Effects of Nifedipine on Renal Vascular Responses to Vasoactive Agents in Rabbits

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SEINO, M., ABE, K., ITO, S., YASUJIMA, M., CHIBA, S., HIWATARI, M., SATO, K., GOTO, T., OMATA, K., TAJIMA, J., TANNO, M. and YOSHINAGA, K. Effects of Nifedipine on Renal Vascular Responses to Vasoactive Agents in Rabbits. Tohoku J. exp. Med., 1984, 142 (1), 67-76. The present study was designed to investigate the effects of the Ca-antagonist, nifedipine, on receptor-mediated renal vascular responses to vasoconstrictors (angiotensin II, norepinephrine and vasopressin) and vasodilators (bradykinin and prostaglandin E2). Renal blood flow was estimated by noncannulating electromagnetic flowmetry in anesthetized rabbits. Vasoactive substances were infused directly into the renal artery. After the intravenous administration of nifedipine (50 μg/kg), decreases in renal blood flow in response to angiotensin II infused at rates of 2.5, 5 and 10 ng/kg/min were attenuated by 64% (p <0.01), 45% (p <0.05) and 42% (p <0.05), respectively. Decreases in renal blood flow in response to vasopressin infused at rates of 10, 20 and 50 mU/kg/min were also attenuated by nifedipine, 50% (p <0.05), 57% (p <0.05) and 38% (p <0.05), respectively. However, renal vasoconstrictor responses to norepinephrine (25, 50 and 100 ng/kg/min) did not change significantly after the administration of nifedipine. Increases in renal blood flow induced by the intrarenal infusion of bradykinin (2.5, 5 and 10 ng/kg/min) and prostaglandin E2 (20, 50 and 100 ng/kg/min) were not affected by nifedipine. These results suggest that receptor-mediated vasoconstrictor responses of the renal vascular bed to angiotensin II and vasopressin are produced mainly by Ca++ influx but that of norepinephrine is not. Furthermore, it is confirmed that the renal vasodilator effect of bradykinin and prostaglandin E2 is not altered by nifedipine in anesthetized rabbits.

Calcium ions (Ca++) play an important role in the regulation of smooth muscle contractility (Somlyo and Somlyo 1970; Johanson 1978). On the other hand, Ca-antagonists such as nifedipine inhibit Ca++ influx into the cell by blockade of the slow Ca-channel (Fleckenstein 1977; Fleckenstein and Flecken-
Fleckenstein 1977). It has been shown that nifedipine inhibits the vasoconstrictor action of norepinephrine in the isolated rabbit aorta or mesenteric artery in vitro (Schümann et al. 1975) and partially inhibits the norepinephrine effects on the canine mesenteric circulation in vivo (Kazimierz et al. 1981). However, the influence of the Ca-antagonist on the renal circulation has not been studied extensively (Klütsch et al. 1972; Ono et al. 1974; Yamaguchi et al. 1974). Little is known regarding the effects of Ca-antagonists on receptor-mediated renal vascular responses to vasoconstrictor substances such as angiotensin II, norepinephrine and vasopressin. If Ca\textsuperscript{++} influx is involved to similar extents in vasoconstrictor responses to these substances, the Ca-antagonists may block the effect to similar extents. One of the aims in present study is to determine whether an increase in transmembrane Ca\textsuperscript{++} influx is a major mechanism involved in receptor-mediated vasoconstrictor responses to angiotensin II, norepinephrine and vasopressin in the renal vascular bed. Further, it is not known whether vasodilators such as bradykinin and prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) are influenced by nifedipine. Another aim of this study is to investigate whether nifedipine modifies the renal vasodilator responses to bradykinin and PGE\textsubscript{2}. In this study, nifedipine was chosen as the Ca-antagonist, since it has been reported to have a higher vascular selectivity than other agents (Narimatsu and Taira 1976).

