Calcium Channel Blockers Reverse the Sustained Elevation of Blood Pressure Induced by Chronic Infusion of Endothelin in Conscious Rats

MINORU YASUJIMA, KEISHI ABE,* MASAYUKI KANAZAWA,† KAZUNORI YOSHIDA,† MAKITO SATO,† KAZUHISA TAKEUCHI,† KAZUO TSUNODA,† KEI KUDO,† MASAHIRO KOHZUKI,† KEN OMATA,† TAMAMI YABE,† MASAO HIWATARI, TOKUTARO SATO and KAORU YOSHINAGA†

Institute of Rehabilitation Medicine, *Department of Clinical Biology and Hormonal Regulation, and †the Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai 980

YASUJIMA, M., ABE, K., KANAZAWA, M., YOSHIDA, K., SATO, M., TAKEUCHI, K., TSUNODA, K., KUDO, K., KOHZUKI, M., OMATA, K., YABE, T., HIWATARI, M., SATO, T. and YOSHINAGA, K. Calcium Channel Blockers Reverse the Sustained Elevation of Blood Pressure Induced by Chronic Infusion of Endothelin in Conscious Rats. Tohoku J. Exp. Med., 1990, 160 (2), 157-165 — To determine whether endothelin could act as a circulating hormone in the regulation of blood pressure and sodium-water excretion, we assessed the chronic effects of synthetic endothelin on systolic blood pressure, urine volume and urinary sodium excretion in conscious rats, and also evaluated the effects of benidipine or nilvadipine, newly developed calcium channel blockers, in rats infused chronically with synthetic endothelin. Continuous infusion of endothelin at a rate of 60 μg/kg/day into the jugular vein via osmotic minipumps induced a significant increase in systolic blood pressure, but did not induce any significant changes in urine volume and urinary sodium excretion, compared to those in vehicle-infused rats. On the contrary, the infusion of endothelin at a rate of 6 μg/kg/day did not induce any significant changes in systolic blood pressure, urine volume and urinary sodium excretion, compared to those in vehicle-infused rats. When 6 mg/kg/day of benidipine or 10 mg/kg/day of nilvadipine was administered simultaneously with 60 μg/kg/day of endothelin, the systolic blood pressure rose on Day 1 to only 137.0 ± 2.4 mmHg (p < 0.05) and 119.7 ± 5.9 mmHg (p < 0.05) compared to the rise to 163.8 ± 4.7 mmHg when endothelin alone was infused. The antihypertensive effect of benidipine or nilvadipine was sustained for the entire experimental period and was not associated with any significant changes in urine volume and urinary sodium excretion. The present results suggest that endothelin can act as a circulating hormone and might be involved in the regulation of blood pressure. In addition, they clearly...
demonstrate that the calcium channel blockers attenuate the elevation of blood pressure induced by endothelin, although the exact mechanisms whereby these drugs could interact remain to be determined.

Endothelin; calcium channel blockers; blood pressure regulation; conscious rats

An endothelium-derived 21-residue peptide vasoconstrictor, endothelin, has been isolated from the culture supernatant of porcine aortic endothelial cells, and also shown to be one of the most potent vasoconstrictors in a variety of blood vessels from various species (Tomobe et al. 1988; Yanagisawa et al. 1988). Moreover, it is suggested that the vasoconstrictor action of endothelin is dependent on the presence of extracellular calcium ion, and is attenuated by the calcium antagonists nicardipine and verapamil (O’Brien et al. 1987; Hirata et al. 1988; Yanagisawa et al. 1988). The vasopressor effect, induced by the administration of synthetic endothelin in vivo (Goetz et al. 1988; Miller et al. 1989), suggests that it could be involved in the regulation of vascular resistance. However, the pathophysiological roles of this substance in the regulation of blood pressure and sodium-water excretion remain to be elucidated.

In the present study, we assessed the chronic effects of synthetic endothelin on systemic blood pressure and sodium-water excretion in conscious rats to determine whether endothelin could act as a circulating hormone in the regulation of blood pressure and sodium-water metabolism, and also evaluated the effects of benidipine (Kubo et al. 1985; Fujii et al. 1988) or nilvadipine (Ohtsuka et al. 1983), newly developed calcium channel blockers, in conscious rats infused chronically with synthetic endothelin.

