Nifedipine Enhances Vasodepressor and Natriuretic Responses to Atrial Natriuretic Peptide in Anesthetized Rats

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ABSTRACT

The interaction between a calcium channel blocker nifedipine and atrial natriuretic peptide (ANP) was examined in normotensive and renal hypertensive rats. The infusion of either ANP or nifedipine produced a significant decrease in mean arterial pressure (MAP). The combined infusion of ANP with nifedipine resulted in a greater fall of MAP than did the infusion of each drug alone. ANP significantly increased urinary volume and excretion of sodium, while nifedipine was without effects. The diuretic/natriuretic effects of ANP were potentiated by the combined infusion with nifedipine. The vasodepressor and renal effects of ANP or nifedipine were qualitatively similar between the normotensive and hypertensive rats. Nifedipine caused an upward and leftward shift of the ANP dose-relaxation curve of the phenylephrine-precontracted thoracic aortic rings isolated from the normotensive rats, suggesting that the vasodilatation sensitivity to ANP is increased in the presence of nifedipine. These results indicate that nifedipine enhances the vasodepressor effect of ANP, the likely mechanisms being attributable to a contraction of effective intravascular volume as a consequence of potentiated renal excretion and a greater peripheral vasodilation.

Key Words: Nifedipine, Atrial natriuretic peptide, Diuretic/natriuretic effect, Peripheral vasodila-

INTRODUCTION

Although intensive effort has been made to elucidate the action mechanism of atrial natriuretic peptide (ANP), it still remains unclear. In addition to its role as a potential inhibitor of intracellular calcium release (Meisneri et al, 1986), ANP has been proposed to interfere with calcium ion movements through the cell membrane (Camargo et al, 1984). The inhibitory effect of ANP on the vascular contraction induced by angiotensin, norepinephrine or potassium (Kleinert et al, 1984) suggests that it could influence calcium influx across the cell membrane, being analogous to calcium channel blockers. Therefore, it is expected that ANP would interact with calcium channel blockers to affect the intracellular calcium level which is critical for the vascular smooth muscle constriction.

In fact, it has been reported that nifedipine enhances ANP-induced natriuresis and vasodepression (Seino et al, 1988). Although the pronounced vasodepression has been attributed to the enhanced diuresis/natriuresis and consequent contraction of effective intravascular volume in their study, the precise mechanism has not been elucidated. Among others, a decrease in peripheral resistance may also play a role, since both ANP and nifedipine are known as vasorelaxants (Ackermann et al, 1984; Godfraind, 1983; Hirata et al, 1985).
In addition, an interaction, if any, between ANP and calcium channel blockers may be modified by the development of hypertension, since it has been suggested that the elevation of blood pressure is related with alterations in the activity of calcium channels (Ishii et al, 1983).

The present study was aimed to explore interactions between ANP and nifedipine, a calcium channel blocker, on the blood pressure and renal excretion in normotensive and hypertensive rats.

**METHODS**

**In vivo experiments**

Male Sprague-Dawley rats were used. Rats weighing 150～200 g were made hypertensive by constricting the left renal artery with a 0.2 mm silver clip under pentobarbital anesthesia, and were used 4～5 weeks later. As controls, rats weighing 200～300 g were provided.

On the experimental day, animals were anesthetized with pentobarbital sodium (50 mg/kg, ip). Polyethylene catheters were introduced into the right femoral artery and vein to record the mean arterial pressure (MAP) and to serve as an infusion route, respectively. For urine collection, bladder catheter was implanted. At least 30 min of equilibrium period was allowed to elapse before the urine collection started.

Each period of urine collection lasted 15 min, which was terminated by flushing the bladder with 1 ml of distilled water followed by 1 ml of air injected through the bladder catheter. Three periods each of basal, experimental and recovery measurements were taken. Drugs were infused only during the experimental periods.

Animals were divided into four groups of normotensive and hypertensive rats, respectively.

1) Control group: Saline (0.9%) solution without any drug was infused throughout the experimental periods (100 μl/kg/min).

2) Nifedipine group: During the experimental periods, nifedipine (1 μg/kg/min) was infused at the same infusion rate (100 μl/kg/min).