**METHODS**

Thirty-two female Japanese white rabbits weighing 3.2 to 3.9 kg were used. They had been allowed free access to normal laboratory chow and drinking water. The animals were anesthetized with intravenous urethane (450 mg/kg) and \( \alpha \)-chloralose (45 mg/kg). Subsequently, small maintenance doses of the anesthetics were given as needed. The trachea was cannulated and polyethylene catheters were inserted into the abdominal aorta (PE 60) through the femoral artery for continuous recording of arterial pressure and into the inferior vena cava through the femoral vein (PE 50) for administration of nifedipine or vehicle. For blood flow measurements, the left renal artery was exposed through a flank incision and a noncannulating electromagnetic flow probe of appropriate diameter (1.5 to 2.5 mm, Nihon Kohden) was placed around the left renal artery. The flow probe was connected to an electromagnetic flow meter (Nihon Kohden, MF-27). The flow probes were calibrated electrically under the condition while they were dipped in the isotonic saline solution. Zero flow was determined by a brief occlusion of the renal arteries distal to the probe immediately after its application and at the end of the experiment. For intrarenal arterial infusion of vasoactive substances, a 27-gauge needle was inserted into the left renal artery proximal to the flow probe and connected to polyethylene tube (PE 50). The lumen of catheter was kept patent by infusion of 5\% dextrose at a rate of 0.057 ml/min with a Harvard infusion pump (Harvard, 940E). Arterial pressure was monitored with a pressure transducer and amplifier (San-Ei, Biophysigraph, 180 system) and renal blood flow was simultaneously recorded on a pen oscillograph. The rabbits were given an infusion of Ringer's lactate solution equal to 2\% of body weight to compensate for surgical losses.

Angiotensin II (Ciba, 0.1 mg, Hypertensin\textsuperscript{®}), DL-norepinephrine (Sankyo, 1 mg/ml), arg-vasopressin (Protein Research Foundation, 550 U/vial), bradykinin (Sandoz, 0.1 mg) and PGE\textsubscript{2} (Ono, 1 mg) were diluted with 5\% dextrose to appropriate concentrations for the administration into the renal artery. Nifedipine (0.2 mg/ml) was a gift from Bayer Co., Ltd. After surgery, at least one hour was allowed to elapse to stabilize the arterial pressure.
and renal blood flow. To study the direct effect of nifedipine on renal circulation, it was infused into the renal artery at 0.5, 1.0 and 2.5 \( \mu g/kg \) per min for 3 min \((n=6)\). In 6 rabbits, angiotensin II was administered similarly. The doses of angiotensin II used in the experiments were 2.5, 5 and 10 ng/kg per min. In other rabbits, norepinephrine was administered into the renal artery at 25, 50 and 100 ng/kg/min \((n=6)\).

In preliminary experiments, large doses of vasopressin (over 10 mU/kg/min) were required to produce renal vasoconstriction. Therefore, vasopressin was administered intrarenally at 10, 20 and 50 mU/kg per min in 5 rabbits.

Bradykinin was infused into the renal artery at 2.5, 5 and 10 ng/kg per min \((n=6)\). These rabbits were used again for intrarenal infusion of PGE\(_2\), because the half life of bradykinin is extremely short. Thirty minutes after the control doses of bradykinin, PGE\(_2\) was infused into the renal artery. The doses of PGE\(_2\) were 20, 50 and 100 ng/kg per min. In 3 rabbits, 5% dextrose was infused as a control, at 0.028, 0.057 and 0.113 ml/min before and after the administration of nifedipine. Each infusion lasted for 3 min.

Recovery periods of 10-20 min were allowed between doses and the sequence of administration was varied randomly. In each experiment, following the determination of the control series of dose-response curves, 50 \( \mu g/kg \) of nifedipine was administered intravenously. Extreme care was taken to reduce the exposure of nifedipine to light. Fifteen minutes after the nifedipine treatment, determinations of dose response curves were repeated again in each experimental group.

In order to determine plasma renin activity, blood (3 ml) was collected into a heparinized plastic tube from the ear artery in conscious animals and from the femoral artery during anesthesia initially before the administration of vasoactive substances and subsequently 15 min after the administration of nifedipine. The blood was replaced immediately with blood collected from other rabbits. Plasma renin activity was determined by radioimmunoassay as described previously (Abe et al. 1972). Renal vascular resistance was calculated by dividing mean arterial pressure by renal blood flow and expressed as mmHg/ml/min. All values are expressed as mean ± s.e. Data were analyzed by Student’s paired t-test.

**Results**

Intrarenal arterial infusions of nifedipine produced a dose-dependent increase in renal blood flow as shown in Table 1. These infusion, however, caused no significant change in systemic arterial pressure. Renal vascular resistance was decreased significantly by each dose.