**Methods**

Male Sprague-Dawley rats weighing from 150-250 g were used. All rats were maintained in a humidity- and temperature-controlled room, each rat being housed in a metabolic cage during the study. The rats were fed a regular diet (Oriental CMF, 0.24% of sodium/g, 0.69% of potassium/g; Oriental Yeast Co., Tokyo) and had free access to tap water. Studies were performed after a 7-day period of acclimatization to the housing, feeding, and drinking conditions. The rats were infused with 6 and 60 μg/kg/day of a synthetic endothelin or vehicle (0.9% physiological saline), delivered via osmotic minipumps (Alzet®, Palo Alto, CA, USA) into the jugular vein for up to 6 days. The vascular catheter (PE 60) was tunneled subcutaneously, and the osmotic minipump was implanted into the interscapular region of the rat's back under pentobarbitone sodium anesthesia (Abbott Laboratories Pty. Ltd., Tokyo). A synthetic endothelin of 21 amino acids purchased from Peptide Institute, Inc. (Osaka) was used in the present experiments and was dissolved in 0.9% physiological saline. Rats in the control group received the vehicle. Assuming that endothelin did not degrade during the study, and that the pumps dispensed fluid at the specified rate of approximately 1 μl/hr, the infusion doses (6 and 60 μg/kg/day) were chosen to be sufficient to induce a significant elevation of circulating levels of this peptide. The stability of endothelin in the osmotic minipump was examined by comparing the blood pressure elevating activity remaining in the solution recovered from the minipump after 6 days of use in the rat to the activities in freshly dissolved endothelin. No difference was observed in the activities between the fresh preparation and the solution.
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recovered from the minipump.

The effect of combined administration of benidipine (Kubo et al. 1985; Fujii et al. 1988) or nilvadipine (Ohtsuka et al. 1983) with endothelin was assessed in rats on a regular diet. Following a 7 day control period, the rats were administered either with benidipine at a rate of 6 mg/kg/day or with nilvadipine at a rate of 10 mg/kg/day dissolved in polyethylene glycol by oral gavage once a day in combination with endothelin infused intravenously at a rate of 60 μg/kg/day for up to 6 days.

Systolic blood pressure in the rats was recorded daily by an indirect tail cuff method (UEDA UR 1000, Ueda Industries Co., Tokyo) without anesthesia (Pfeffer et al. 1971). Direct blood pressure measurements were assessed on the final day of experiments in the selected rats of each group for comparison with data obtained by the indirect method. With the rats under ether anesthesia, the left femoral artery was cannulated using polyethylene tubing. The catheter was tunneled subcutaneously and exteriorized at the nape of the neck. They were filled with heparinized (1,000 U/ml) saline solution and sealed by heating. Blood pressure measurements were performed using fully conscious rats. Rats were placed in rectangular boxes with no restriction of movement. The catheter was connected to an electronic transducer and amplifier (RMP 6004, Nihon Kohden, Tokyo) for blood pressure measurement. We confirmed that there was a significant correlation (p <0.05) between data obtained by both methods. The daily fluid intake, urine volume and urinary sodium excretion were also determined. Urinary sodium was measured with a flame photometer.

Data are expressed as means ± s.e. Statistical analysis of the data between groups was performed by two-way analysis of variance for repeated measurements. Statistically significant differences on each day were isolated by the Newman-Keuls test for multiple comparisons of each value.

RESULTS

Body weight, systolic blood pressure, fluid intake, urine volume and urinary sodium excretion were not significantly different between the groups prior to the infusion of endothelin or vehicle.

As shown in Table 1, continuous infusion of endothelin at a rate of 60 μg/kg/day into the jugular vein via osmotic minipumps induced a significant increase in systolic blood pressure but not any significant changes in urine volume and urinary sodium excretion for up to 6 days, compared to those in vehicle-infused rats. On the contrary, the infusion of endothelin at a rate of 6 μg/kg/day did not induce any significant changes in systolic blood pressure, urine volume and urinary sodium excretion for up to 6 days, compared to those in vehicle-infused rats.