3) ANP group: ANP was infused (60 ng/kg/min) during the experimental periods.

4) ANP+Nifedipine group: After 15 min infusion of nifedipine, ANP infusion was superimposed for 3 additional periods.

**In vitro experiments**

Aortic rings were prepared as described previously (Lee et al, 1988). Thoracic aorta was removed under pentobarbital anesthesia and its vascular rings 5 mm long each were prepared in ice-cold saline solution under stereoscopic microscope. A special care was taken not to inflict damage to the endothelium.

Each ring was mounted via fine stainless steel wires suspended in a 20 ml tissue bath containing physiological salt solution at 37 ± 0.05°C and being continuously bubbled with 95% O₂, 5% CO₂. The bath was raised such that the lower end of the ring-suspending rod was immersed into the bathing medium without contacting the bottom of the bath chamber. The other end of the ring was attached to a force-displacement transducer (Grass FT 03). Baseline load placed on the rings was 2.0 g. After 1.5 to 2-h of equilibrium, aortic rings were subjected to the first exposure to phenylephrine.

Relaxations were calculated as percent reductions of the phenylephrine (10⁻⁵ M)-induced contraction of the aortic rings. IC₅₀ values were obtained by linear regression using log transformation of the concentration.

The composition (in mM) of the physiological salt solution used was NaCl 112, KCl 5, NaHCO₃ 25, KH₂PO₄ 1, MgSO₄ 1.2, CaCl₂ 2.5, and glucose 1.5.

Drugs were purchased from Sigma Chemical Company. ANP used was atriopeptin III. Statistical comparisons were made using Student’s t-test.
RESULTS

Normotensive rats

Fig. 1 shows the MAP in normotensive rats. Infusion of either nifedipine or ANP resulted in a significant decrease of MAP. The superimposed infusion of ANP on an ongoing infusion of nifedipine caused a greater and more prolonged decrease of MAP compared to the ANP infusion alone. No significant changes were observed in the control group (data not shown).

Urinary volume (UV) and excretion of sodium ($U_{Na}V$) are shown in Fig. 2. Overall, changes in $U_{Na}V$ and UV were parallel. In the control group, no significant changes were observed in either UV or $U_{Na}V$. Although UV appeared to be increased by nifedipine infusion, it was not statistically significant. ANP produced significant increases in UV and $U_{Na}V$, which returned to the basal level during the recovery periods. ANP+Nifedipine group showed significantly enhanced (during the third infusion period) and more protracted increase in urinary excretion than did the ANP alone group.

Hypertensive rats

Fig. 3 shows the MAP in hypertensive rats. The vasodepressor effects of nifedipine, ANP alone or combined with nifedipine were qualitatively similar to those observed in normotensive rats. The vasodepression caused by the combined infusion was greater than that obtained by either drug.

The urinary excretion data in hypertensive rats are shown in Fig. 4. Nifedipine alone was without significant effect. On the other hand, ANP significantly increased UV and $U_{Na}V$ from the second experimental period, which lasted into the first recovery period. In the ANP+Nifedipine group, urinary excretion increased significantly from the first experimental period and the effect went on up to the second recovery period.

Magnitude of vasodepressor effects

Table 1 shows the percent decrease in MAP from the basal value at 30 min of drug infusion. In both normotensive and hypertensive rats, the magnitude of decreases in MAP was greater in the ANP+
Fig. 2. Urinary volume and excretion of sodium (U_{Na}V) in normotensive rats. E1-E3 represent the first through the third experimental periods during which the drug was infused. R1 and R2 denote the first and the second recovery periods following termination of the drug infusion. ★p < 0.05, ★★p < 0.01; compared to the basal value (B) in each group. ♠p < 0.05, compared to the ANP group at the corresponding period.

Nifedipine group than in either Nifedipine or ANP group. Nifedipine caused a more pronounced decrease of MAP in hypertensive than in normotensive rats. However, the magnitude of the ANP (either alone or combined with nifedipine)-induced depression was not significantly different between normotensive and hypertensive rats.