The plasma renin activity in the conscious animals was 0.7±0.1 ng/ml/hr. Under anesthesia, it markedly increased (Fig. 1). A further significant increase in

| Table 1. Effects of intrarenal arterial administration of nifedipine on mean arterial pressure (MAP), renal blood flow (RBF) and renal vascular resistance (RVR) |
|---|---|---|---|
| Control | Nifedipine (\( \mu g/kg/min \)) | 0.5 | 1.0 | 2.5 |
| MAP (mmHg) | 116±1.9 | 116±1.9 | 115±1.8 | 114±1.7 |
| RBF (ml/min) | 40.8±1.8 | 44.5±2.3* | 47.8±2.3† | 52.5±2.7‡ |
| RVR (mmHg/ml/min) | 2.85±0.09 | 2.61±0.10† | 2.43±0.09‡ | 2.19±0.09‡ |

Values are expressed as mean ± s.e. *p < 0.05, †p < 0.01, ‡p < 0.001 as compared with control value. \( n=6 \).
Fig. 1. PRA levels in conscious state, during anesthesia and after the administration of nifedipine in 6 rabbits.

Table 2. Changes in mean arterial pressure (MAP), renal blood flow (RBF) and renal vascular resistance (RVR) before and after the intravenous administration of nifedipine in each experimental group

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>MAP (mmHg)</th>
<th>RBF (ml/min)</th>
<th>RVR (mmHg/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II (n = 6)</td>
<td>Before 114±4.6</td>
<td>39.0±4.4</td>
<td>3.00±0.24</td>
</tr>
<tr>
<td></td>
<td>After 108±4.6†</td>
<td>36.5±3.3</td>
<td>2.99±0.18</td>
</tr>
<tr>
<td>Norepinephrine (n = 6)</td>
<td>Before 112±5.4</td>
<td>38.0±3.4</td>
<td>2.98±0.16</td>
</tr>
<tr>
<td></td>
<td>After 108±4.1</td>
<td>34.5±2.2</td>
<td>3.15±0.13</td>
</tr>
<tr>
<td>Vasopressin (n = 5)</td>
<td>Before 112±3.4</td>
<td>41.5±6.6</td>
<td>2.91±0.48</td>
</tr>
<tr>
<td></td>
<td>After 106±3.5*</td>
<td>41.0±6.9</td>
<td>2.89±0.64</td>
</tr>
<tr>
<td>Bradykinin (n = 6)</td>
<td>Before 112±4.0</td>
<td>39.4±4.7</td>
<td>2.96±0.25</td>
</tr>
<tr>
<td></td>
<td>After 108±4.6</td>
<td>38.0±4.1</td>
<td>2.92±0.19</td>
</tr>
<tr>
<td>Prostaglandin E2 (n = 6)</td>
<td>Before 113±3.8</td>
<td>38.8±4.4</td>
<td>3.03±0.25</td>
</tr>
<tr>
<td></td>
<td>After 111±3.9</td>
<td>36.0±2.6</td>
<td>3.13±0.16</td>
</tr>
<tr>
<td>Control (n = 3)</td>
<td>Before 117±2.4</td>
<td>40.1±3.2</td>
<td>2.91±0.19</td>
</tr>
<tr>
<td></td>
<td>After 115±1.6</td>
<td>37.0±2.5</td>
<td>3.16±0.27</td>
</tr>
</tbody>
</table>

Values are expressed as mean±s.e. *p<0.02, †p<0.01 as compared with the value before the administration of nifedipine. Before, Before the administration of nifedipine; After, after the administration of nifedipine.
plasma renin activity was noted after the intravenous administration of nifedipine (Fig. 1).

Table 2 shows the changes in mean arterial pressure, renal blood flow and renal vascular resistance before and after the intravenous administration of nifedipine. In all experimental groups, mean arterial pressure tended to decrease after the intravenous administration of nifedipine. However, the decrease reached statistical significance only in the infusion group of angiotensin II and vasopressin. Renal blood flow and renal vascular resistance were unchanged after the administration of nifedipine. During the determination of dose-response curves to vasoactive substances, basal arterial pressure and renal blood flow were stable without irregular fluctuations.

