced and reached a peak value before abruptly recovering to the prestimulated level within 2 or 3 minutes. The NE dose (0.3–1 μg) and the RNS frequency (4–7 Hz) were adjusted to decrease the RBF to about one half of its initial level in the control period, and these fixed levels of NE dosage and RNS frequency were challenged in all four periods. The drug injections and RNS were applied at 5- to 7-minute intervals in random order.

Group 2

Group 2 (n = 5) underwent the same procedures as described for Group 1, except that only vehicle, 0.1 ml/min, was infused during the four periods.

Group 3

Nine dogs received a series of intrarenal bolus injections of nifedipine (1, 3, and 10 μg) and acetylcholine (0.1 and 0.3 μg) into the right kidney during the following three periods: 1) a 40-minute control period, 2) a 60-minute period during the reduction of left renal arterial pressure to about 50 mm Hg with the aortic constrictor, and 3) a 40-minute recovery period when the aortic constrictor was removed. In the second and third periods, the series of drug injections were started 20 minutes after the initiation of aortic constriction and 60 minutes after the removal of the aortic constriction, respectively. Bolus injection of nifedipine or acetylcholine caused an immediate increase in RBF, which reached a maximum level and then abruptly returned to the preinjection level within 3 or 4 minutes. Nifedipine and acetylcholine were injected in random order at about 5-minute intervals. Arterial blood samples were collected just before and 10 minutes after the last dosing in the series of drug injections for each period. Arterial plasma renin activity (PRA), plasma ANG II concentration (in 5 of 9 animals), and plasma NE concentration were determined.

Measurements

The blood samples were transferred to chilled tubes containing disodium ethylenediaminetetraacetic acid (6 mg/ml of blood) and centrifuged to obtain plasma samples. The PRA was measured by radioimmunoassay according to the methods of Fyhrquist et al., as...
previously described. Plasma ANG II concentration was measured by radioimmunoassay following extraction of plasma on Dowex resin by the methods of Page et al. The ANG II antibody used in the assay cross-reacts 100% with ANG III and 0.1% with ANG I. The intra-assay and interassay coefficients of variation average 2.4 and 10.4%, respectively. Recovery of ANG II during the extraction procedure averages 75.6%. Plasma NE concentration was measured with an amperometric detector after catecholamine separation by high-performance liquid chromatography (Model LC 304; Bioanalytical Systems, West Lafayette, IN, USA) according to the methods described by Lanier and Malik.

Drugs

The ANG II (Osaka Protein Research Foundation, Osaka, Japan), L-NE hydrochloride (Sigma Chemical, St. Louis, MO, USA), and acetylcholine chloride (Daiichi Pharmaceutical, Tokyo, Japan) were dissolved in 0.9% saline. Nifedipine (Bayer Yakuhin, Osaka, Japan) was dissolved in 0.9% saline/polyethylene glycol 400/ethanol (70:15:15, vol/vol).

Statistics

Differences were considered significant at a $p$ level of less than 0.05 using Student's $t$ test and analysis of variance for multiple comparisons. When multiple comparisons were made with a single control, Dunnett's test was used to determine significance levels. Regression coefficients were calculated using the method of least squares. All values are presented as the mean ± SEM.

Results

Group 1

Ten minutes after the start of nifedipine infusions, RBF increased from 273 ± 19 to 280 ± 19 ml/min (0.3 $\mu$g/min; $p < 0.01$), from 280 ± 22 to 288 ± 23 ml/min (1 $\mu$g/min; $p < 0.05$), and from 294 ± 24 to 305 ± 26 ml/min (3 $\mu$g/min; $p < 0.05$). The MBP was not altered by the nifedipine infusions of 0.3 and 1 $\mu$g/min, but it was slightly reduced from 123 ± 5 to 120 ± 6 mm Hg 10 minutes after the start of the 3 $\mu$g/min infusion. Administration of ANG II (0.03, 0.05, and 0.1 $\mu$g) produced a dose-dependent reduction of RBF, while the injection of NE (0.3-1 $\mu$g) and application of RNS (4-7 Hz) decreased RBF by 52.3 ± 3.9 and 52.3 ± 2.3%, respectively.

The MBP did not change in response to vasoconstrictor stimuli. During the nifedipine infusion, the decrease in RBF produced by ANG II was markedly suppressed. This phenomenon was observed even at the lowest dose of nifedipine. The NE-induced vasoconstriction was significantly reduced by nifedipine only at the highest dose used (Figure 1). From these observations, there was no doubt that the inhibitory effect of nifedipine on ANG II-induced renal vasoconstriction was greater than its effect on NE-induced renal vasoconstriction. The RNS-induced vasoconstriction was not affected by nifedipine even at the highest dose (see Figure 1).

