Nifedipine Enhances the Vasodepressor and Natriuretic Effects of Atrial Natriuretic Peptide

MASAHIDE SEINO, KEISHI ABE, NOBORU NUSHIRO, AND KAORU YOSHINAGA

SUMMARY We examined a possible interaction between the calcium entry blocker nifedipine and atrial natriuretic peptide on blood pressure and natriuresis in anesthetized rabbits. The administration of atrial natriuretic peptide (0.05 μg/kg/min) produced a significant decrease in mean arterial pressure. Similar reductions in blood pressure were obtained during the administration of nifedipine (1.0 μg/kg/min). Atrial natriuretic peptide produced a consistent increase in glomerular filtration rate that was higher than the increase in renal blood flow; hence, the filtration fraction was significantly elevated. Atrial natriuretic peptide also elicited a significant increment in urine volume and urinary sodium excretion, while nifedipine was devoid of any significant effects on renal hemodynamics and renal excretory function during the experimental period. The administration of atrial natriuretic peptide superimposed on an ongoing infusion of nifedipine resulted in a greater fall of blood pressure than that seen during the administration of atrial natriuretic peptide or nifedipine alone. Sodium excretion was also potentiated, but there were no changes in renal hemodynamics or in the filtration fraction. These results suggest that calcium entry blockers potentiate the vasodepressor and the natriuretic effects of atrial natriuretic peptide but prevent its renal hemodynamic effects. (Hypertension 11: 34-40, 1988)

KEY WORDS calcium entry blockers • renal hemodynamics • natriuresis

MAMMALIAN atria contain peptides with potent diuretic, natriuretic, and vasorelaxant properties, and atrial natriuretic peptides (ANPs) have been purified and synthesized. However, it remains unclear whether the natriuretic effect of these peptides is due to their direct action on renal tubules or to indirect actions through renal hemodynamic changes, or to both. The administration of ANP induces a reduction in arterial pressure. Although the mechanism for this action is not clear, it has been reported that the reduction in arterial pressure results from a decrease in either cardiac output or systemic vascular resistance.

On the other hand, it has been proposed that one of the mechanisms in the actions of ANP is an interference with calcium ion movements through the cell membrane. Renal hemodynamic, diuretic, and natriuretic effects of atrial extracts have been reported to be blunted by pretreatment with verapamil or perfusion with low concentrations of Ca²⁺ in the isolated, perfused rat kidney. ANP also inhibits vascular contraction induced by angiotensin, norepinephrine, and potassium. Thus, ANPs could influence Ca²⁺ influx across the membrane.

In the present study, we examined a possible interaction between the Ca²⁺ entry blocker nifedipine and α-human ANP on blood pressure, renal hemodynamics, and natriuresis in anesthetized rabbits.

Materials and Methods

Experiments were performed on 22 female albino rabbits (weight, 3.0–3.5 kg; age, 6–7 months) supplied by Oriental Kohbo (Tokyo, Japan). The rabbits fasted overnight but were allowed free access to water. Animals were anesthetized with urethan (450 mg/kg) and α-chloralose (45 mg/kg) and given supplementary anesthetics as required. The rabbits were tracheotomized, but not ventilated artificially. The left jugular vein and the femoral artery and vein were cannulated with polyethylene catheters. The arterial catheter was attached to a transducer and recorder (Biophysigraph, 180 system, Sän-Ei, Tokyo, Japan) to monitor arterial pressure. A left flank incision was performed to isolate the left renal artery. For measurement of renal blood
flow (RBF), a noncannulating electromagnetic flow probe was placed around the left renal artery and connected to an electromagnetic flowmeter (Model MF27, Nihon Kohden, Tokyo, Japan). For urine collection, the left ureter was catheterized with silicon tubing.

After the surgical preparation, at least 1 hour was allowed to elapse to stabilize the arterial pressure and RBF. Rabbits were given an infusion of 5% dextrose solution in a dose corresponding to 2% of body weight in the recovery period. Collected urine volume was replaced with twice the volume of 5% dextrose solution through the femoral vein catheter. The replacement was performed slowly and had no effect on blood pressure and RBF. For measurement of creatinine clearance ($C_C$), a priming dose of creatinine (50 mg/kg) was injected at the end of the recovery period and followed by a constant infusion of creatinine at a rate of 0.5 mg/kg/min (0.23 ml/min) through the jugular vein catheter throughout the experiments.