Infusions of angiotensin II, norepinephrine, bradykinin or PGE₂ into the renal artery caused no significant change in systemic arterial pressure. However, the intrarenal arterial infusion of vasopressin elicited a significant increase in systemic arterial pressure from 111±3.4 to 114±3.5 (p<0.05), 117±3.7 (p<0.01) and 120±4.1 mmHg (p<0.01) at the doses of 10, 20 and 50 mU/kg per min, respectively.

Fig. 2 shows the effects of nifedipine on renal vascular responses to intrarenal arterial infusion of angiotensin II. As expected, an infusion of angiotensin II into the renal artery produced a decrease in renal blood flow and an increase in renal vascular resistance. After the intravenous infusion of nifedipine, the decrease in renal blood flow induced by angiotensin II was suppressed significantly from 5.0±1.0, 7.3±1.5 and 11.8±1.6 ml/min to 1.8±0.3 (p<0.01), 4.0±0.4 (p<0.05) and 6.8±0.7 ml/min (p<0.05), respectively at each dose of angiotensin II. The increase in renal vascular resistance induced by angiotensin II was significantly attenuated (Fig. 2).

![Fig. 2.](image-url)

Fig. 2. Effects of nifedipine on the change in renal blood flow (RBF) and renal vascular resistance (RVR) induced by the intrarenal infusion of angiotensin II. n=6. Values are mean±s.E.

- ● ● , before the administration of nifedipine;
- ○ ○ ○ ○ , after the administration of nifedipine. Reference marks indicate significant differences (* p<0.05, † p<0.01) from the value before the administration of nifedipine.
Fig. 3 shows the effect of nifedipine on the change in renal blood flow (RBF) and renal vascular resistance (RVR) induced by the intrarenal infusion of norepinephrine. n = 6. Values are mean ± s.e. •• - - - , before the administration of nifedipine; ○ - - - ○, after the administration of nifedipine.

Fig. 3 shows the effect of nifedipine on the change in renal blood flow and renal vascular resistance induced by norepinephrine. The renal vasoconstrictor action of norepinephrine was not affected by the administration of nifedipine.

Intrarenal infusion of vasopressin elicited a significant increase in systemic
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As shown in Fig. 4, the increase in mean arterial pressure induced by vasopressin was significantly attenuated after the administration of nifedipine. The infusion of vasopressin into the renal artery caused a decrease in renal blood flow and an increase in renal vascular resistance in a dose-dependent manner. After the administration of nifedipine, the decrease in renal blood flow was suppressed (Fig. 4). The increase in renal vascular resistance was attenuated at doses of 20 and 50 mU/kg per min, but not at a dose of 10 mU/kg per min (Fig. 4).

The intrarenal arterial infusion of bradykinin produced an increase in renal blood flow and a decrease in renal vascular resistance. The changes in renal blood flow induced by bradykinin were 2.6±0.4, 5.4±0.8 and 7.8±0.9 ml/min, respectively, at the three different doses. The increase in renal blood flow and the decrease in renal vascular resistance induced by bradykinin were not affected by the administration of nifedipine (Fig. 5).

After the control dose-response curves to bradykinin had been determined, the animals were allowed to equilibrate for 30 min before the intrarenal arterial infusion of PGE₂ started. Systemic arterial pressure and basal renal blood flow were stable during this period; 113±3.8 mmHg and 38.4±4.4 ml/min, respectively. Intrarenal arterial infusion of PGE₂ produced an increase in renal blood flow and a decrease in renal vascular resistance in a dose-dependent manner (Fig. 5). After the administration of nifedipine, the increase in renal blood flow induced by PGE₂ was not altered significantly as shown in Fig. 5. The decrease in renal vascular resistance induced by PGE₂ infusion was not affected by the
administration of nifedipine.

In 3 control rabbits, 5% dextrose was infused intrarenally. No significant changes in systemic arterial pressure, renal blood flow or renal vascular resistance were noted either before or after the administration of nifedipine.