Group 2

The time control study demonstrated the reproducibility of the responses to renal vasoconstrictor stimuli (Table 1).
TABLE 1. Decrease in Renal Blood Flow Induced by Intrarenal Angiotensin II or Norepinephrine Injections or Renal Nerve Stimulation

<table>
<thead>
<tr>
<th>Period</th>
<th>Angiotensin II</th>
<th>NE (0.2–0.5 µg)</th>
<th>RNS (3–7 Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.03 µg</td>
<td>0.05 µg</td>
<td>0.1 µg</td>
</tr>
<tr>
<td>1 (control)</td>
<td>36 ± 8</td>
<td>75 ± 12</td>
<td>131 ± 20</td>
</tr>
<tr>
<td></td>
<td>(191 ± 23)</td>
<td>(196 ± 22)</td>
<td>(204 ± 21)</td>
</tr>
<tr>
<td>2</td>
<td>42 ± 12</td>
<td>76 ± 18</td>
<td>123 ± 20</td>
</tr>
<tr>
<td></td>
<td>(194 ± 36)</td>
<td>(191 ± 27)</td>
<td>(192 ± 23)</td>
</tr>
<tr>
<td>3</td>
<td>42 ± 14</td>
<td>77 ± 17</td>
<td>136 ± 19</td>
</tr>
<tr>
<td></td>
<td>(183 ± 26)</td>
<td>(186 ± 23)</td>
<td>(189 ± 24)</td>
</tr>
<tr>
<td>4</td>
<td>44 ± 11</td>
<td>74 ± 13</td>
<td>132 ± 17</td>
</tr>
<tr>
<td></td>
<td>(184 ± 30)</td>
<td>(183 ± 27)</td>
<td>(182 ± 27)</td>
</tr>
</tbody>
</table>

Values are means ± SEM in five dogs. Basal renal blood flow levels (ml/min) are shown in parentheses.

The dose of norepinephrine (NE) and the frequency of renal nerve stimulation (RNS) were adjusted so that renal blood flow was decreased to about one half of its initial level in the first period.

TABLE 2. Changes in Mean Systemic and Renal Arterial Blood Pressure and Renal Blood Flow Induced by Aortic Constriction Before and After a Series of Injections of Nifedipine and Acetylcholine in Nine Dogs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Aortic constriction</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>129.1 ± 7.2</td>
<td>132.3 ± 7.9</td>
<td>148.7 ± 7.3*</td>
</tr>
<tr>
<td>Mean left RAP (mm Hg)</td>
<td>127.2 ± 7.0</td>
<td>130.2 ± 7.4</td>
<td>49.2 ± 2.0*</td>
</tr>
<tr>
<td>Renal blood flow (ml/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>161.7 ± 15.8</td>
<td>154.2 ± 16.8</td>
<td>131.0 ± 16.4†</td>
</tr>
<tr>
<td>Left</td>
<td>156.4 ± 20.0</td>
<td>150.1 ± 23.4</td>
<td>67.9 ± 14.5†</td>
</tr>
</tbody>
</table>

All values are means ± SEM. MBP = mean systemic blood pressure; RAP = renal arterial pressure.

* p < 0.001, † p < 0.05, compared with control values (before).

Group 3

During aortic constriction, left and right RBF and left renal arterial pressure were reduced while the MBP was significantly increased (Table 2). During the constriction, RBF was maintained at a relatively constant level. Under these conditions, PRA and plasma ANG II concentration increased from 4.0 ± 0.4 to 4.8 ± 0.8 ng ANG I/ml/hr ( p < 0.02) and from 28.5 ± 8.6 to 62.9 ± 16.4 pg/ml ( p < 0.02), respectively. The plasma NE concentration was not altered after this procedure (Figure 2). The administration of nifedipine (1, 3, and 10 µg) and acetylcholine (0.1 and 0.3 µg) into the right renal artery produced a dose-dependent increase in blood flow to the kidney. During the reduction in perfusion pressure of the contralateral kidney, nifedipine-induced renal vasodilation was notably augmented. Bolus injection of nifedipine or acetylcholine into the right renal artery did not affect the MBP and left RBF. However, during the reduction in perfusion pressure of the contralateral kidney, nifedipine-induced renal vasodilation was notably augmented. Bolus injection of nifedipine or acetylcholine into the right renal artery did not affect the MBP and left RBF. Also, there were no statistically significant differences between PRA, plasma ANG II concentration, or plasma NE concentration before or after the series of drug injections in each period (see Figure 2).