Rabbits were divided into the following four groups.

**Group 1**

Group 1 ($n = 5$) was used as a vehicle control. A solution of 5% dextrose was infused continuously throughout the experiment through the femoral vein catheter at a rate of 0.23 ml/min for 120 minutes. Every 30 minutes, urine was collected into a plastic test tube. Blood (4 ml) was drawn at the end of each 30-minute collection period.

**Group 2**

After completion of the recovery period, a solution of 5% dextrose was infused for 30 minutes as a baseline period and continued for another 30 minutes as a control period in the ANP infusion group (Group 2, $n = 6$). At the end of each 30-minute urine collection in baseline and control periods, 4 ml of blood was obtained. Then, a synthetic α-human ANP (Protein Research Foundation, Osaka, Japan) was administered intravenously at a rate of 0.05 μg/kg/min for 60 minutes. Two 30-minute clearances (Periods 1 and 2) were measured, and 4 ml of blood was drawn at the end of each period. After the ANP infusion was stopped, clearance was again measured for 30 minutes (recovery phase).

**Group 3**

After recovery from the operation, a solution of 5% dextrose was infused through the femoral vein catheter at a rate of 0.23 ml/min for 30 minutes as a baseline period. Blood (4 ml) was drawn at the end of the 30-minute urine collection. Then, nifedipine (Bayer) was infused intravenously at a rate of 1.0 μg/kg/min (0.23 ml/min) for 30 minutes as a control period in the nifedipine infusion group (Group 3, $n = 5$). At the end of the 30-minute urine collection, 4 ml of blood was obtained again. The infusion of nifedipine was continued for 60 minutes. Two 30-minute urine collections were performed with blood drawing (4 ml) at the end of 30 minutes (Period 1) and 60 minutes (Period 2) of the nifedipine infusion. After the infusion of nifedipine was stopped, clearance again was measured for 30 minutes (recovery phase).

**Group 4**

The protocol for Group 4 ($n = 6$) was similar to that for Group 3. However, after the 30-minute control infusion of nifedipine, ANP at the rate given in Group 2 was superimposed on the continuous infusion of nifedipine and the infusion was continued for 60 minutes. During the infusion of ANP and nifedipine, two 30-minute clearances were performed as already described. After the infusion was stopped, clearance again was measured for 30 minutes (recovery period).

In each experiment, a 4-ml blood sample was obtained through the arterial catheter for measurement of plasma sodium, creatinine, and hematocrit. Collected blood was replaced immediately with an equal volume of donor blood.

Glomerular filtration rate was estimated by $C_C$. Sodium concentrations in plasma and urine were measured by an autoanalyzer (Type II, Nihon Technikon, Tokyo, Japan). Creatinine levels in plasma and urine were also measured by the same technique (Model VS-700S, Nihondensi, Tokyo, Japan). Renal vascular resistance (RVR) was calculated by a standard formula and presented as mm Hg/ml/min. Statistical comparisons were made with analysis of variance and with Student's $t$ tests, where appropriate. Values are presented as means ± SE.

**Results**

Figure 1 shows the changes in mean arterial pressure (MAP) in the four groups. No significant differences in MAP were observed at baseline among these groups. In Group 1 (vehicle control), the infusion of 5% dextrose solution did not induce any significant changes in MAP. In Group 2 (ANP infusion alone), the infusion of ANP resulted in a significant decrease in MAP at 10

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Renal hemodynamic changes in the four groups are summarized in Table 1. RBF, RVR, and Cc in Group 1 were unchanged throughout the infusion of 5% dextrose solution. In Group 2, after the infusion of ANP, RBF increased significantly in Period 1 (first 30 minutes of infusion) and Period 2 (second 30 minutes of infusion), with a concomitant decrease in RVR compared with baseline values. These changes in RBF and RVR were also significant when compared with those of Group 1 (vehicle control). Cc increased during the infusion of ANP compared with the baseline values, but there were no significant differences when compared with Group 1 values. In Group 3, during the infusion of nifedipine in the control period, RBF increased from 26 ± 2 to 29 ± 3 ml/min (p < 0.05). This increase in RBF was also significant compared with the control values in Group 1. In Group 4, during the continuous infusion of nifedipine with ANP, RBF returned to the preinfusion level. RVR decreased from 4.4 ± 0.5 to 3.7 ± 0.6 mm Hg/ml/min (p < 0.05) during the infusion of nifedipine in Group 4, which was also significant when compared with Group 1 values. However, the additional infusion of ANP with nifedipine did not induce any significant changes in RVR when compared with Group 1. Cc remained constant throughout the infusion of nifedipine with ANP.