**In vitro experiments**

ANP relaxed the aortic rings precontracted with phenylephrine (10^{-5} M) in a dose-dependent manner (Fig. 5). The dose-relaxation curve was shifted upward and leftward in the presence of nifedipine, significantly reducing IC_{50} from (20.3 ± 7.2) × 10^{-10} (n = 25) to (2.84 ± 1.6) × 10^{-10} M (n = 21, p < 0.05).

However, IC_{50} of ANP was not different between the hypertensive and normotensive preparations ((28.2 ± 6.3) × 10^{-10} M, n = 24 vs (20.3 ± 7.2) × 10^{-10} M, n = 25).

Nifedipine also dose-dependently relaxed the aor-
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Table 1. Percent decreases in mean arterial pressure from the basal values at 30 min during the drug infusion

<table>
<thead>
<tr>
<th></th>
<th>Nifedipine</th>
<th>ANP</th>
<th>ANP + Nife</th>
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<tbody>
<tr>
<td>NTR</td>
<td>8.4±1.6(5)</td>
<td>18.8±3.4(11)</td>
<td>30.1±4.1*(8)</td>
</tr>
<tr>
<td>GHR</td>
<td>15.8±2.8*(10)</td>
<td>25.3±2.8(5)</td>
<td>37.0±4.3*(7)</td>
</tr>
</tbody>
</table>

NTR, normotensive rats. GHR, hypertensive rats. Numerals in the parentheses are the numbers of experiments. *p<0.05, compared to NTR. *p<0.05, compared to either nifedipine- or ANP-infused group.

tic rings precontracted with phenylephrine (10⁻⁵ M). IC₉₀ was significantly lower in the hypertensive \((2.3±0.9)×10^{-8} \text{ M, } n=7\) than in the normotensive preparations \((3.6±1.5)×10^{-8} \text{ M, } n=7, \ p<0.05\).

**DISCUSSION**

The infusion of nifedipine or ANP elicited a potent hypotensive effect in both normotensive and hypertensive rats. The combined infusion of the two drugs resulted in a greater fall of MAP along with potentiated diuretic/natriuretic effects than did the infusion of ANP alone. These results are in agreement with those observed by Seino et al (1988) in normotensive rats.

Fig. 5. ANP dose-relaxation curves of phenylephrine-precontracted aortic rings isolated from normotensive rats in the absence (−Nife) and presence (+Nife) of nifedipine (10⁻¹¹ M).

On the contrary, in the isolated perfused kidney, the renal hemodynamic and natriuretic effects of atrial extracts were reported blunted by verapamil pretreatment (Camargo et al, 1984). Although the discrepancy may not be fully accounted for, these
results indicate an interaction between calcium channel blockers and ANP on the kidney and vascular system.

One possible mechanism for the enhanced ANP-induced vasodepression due to nifedipine is a more pronounced diuresis/natriuresis, as has been suggested by Seino et al (1988). In fact, the exaggerated urinary excretion despite the marked fall of MAP may provide a clue to resolve cause-and-effect relationship between the two parameters. The enhanced urinary excretion could not be a reflection of the vasodepression, whereas the potentiated vasodepression could be ascribed to a contraction of effective intravascular volume as a consequence of the increased renal excretion.

However, the mechanism for the potentiation of nifedipine on the renal effects of ANP is not clear. Either an increase in renal hemodynamics or a decrease in tubular reabsorption (or both) may be responsible. Since ANP has been demonstrated to increase the renal papillary plasma flow (Salazar et al, 1986), it is possible that a redistribution of renal blood flow has contributed to the diuresis/natriuresis. Although previous studies have postulated that ANP has a direct tubular action (Lee and Malvin, 1987; Salazar et al, 1986), it is still uncertain which portion of the tubules may be affected by ANP. The potentiation may also have been due to the summation of different renal actions of the two drugs.

Alternatively, the potentiation of ANP-induced vasodepression by nifedipine may have been due to a greater peripheral vasodilation. Seino et al (1988) found that nifedipine was without effect on renal hemodynamics and furthermore abolished the vasodilation induced by ANP in renal vasculature. However, in the present study, we observed that the ANP-induced relaxation of isolated aortic rings was more profound in the presence of nifedipine, indicating that nifedipine potentiates the ANP-vasodilation.