Discussion

The present experiments show that nifedipine effectively attenuates ANG II–induced and, to a lesser degree, NE-induced vasoconstriction in the canine renal vascular bed. In this experiment, injection of NE (0.3–1 µg) caused about a 50% reduction in RBF (see Figure 1). A partially inhibitory effect of nifedipine on NE-induced vasoconstriction has been reported in canine mesenteric circulation.14 Nifedipine is commonly believed to interfere with the slow inward current (i.e., transmembrane Ca2+ influx),1 but an effect on intracellular Ca2+ release has also been proposed.15 The present result, therefore, suggests that the mechanisms by which ANG II and NE interact with Ca2+ to induce renal vasoconstriction are different. In isolated vascular preparations, the vasoconstriction induced by NE has been shown primarily to involve intracellular Ca2+ release14, 15 and transmembrane Ca2+ influx.14 15 Also, in isolated rabbit aortic strips, NE-induced contraction was not affected by nifedipine.16 These results may be
explained in terms of the involvement of intracellular Ca\(^{2+}\) in response to NE and also the preferential blockade by nifedipine of the transmembrane influx of extracellular Ca\(^{2+}\). Ackerly et al.\(^\text{17}\) found that ANG II-induced contractions in isolated rabbit aorta were partially blocked by verapamil, another calcium entry blocker, and more effectively blocked on the removal of Ca\(^{2+}\) from the extracellular environment. These authors suggested that ANG II uses extracellular Ca\(^{2+}\) and loosely bound Ca\(^{2+}\) to elicit the contraction.

Freer\(^\text{18}\), on the other hand, reported that ANG II-induced contraction in isolated rabbit aorta remained virtually unchanged despite the presence of verapamil. Our in vivo study showed that nifedipine exerts a greater inhibitory effect on ANG II-induced than on NE-induced renal vasoconstriction. Although we cannot reconcile in vitro findings for relatively larger vessels (i.e., those at the arteriolar level) with our in vivo findings for those at the arteriolar level, if nifedipine does primarily block the transmembrane influx of extracellular Ca\(^{2+}\) then our results suggest that ANG II-induced vasoconstriction is much more dependent than NE-induced vasoconstriction on the influx of Ca\(^{2+}\) from the extracellular environment.

On the other hand, nifedipine failed to alter the vasoconstrictor response to RNS. This finding suggests that extracellular Ca\(^{2+}\) influx into the cytoplasm in the coupling of the nerve impulses with the consequent release of NE at adrenergic terminals may not be affected by nifedipine. The same line of evidence was reported by Haeusler\(^\text{19}\), who showed that verapamil had no effect on the Ca\(^{2+}\)-dependent release of NE from adrenergic nerve terminals in the isolated cat heart.

Furthermore, we found that nifedipine-induced renal vasodilation was significantly augmented during the reduction in perfusion pressure of the contralateral kidney. In this situation, arterial PRA and concomitant plasma ANG II concentration were elevated, possibly because of baroreceptor-mediated renin release from the juxtaglomerular cells of the affected kidney. It is conceivable that endogenously increased ANG II concentration continuously stimulates the renal vascular ANG II receptor, thereby contributing detectably to

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**Figure 2.** Changes in plasma renin activity (PRA) and plasma angiotensin II (ANG II) and norepinephrine (NE) concentrations as a result of aortic constriction (AC) in nine dogs. Values are means ± SEM. C = control period; R = recovery period; Bef = before the series of drug injections; Aft = after the series of drug injections.

**Figure 3.** Influence of reduction in contralateral renal arterial pressure by aortic constriction (AC) on the nifedipine (Nif)-induced or acetylcholine (Ach)-induced increase in ipsilateral renal blood flow (RBF) in nine dogs. Values are means ± SEM. C = control period; R = recovery period.
renal vascular tone. In fact, ipsilateral RBF was decreased with an accompanying increase in MBP during this procedure. Other investigators have also demonstrated a decrease in RBF accompanied by a reduction of contralateral renal arterial pressure in conscious dogs. The resulting increase in transmembrane Ca²⁺ influx may be associated with the increase in plasma ANG II concentration. Under these circumstances, extracellular Ca²⁺-dependent vascular tone would be effectively unmasked by the blockade of the Ca²⁺ channel with nifedipine. Thus, the vasodilation induced by nifedipine would be greater.

In a situation in which hypertension is ascribable to an increase in renin release and hence an increased ANG II concentration in the blood, the inhibitory effect of nifedipine on ANG II–induced renal vasoconstriction may be an important factor in the hypotensive effect of nifedipine. It has also been reported that nifedipine reduces the pressor effect and aldosterone secretion induced by ANG II in humans. Based on the present results using dog renal vasculature, the hypotensive response to nifedipine would be expected to be correlated with PRA. However, studies in humans suggest that the hypotensive response to nifedipine is effective in patients with low PRA and that it is inversely correlated to PRA. At present, too large a gap exists to allow an explanation of the discrepancy between the experimental and clinical results.
in relation to PRA. It is a matter of conjecture whether the discrepancy between animal experimental and clinical results is related to species difference, to the difference between blood pressure response in hypertensive patients and renal vascular response in normotensive dogs, or to other undefined factors. Thus, further study appears to be necessary to assess the role of calcium entry blockers, including nifedipine, in anti-hypertensive therapy.

Acknowledgment

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