Figure 2 shows the changes in filtration fraction (FF) in the present experiments. In Group 1, FF increased significantly in Periods 1 and 2 compared with the value in the baseline period. ANP infusion alone in Group 2 also caused a significant increase in FF in Periods 1 and 2. Even during the recovery period, FF increased significantly. However, there were no significant differences in FF between Groups 1 and 2. In Group 3, during the infusion of nifedipine alone, no significant changes in FF were observed. The additional infusion of ANP with nifedipine in Group 4 also did not cause any significant changes in FF until the end of the infusion. However, as shown in Figure 2, significant differences in FF were observed between Groups 2 (ANP) and 4 (nifedipine + ANP) in Period 2 and in the recovery period. Thus, the increase in FF elicited by ANP was blunted by the pretreatment with nifedipine.

Figure 3 shows the changes in urine volume (UV) in the present experiments. In Group 1, a continuous infusion of 5% dextrose solution did not induce any changes in UV. The infusion of ANP alone in Group 2 produced a marked increase in UV from 0.43 ± 0.06 to 2.22 ± 0.12 ml/min in Period 1 (p < 0.001) and to 1.74 ± 0.15 ml/min in Period 2 (p < 0.01). In the recovery period, UV returned to control levels. In Group 3, during the infusion of nifedipine in the control period, UV tended to increase, although the value did not reach statistical significance. The continuous infusion of nifedipine did not induce any significant changes in UV. In Group 4, during the infusion of nifedipine in the control period, a slight increase in UV was observed. The additional infusion of ANP with nifedipine caused significant increases in UV from 0.48 ± 0.09 ml/min to 1.77 ± 0.06 ml/min (p < 0.01) in Period 1 and to 1.89 ± 0.06 ml/min (p < 0.01) in Period 2. However, these changes in UV did not show any significant differences when compared with ANP infusion alone. During the recovery period, UV returned to the baseline values.

Urinary sodium excretion (UnV) in the four groups is illustrated in Figure 4. In Group 1, UnV was constant throughout the infusion of 5% dextrose solution. The administration of ANP alone in Group 2 induced significant increases in UnV from 4.0 ± 1.6 to 17.4 ± 5.5 µEq/min (p < 0.05) in Period 1 and to 17.3 ± 5.2 µEq/min (p < 0.05) in Period 2. In Group 3, during the infusion of nifedipine in the control period, UnV tended to increase (from 4.4 ± 0.6 to 8.8 ± 2.8 µEq/min), but there were no significant differences when compared with baseline values. These changes in UV and UnV were also significant when compared with Group 1 values. However, the additional infusion of ANP with nifedipine did not induce any significant changes in UV in Group 4, during the infusion of nifedipine in the control period.

Table 1. Changes in Renal Blood Flow, Renal Vascular Resistance, and Creatinine Clearance in the Four Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>RBF (ml/min)</th>
<th>RVR (mm Hg/ml/min)</th>
<th>Cc (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>C</td>
<td>1</td>
</tr>
<tr>
<td>1 (5% dextrose)</td>
<td>±3</td>
<td>±2</td>
<td>±2</td>
</tr>
<tr>
<td>2 (ANP)</td>
<td>±2</td>
<td>±2</td>
<td>±2</td>
</tr>
<tr>
<td>3 (nifedipine)</td>
<td>±2</td>
<td>±2</td>
<td>±2</td>
</tr>
<tr>
<td>4 (nifedipine + ANP)</td>
<td>±2</td>
<td>±2</td>
<td>±2</td>
</tr>
</tbody>
</table>

Values are means ± SE. RBF = renal blood flow; RVR = renal vascular resistance; Cc = creatinine clearance; B = baseline; C = control period; 1 = Period 1 (first 30 minutes of infusion); 2 = Period 2 (second 30 minutes of infusion); R = recovery period.

*p < 0.05, compared with baseline values; †p < 0.05, compared with values in Group 1.
**Figure 2.** Changes in filtration fraction (FF) in the four groups. Asterisk indicates significant difference (p<0.05) compared with control value in each group.