**DISCUSSION**

In the present study, nifedipine infused intrarenally, produced a renal vasodilator response without any change in systemic arterial pressure. The present findings are consistent with those of previous workers (Ono et al. 1974). Yamaguchi et al. (1974) also reported in dogs that another Ca-antagonist, CRD-401 (diltiazem), produced an increase in renal blood flow, when it was administered systemically or into the renal artery. However, the systemic administration of nifedipine (50 μg/kg) caused a fall in systemic arterial pressure, and this hypotensive dose of nifedipine failed to increase basal renal blood flow. This may be accounted for by changes in sympathetic tone or the release of vasoconstrictor hormones which occur with a decline in arterial pressure, masking the vasodilative response to nifedipine. Plasma renin activity increased significantly after the administration of nifedipine. It has been reported that plasma renin activity was suppressed by the administration of calcium chloride (Kotchen et al. 1974). However, Okahara et al. (1980) showed that the administration of calcium ionophore, A 23187, elicited the renin release in dogs. In the present study, after the administration of Ca-antagonist, nifedipine, a further increase in plasma renin activity was observed. It is unclear whether the increase in plasma renin activity is due to the inhibition of Ca++ influx which was related to the renin secretion mechanism or the decrease in renal perfusion pressure accompanied with the reduction in arterial pressure.

It is commonly believed that nifedipine interferes with the slow inward current, i.e. transmembrane Ca++ influx (Fleckenstein 1977; Fleckenstein and Fleckenstein 1977), although an inhibitory effect on intracellular Ca++ release has also been proposed (Church and Zoster 1980). Moreover, an increase in Ca++ efflux is also one of mechanisms involved in vascular relaxation induced by nifedipine (Fleckenstein 1977; Church and Zoster 1980). However, Godfraind (1982) has shown that in the rat aorta, nifedipine blocks only Ca++ influx and Ca++ efflux is not affected by this Ca++ antagonist. Accordingly, the present study strongly suggests that the renal vascular tone depends on Ca++ influx and the renal vasodilator mechanism of nifedipine is attributed to its interference in this process.

The renal vasoconstriction induced by angiotensin II or vasopressin, but not norepinephrine was attenuated by the administration of nifedipine (Figs. 2 and 4). These results would suggest that the receptor-mediated renal vasoconstrictor responses to these 3 agents is not mediated by a Ca++ mobilization pathway common to all three. It has been reported that norepinephrine stimulates the
release of Ca++ from intracellular store sites as well as Ca++ influx (Hudgins and Weiss 1968; Seidel and Bohr 1971). In canine mesenteric circulation, norepinephrine induced constriction is only partly inhibited by nifedipine (Kazimierz et al. 1981). In the rabbit aorta, Ca-antagonists have been shown to have little effect on norepinephrine-induced contraction (Broekaert and Godfraind 1979). Furthermore, CRD-401 also failed to affect the renal vaso-constriction induced by adrenalin in dogs (Yamaguchi et al. 1974). These results suggest that the vasoconstrictor response induced by norepinephrine depends on mainly Ca++ release from intracellular store sites and Ca-antagonists do not affect this mechanism. In the present experiments, it is thought that norepinephrine mediated renal vasoconstrictor response could be evoked by mainly intracellular Ca++ release. Recently, Van Zwieten et al. (1982) reported that various Ca-antagonists have little influence on the vasoconstriction provoked by stimulation of vascular postsynaptic \( \alpha_1 \)-adrenoceptors. In the present study, there is a possibility that renal vasoconstriction induced by norepinephrine is stimulated through the action of \( \alpha_1 \)-adrenergic receptor.

Nifedipine attenuated the vasoconstrictor response of angiotensin II or vasopressin, suggesting that the renal vasoconstrictor effects of both drugs are mediated through a similar Ca++ influx into the cytosol. Nifedipine also attenuated the increase in blood pressure induced by vasopressin. This indicates that the increase in peripheral vascular resistance by this peptide is mediated by Ca++ influx into vascular smooth muscle cells.

When exogenous bradykinin and PGE\(_2\) were infused intrarenal arterially, a dose-dependent increase in renal blood flow was produced without any change in systemic arterial pressure. After the administration of nifedipine, the increase in renal blood flow produced by both vasodilator drugs were unchanged, suggesting that nifedipine does not influence the vasodilator action of bradykinin and PGE\(_2\).

In summary, the renal vasoconstrictor responses to angiotensin II and vasopressin were attenuated by nifedipine to a similar degree, but the responses to norepinephrine were not. This indicates that vasoconstrictor responses to these substances are not mediated through the same mechanism of Ca++ mobilization. It was confirmed that the renal vasodilator effect of bradykinin and PGE\(_2\) is not modified by nifedipine in anesthetized rabbits.

References