The magnitude of vasodepression caused by ANP was not different between normotensive and hypertensive rats. This was further substantiated by the finding that the vasorelaxant sensitivity (IC_{50}) to ANP was not modified in the hypertensive vascular preparations.

On the other hand, as we have previously reported (Lee et al, 1989), the depressor response to nifedipine was exaggerated in hypertensive rats. Furthermore, the nifedipine-induced vasorelaxation was more sensitive in the hypertensive than in the normotensive preparations. Such a difference in nifedipine-sensitivity between normotensive and hypertensive rats may be attributable to an alteration in the calcium channel activity following the development of hypertension (Ishii et al, 1981). However, the depressor magnitude in the ANP+Nifedipine groups was not different between normotensive and hypertensive rats. The explanation for such a dissociation awaits further clarification.

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REFERENCES


Ishii K, Kano T, Kurobe Y & Ando J (1983). Binding of 
$[^{3}H]$ nitrendipine to heart and brain membranes from normotensive and spontaneously hypertensive 
rats. Eur J Pharmacol 88, 277–278
Kleinert HD, Maack T, Atlas SA, Januszewicz A, Sealey 
JE & Laragh JH (1984). Atrial natriuretic factor 
inhibits angiotensin-, norepinephrine-, and 
kangium-induced vascular contractility. Hypertension 
6(Suppl 1), 1143–1147
(1988). Comparison of vasorelaxant potency and 
mode of action between atrial natriuretic peptide 
and nitroprusside. Chonnam J Med Sci 1, 102–105
homologous heart extract in aglomerular toadfish.
Am J Physiol 252, R1055–R1058
Central cardiovascular effects of nifedipine and Bay 
K 8644 in chronic 2-kidney, 1-clip hypertensive rats. 
Chonnam J Med Sci 2, 26–30
Meisner KD, Taylor CJ & Sanei H (1986). Synthetic 
atrial natriuretic peptide inhibits intracellular calcium 
release in smooth muscle. Am J Physiol 250, 
C171–C174
Salazar FJ, Fiksen-Olsen MJ, Opgenorth TJ, Granger JP, 
Burnett JC & Romero JC (1986). Renal effects of 
ANP without changes in glomerular filtration rate and 
blood pressure. Am J Physiol 251, F532–F536
Nifedipine enhances the vasodepressor and natriuretic 
effects of atrial natriuretic peptide. Hypertension 
11, 34–40

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Nifedipine과 Atrial Natriuretic Peptide의 혈압내림효과에 미치는 영향

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Pentobarbital 마취한 정상혈압 및 신경 고혈압 천쥐에서 calcium channel 봉쇄약물 nifedipine과 atrial natriuretic peptide (ANP)의 상호작용을 조사하였다. 정상혈압 천쥐에서 nifedipine (1.0 µg/kg/min) 또는 ANP (60 ng/kg/min)의 주입은 각각 유의하게 혈압을 내렸으며 두 약물의 동시 주
입시에 개별적으로 주입하였을 때보다 그 혈압내림의 정도가 더욱 뛰었다. Nifedipine는 단독 주입
하였을 때에 신기능에 유의한 영향을 미치지 않았으나 ANP와 동시에 주입하였을 때에는 ANP의
요량 및 Na 배설 증가 효과를 향상시켰다. 한편 고혈압 천쥐에서도 ANP의 혈압내림효과와 신장
효과는 nifedipine과 함께 주입하였을 때에 더 뛰었다. 격추 홍부대동맥 표본을 phenylephrine으로
미리 수축시킨 후 ANP를 첨가하면 용량의존 이완반응을 보였고 nifedipine 존재하에서 더 예민하
였다. 이상의 실험결과는 calcium channel 봉쇄약물이 ANP의 혈압내림효과를 향상시킬음을 보인
것이며 그 기전으로 요즘 배설 증가 및 혈관이완효과 증가 등이 관여함을 시사하였다.