In the subsequent experiments, the effects of benidipine or nilvadipine on blood pressure, urine volume and urinary sodium excretion were assessed in rats infused with endothelin at a rate of 60 μg/kg/day. When 6 mg/kg/day of benidipine were administered simultaneously with 60 μg/kg/day of endothelin, the tail systolic blood pressure of conscious rats rose on Day 1 to only 137.0 ± 2.4 mmHg (p <0.05), compared to the rise to 163.8 ± 4.7 mmHg when endothelin alone was infused (Fig. 1). The antihypertensive effect of benidipine was sustained for the entire experimental period in rats infused with endothelin (Fig. 1). This was not associated with any significant changes in urine volume and urinary sodium
<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
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<tr>
<td>SBP (mmHg)</td>
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<tr>
<td>ET (6 μg/kg/day)</td>
<td>(n = 7)</td>
<td>143.0 ± 2.3</td>
<td>138.5 ± 0.8</td>
<td>133.5 ± 1.3</td>
<td>132.0 ± 1.8</td>
<td>130.0 ± 1.3</td>
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<tr>
<td>ET (60 μg/kg/day)</td>
<td>(n = 8)</td>
<td>143.7 ± 2.0</td>
<td>163.8 ± 4.7*</td>
<td>149.1 ± 1.2</td>
<td>149.4 ± 1.1*</td>
<td>152.3 ± 1.7*</td>
<td>152.0 ± 2.4*</td>
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<tr>
<td>Vehicle</td>
<td>(n = 6)</td>
<td>135.5 ± 7.9</td>
<td>140.5 ± 8.0</td>
<td>144.0 ± 4.8</td>
<td>139.5 ± 1.0</td>
<td>141.8 ± 2.3</td>
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<td>UV (ml/day)</td>
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<tr>
<td>ET (6 μg/kg/day)</td>
<td>(n = 7)</td>
<td>7.0 ± 0.7</td>
<td>7.3 ± 1.3</td>
<td>7.3 ± 0.8</td>
<td>6.8 ± 0.5</td>
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<td>ET (60 μg/kg/day)</td>
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<td>7.6 ± 1.1</td>
<td>8.6 ± 1.4</td>
<td>6.7 ± 1.0</td>
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<td>U_{Na}V (mEq/day)</td>
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<tr>
<td>ET (6 μg/kg/day)</td>
<td>(n = 7)</td>
<td>0.72 ± 0.05</td>
<td>0.65 ± 0.03</td>
<td>0.69 ± 0.03</td>
<td>0.65 ± 0.08</td>
<td>0.75 ± 0.10</td>
<td>0.93 ± 0.19</td>
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<tr>
<td>ET (60 μg/kg/day)</td>
<td>(n = 8)</td>
<td>0.64 ± 0.05</td>
<td>0.52 ± 0.11</td>
<td>0.72 ± 0.08</td>
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<td>0.76 ± 0.50</td>
<td>0.56 ± 0.16</td>
<td>0.69 ± 0.18</td>
<td>0.87 ± 0.03</td>
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Values are expressed as means ± s.e. SBP, systolic blood pressure; UV, urine volume; U_{Na}V, urinary sodium excretion; ET, endothelin. Analysis of variance for repeated measurements revealed a significant change in systolic blood pressure in rats given endothelin alone at a rate of 60 μg/kg/day (p < 0.05) compared to that in rats given vehicle alone. *p < 0.05 compared to the values in rats given vehicle alone.
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When 10 mg/kg/day of nilvadipine were administered simultaneously with 60 \(\mu g/kg/day\) of endothelin, the tail systolic blood pressure of conscious rats also rose on Day 1 to only 119.7 \(\pm\) 5.9 mmHg \((p < 0.05)\), compared to the rise to 163.8 \(\pm\) 4.7 mmHg when endothelin alone was infused (Fig. 2). The antihypertensive effect of nilvadipine was sustained during the experimental period in rats infused with endothelin (Fig. 2). Systolic blood pressure in the group given nilvadipine and endothelin was also significantly lower than that in vehicle-infused rats. This was not associated with any significant changes in urine volume and urinary sodium excretion.

**DISCUSSION**

In the present study, we demonstrated that chronic infusion of a synthetic endothelin of 21 amino acids at a rate of 60 \(\mu g/kg/day\) for 6 days induced a sustained elevation of blood pressure whereas it failed to induce any significant changes in urine volume and urinary sodium excretion. In addition, it is interesting to note that calcium channel blockers were effective in counteracting the
The blood-pressure elevating action of endothelin might be due to the increased vascular resistance because it has been shown that in vitro preparations endothelin induces a potent vasoconstrictive effect on a variety of blood vessels from various species (Tomobe et al. 1988; Yanagisawa et al. 1988). It has also been suggested that the vascular effect of endothelin is dependent on extracellular calcium ion (Hirata et al. 1988; Yanagisawa et al. 1988). In addition, it has been shown that nicardipine, a dihydropyridine derivative, counteracts the vasoconstrictive effect of endothelin in vitro. To our knowledge, the sustained vasopressor effect of endothelin has not been previously reported, although it has been well documented that in short-term experiments endothelin induces an elevation of blood pressure (Goetz et al. 1988; Miller et al. 1989; Yanagisawa et al. 1988). The attenuation of the blood-pressure elevating action of endothelin by benidipine or nilvadipine, the other dihydropyridine derivatives, was not due to tachyphylaxis per se, since endothelin alone induced a sustained increase in systolic blood pressure in the absence of calcium channel blockers.