**Figure 3.** Changes in urine volume (UV) in the four groups. Double (p<0.01) and triple (p<0.001) asterisks indicate significant difference compared with control value in each group.

**Figure 4.** Changes in urinary sodium excretion (UNaV) in the four groups. Single (p<0.05), double (p<0.01), and triple (p<0.001) asterisks indicate significant difference compared with control value in each group.

Although these changes were not significant, UNaV tended to increase during the continuous infusion of nifedipine, but these changes did not reach statistical significance. In Group 4, during the infusion of nifedipine in the control period, UNaV also tended to increase, as in Group 3. The additional infusion of ANP with nifedipine caused a marked increase in UNaV from 5.9 ± 2.4 to 23.3 ± 6.4 μEq/min (p < 0.01) in Period 1 and to 30.4 ± 8.4 μEq/min (p < 0.001) in Period 2. A significant increase in UNaV was also observed in Period 2 when compared with Group 2 values (ANP infusion alone). Thus, nifedipine pretreatment did not attenuate the increase in UNaV induced by ANP. In the recovery period, UNaV returned to control values.

As shown in Figure 5, fractional excretion of sodium (FENa) showed a pattern similar to that of UNaV. In Group 1, the infusion of 5% dextrose solution did not induce any significant changes in FENa. The infusion of
FIGURE 5. Changes in fractional excretion of sodium (\(\text{FE}_{\text{Na}}\) ) in the four groups. Single (p<0.05), double (p<0.01), and triple (p<0.001) asterisks indicate significant difference compared with control value in each group.

ANP alone in Group 2 produced a marked increase in \(\text{FE}_{\text{Na}}\), from 0.57 ± 0.22 to 3.24 ± 1.05% (p<0.001) in Period 1 and to 1.59 ± 0.61% (p<0.01) in Period 2. Even in the recovery period, \(\text{FE}_{\text{Na}}\) was increased significantly compared with baseline values. During the infusion of nifedipine alone in Group 3, \(\text{FE}_{\text{Na}}\) tended to increase; however, these changes in \(\text{FE}_{\text{Na}}\) were not statistically significant. In Group 4, during the infusion of nifedipine, \(\text{FE}_{\text{Na}}\) also tended to increase, as in Group 3, although this increase did not reach statistical significance. The additional infusion of ANP with nifedipine induced significant increases in \(\text{FE}_{\text{Na}}\), from 0.51 ± 0.18 to 2.99 ± 0.68% (p<0.001) in Period 1 and to 3.70 ± 1.04% (p<0.001) in period 2. In the recovery period, \(\text{FE}_{\text{Na}}\) was still increased significantly. A significant difference in \(\text{FE}_{\text{Na}}\) was also observed in Period 2 when compared with Group 2 values (ANP infusion alone).

Discussion

In the present study, the infusion of nifedipine or ANP exerted a potent hypotensive effect, as expected. However, the administration of ANP superimposed on the infusion of nifedipine resulted in a greater fall of arterial pressure than that observed during the administration of ANP or nifedipine alone. The mechanism of the vasodepressor effect of ANP, which was potentiated by nifedipine, is not clear. However, some possible mechanisms could be considered. One of the possibilities is a large natriuresis induced by the additional infusion of ANP with nifedipine. Another possibility is a fall in cardiac output, since ANP-mediated hypotension reportedly results from a reduction in cardiac output.16-18 However, the administration of ANP alone induced less of a decrease in MAP than did combined ANP and nifedipine infusion. Therefore, this possibility is not likely, but an unknown interaction of the two drugs could occur to affect the cardiac output. The potentiation by nifedipine also could have been due to a greater peripheral vasodilation. However, nifedipine abolishes vasodilation in renal vasculature, making it unlikely that nifedipine potentiates only extrarenal vaso-dilation. Thus, the marked natriuresis induced by ANP with nifedipine could contribute mainly to the greater fall in arterial pressure.

A well-known potent natriuretic response of ANP was also accompanied by the increments in RBF and \(C_v\). Others have reported that changes in renal hemodynamics participate in the increase in urinary fluid and electrolyte excretions.11-12 The administration of ANP produced a consistent increase in \(C_v\) that was higher than the increase in RBF, raising the FF significantly. The FF was also increased in Group 1 (vehicle control), probably as a result of the anesthesia or volume status.