It remains to be determined whether endogenous endothelin could act as a circulating or local hormone, since we have not checked the levels of circulating endothelin during the infusion of this peptide. We have no definite explanation
for lack of blood-pressure elevating effect of endothelin infused at a rate of 6 \( \mu \text{g/kg/day} \). It is not likely that lack of changes in blood pressure and urinary sodium excretion can be explained by an insufficient infusion rate of endothelin in the present study, since we chose the effective doses to induce vascular actions on the basis of in vitro and in vivo experiments on this peptide and its related vasoconstrictors. The systolic blood pressure of rats given endothelin at a dose of 6 \( \mu \text{g/kg/day} \) seems to be lower, but not significantly than that of vehicle-infused rats. The blood pressure response induced by a low dose of endothelin is most likely due to an indirect effect of endothelin since this peptide has direct contractile effects on isolated cardiac and vascular smooth muscle (Hirata et al. 1988; Ishikawa et al. 1988; Tomobe et al. 1988; Yanagisawa et al. 1988). A possible explanation might be that endothelin activates the buffering mechanisms including the enhanced baroreflex sensitivity, thereby reducing vascular tone. Similar phenomenon is known when vasopressin is administered in conscious animals (Johnston et al. 1981). Another possible explanation would be due to the release of vasodilatory substance(s) induced by endothelin (de Nucci et al. 1988; Lippton et al. 1988). Recently, Winquist et al. (1989) reported that endothelin appears to cause a direct release of immunoreactive atrial natriuretic factor from rat right atria in vitro. Goetz et al. (1988) also reported that infusions of endothelin increased plasma levels of atrial natriuretic factor in conscious dogs. These evidence raised the possibility that the blood pressure response induced by a low dose of endothelin may be modulated indirectly, in part, by the actions of atrial natriuretic factor, since it has been reported to effect vasodilation in rats (Garcia et al. 1985). However, the exact mechanisms remain to be elucidated in detail.

The mechanism by which calcium channel blockers attenuated the blood-pressure elevating action of chronic infusion of endothelin can not be determined in detail from the present experiments. However, our results are consistent with the observation of Yanagisawa et al. (1988) that the vasoconstrictive effect of endothelin is dependent on extracellular calcium ion. Furthermore, Hirata et al. (1988) reported that the sustained increase in cytosolic free calcium ion concentrations stimulated by endothelin is entirely dependent on calcium ion influx through the voltage-dependent calcium ion channels. On the contrary, there have also several reports that endothelin may not act by voltage-dependent calcium channels (Auguet et al. 1988; Gu et al. 1989). Therefore, its exact mechanisms remain unclear. Although calcium channel blockers exert its antihypertensive effects in various types of hypertension via complex mechanisms (Stone et al. 1980; Van Zwieten 1984), we could not find any significant differences in urine volume and urinary sodium excretion between the groups infused with endothelin alone and in combination with calcium channel blockers. Therefore, it is unlikely that the antihypertensive effect of calcium channel blockers is due to loss of water and sodium. Further study will be needed to clarify the exact mechanisms by which calcium channel blockers attenuate the
hypertensive effect induced by chronic infusion of endothelin.

In conclusion, the result of the present study that elevated levels of circulating endothelin could induce a sustained increase in systemic blood pressure, suggests that endothelin may be involved in the regulation of blood pressure by acting as a circulating hormone. However, its actual physiological relevance remains to be elucidated, since the release of endothelin into the circulation has not yet been shown.

Acknowledgments

We wish to acknowledge the excellent technical assistance of Keiko Shiraishi, Michiko Okamoto, and Naeko Hatanaka, and the secretarial assistance of Junko Okazaki. We are also grateful to Kyowa Hakko Kogyo Co., Tokyo, Japan and Fujisawa Pharmaceut., Co., Tokyo, Japan for supplying benidipine and nilvadipine, respectively. This study was supported by the Grant-in-Aid for Cardiovascular Disease from the Ministry of Health and Welfare of Japan (60-C-6) and for Scientific Research (61132005 and 62570276).

References


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