One of the proposed mechanisms of ANP-induced natriuresis is dependent on Ca\(^{2+}\).13 Therefore, in the present study, we aimed to examine a possible role of Ca\(^{2+}\) in modulating the natriuretic and hemodynamic effects of ANP in vivo by using a Ca\(^{2+}\) entry blocker, nifedipine. The infusion of nifedipine alone induced a significant increase in RBF during the control infusion period. During the sustained infusion of nifedipine, the initial increase in RBF returned to the baseline level. The mechanism returning RBF toward the baseline level may be the fall in MAP and tachyphylaxis of nifedipine to renal vasculatures. During the continuous infusion of nifedipine, \(U_{\text{Na,V}}\) tended to increase without significant changes in renal hemodynamics, suggesting that nifedipine possesses a direct tubular effect on sodium reabsorption. Administration of Ca\(^{2+}\) entry blockers in an amount that does not affect renal hemodynamics was reported to produce natriuresis.22 '23 Our present results are compatible with these reports. However, we cannot rule out the possibility that nifedipine per se may exert effects in renal tubules that are independent of inhibition of Ca\(^{2+}\) fluxes. Although the recognized effect of nifedipine is blockage of Ca\(^{2+}\) movements through voltage-activated channels in excitable tissues, the effect of nifedipine may be mediated by Ca\(^{2+}\) movements through nifedipine-insensitive pathways and intracellular Ca\(^{2+}\) homeostasis.

The ANP-induced increases in RBF, \(C_v\), and FF
were blocked by pretreatment with nifedipine, suggesting that ANP-induced renal hemodynamic responses are dependent on the availability of extracellular Ca\(^2+\). The increase in FF, reflecting a preferential renal efferent arteriolar vasoconstriction, could be mediated through Ca\(^2+\). The blunted response in RBF and C\(_{\text{in}}\) during the infusion of ANP with nifedipine is unexplained in the present study, but it could be partly affected by alterations in autoregulation induced by the fall in blood pressure, by a tubuloglomerular feedback mechanism through the marked natriuresis produced by the superimposition of ANP on nifedipine, or by both.

In spite of the marked fall in arterial pressure, the administration of ANP with nifedipine elicited a greater rise in U\(_{\text{Na}}\)V than that elicited by the ANP infusion alone, although renal hemodynamics were unchanged. This finding was unexpected. Several studies have postulated that ANP has a direct action on renal tubules, but it is still unclear which portion of the renal tubules is affected by ANP. The superimposition of ANP on nifedipine induced a synergistic increase in U\(_{\text{Na}}\)V, suggesting that different portions were involved in the renal tubular effects of these substances. In the present study, we cannot rule out that the redistribution of RBF could contribute to the natriuresis, since ANP has been demonstrated to increase the renal papillary plasma flow. Furthermore, renal tubules reportedly have many sympathetic nerve fibers. These sympathetic nerve activities in the kidney could be affected by anesthesia and surgical procedures. Therefore, it is also possible that the marked natriuresis observed by ANP infusion with nifedipine is due to an inhibition of the sympathetic nerve activity in renal tubules. Thus, the potentiating effect of nifedipine on ANP-induced natriuresis could be caused by the results of alterations in the tubular handling of sodium.

Camargo et al. observed that renal hemodynamic and natriuretic effects of atrial extracts were blunted by verapamil pretreatment in isolated, perfused rat kidney. In the present whole-animal study, ANP-induced natriuresis was not abolished by nifedipine pretreatment, whereas renal hemodynamic changes were blocked. This discrepancy between their and our results could be due to the differences in experimental conditions; namely, hemodynamic regulation in the isolated, perfused kidney may be different from that in whole-animal kidney. One obvious difference is the lack of innervation, another is the material used — whole-animal kidney. One obvious difference is the material used — whole-animal kidney. In the present whole-animal study, ANP-induced natriuresis could be caused by the results of alterations in autoregulation induced by the fall in blood pressure, by a tubuloglomerular feedback mechanism through the marked natriuresis produced by the superimposition of ANP on nifedipine, or by both.

In conclusion, our results suggest that Ca\(^2+\) entry blockers potentiate the vasodepressor and the natriuretic effects of ANP, whereas they inhibit the renal hemodynamic effects of ANP in anesthetized rabbits.

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Nifedipine enhances the vasodepressor and natriuretic effects of atrial natriuretic peptide.

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