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Effect of chronically infused adrenomedullin in two-kidney, one-clip hypertensive rats

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Abstract

The hypotensive effect of chronically infused adrenomedullin, a potent vasodilator peptide, was examined in conscious two-kidney, one-clip (2K-1C) hypertensive and sham-operated rats. They were infused with 1.0 μg/h of synthetic human adrenomedullin for 14 days by means of osmotic minipumps. Control groups were infused on the same schedule with 0.9% saline. Systolic blood pressure was measured before and during the infusion. Plasma renin activity, aldosterone and human adrenomedullin concentrations were determined at day 14 of the infusion. A significant reduction of systolic blood pressure was observed in the adrenomedullin-infused 2K-1C rats at day 4, and systolic blood pressure remained significantly lower throughout the experiment compared to that of the control 2K-1C. A similar hypotensive effect was seen in the adrenomedullin-infused sham-operated rats. Both the plasma renin activity and aldosterone concentrations of the adrenomedullin-infused 2K-1C and sham groups were significantly reduced compared to those of the respective control, whereas, the plasma human adrenomedullin concentration in the adrenomedullin-infused groups was found to be within the physiological range. These findings demonstrated that chronically infused adrenomedullin had a hypotensive effect accompanied by significant reductions of plasma renin activity and plasma aldosterone concentration in 2K-1C hypertensive and sham-operated rats.

Keywords: Adrenomedullin; Blood pressure; Renovascular hypertension; (Rat); Renin; Aldosterone

1. Introduction

Adrenomedullin is a potent vasodilator peptide recently isolated from a tissue extract of human pheochromocytoma by monitoring the elevation of cyclic AMP (cAMP) in rat platelets (Kitamura et al., 1993). Adrenomedullin consists of 52 amino acids with an intramolecular disulfide bridge (Kitamura et al., 1993), having a slight structural homology with calcitonin gene-related peptide (CGRP), an established vasodilator peptide. A potent long-lasting hypotensive effect with a marked reduction of total peripheral resistance was observed following the intravenous administration of adrenomedullin to anesthetized rats (Ishiyama et al., 1993). The development of a radioimmunoassay (RIA) revealed the presence of considerable concentrations of adrenomedullin in normal rat and human tissues such as adrenal medulla, kidney, cardiac atrium and lung (Ichiki et al., 1994; Sakata et al., 1994). We reported that adrenomedullin circulates in the blood and that the plasma concentration of adrenomedullin was increased in patients with essential hypertension or primary aldosteronism compared to that in normotensive controls (Kitamura et al., 1994; Kato et al., 1995). While these findings imply a role of adrenomedullin in regulating blood pressure, the effect of adrenomedullin on the renin–angiotensin–aldosterone system remains unclear at present. To examine the effect of chronically infused adrenomedullin on this system, synthetic adrenomedullin was administered intravenously for two weeks to two-kidney, one-clip (2K-1C) renovascular hypertensive rats. In these rats the activated renin–angiotensin–aldosterone system plays an important role in the maintenance of high blood pressure under the conditions of the present study.

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2. Materials and methods

2.1. Experimental protocols

Four-week-old male Wistar rats were obtained from Charles River (Atsugi, Japan) and synthetic human adrenomedullin was purchased from the Peptide Institute (Osaka, Japan). The rats were housed in a temperature- and light-controlled environment, were maintained on standard rat chow containing 0.85% NaCl and had free access to tap water. At five weeks of age, a silver clip with an internal gap of 0.2 mm was placed on the left renal artery to produce a 2K-1C model of renovascular hypertension. Systolic blood pressure was measured at periodic intervals for four weeks to check whether the blood pressure had reached a satisfactory level.

Thereafter, the rats were randomly divided into two groups: one for adrenomedullin infusion ($n = 12$) and the other for control ($n = 12$). They were subcutaneously implanted under light ether anesthesia with osmotic minipumps (model 2002; Alza, Palo Alto, CA, USA), which had been filled with either synthetic human adrenomedullin dissolved in 0.9% saline to release 1.0 μg/h of the peptide over two weeks or only 0.9% saline for the controls. A single dose of adrenomedullin was used in the present study, while a dose–response relationship of adrenomedullin and blood pressure has been examined by a short time-period experiment (Ishiyama et al., 1995). The pumps were connected to the left jugular vein by a polyethylene catheter (PE-50) and positioned in a pocket constructed in the subcutaneous tissue. Systolic blood pressure and heart rate were measured at 9 a.m. on alternate days before and after the start of infusion by the tail-cuff method without anesthesia. Another group of rats underwent a sham operation except for the clipping, followed by identical experimental procedures (adrenomedullin infusion, $n = 8$; control, $n = 7$).

At the end of the infusion, the animals were killed by decapitation and the blood was collected into chilled tubes containing 500 kallikrein inhibitory units/ml of aprotinin and 1.0 mg/ml of EDTA-2Na. The blood was then immediately centrifuged at 3000 rpm for 10 min at 4°C and the plasma was stored at −30°C until assayed.

2.2. Assay procedures

The plasma human adrenomedullin concentration was measured with a specific RIA that has no cross-reactivity with rat adrenomedullin peptide, after the extraction of plasma as described previously (Kitamura et al., 1994). Plasma renin activity was determined by means of a RIA kit supplied by Incstar (Stillwater, MN, USA) (Ryan et al., 1994), and plasma aldosterone concentrations with the SPAC-S Aldosterone kit from Daiichi Radioisotope Laboratories (Tokyo, Japan) (Shionoiri et al., 1989).

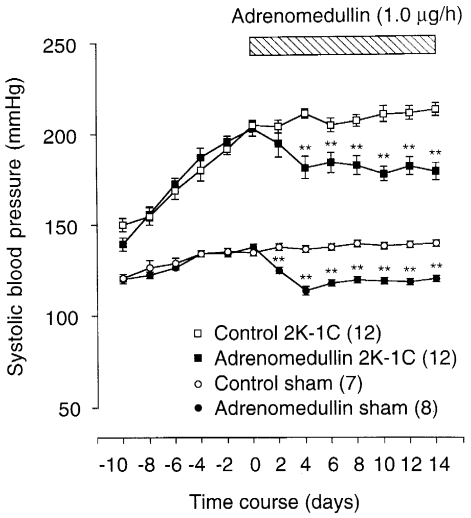

Fig. 1. Systolic blood pressure before and during infusion of 1.0 μg/h adrenomedullin (closed symbols) and 0.9% saline (open symbols) in 2K-1C and sham-operated groups. Values are the means ± S.E.M., and the numbers of rats examined are indicated in parentheses. ** $P < 0.01$, compared to respective control group.

2.3. Statistical analysis

All data are expressed as the means ± S.E.M. Multiple comparisons were evaluated with two-way analysis of variance (ANOVA) for repeated measures. The difference between two variables was examined by means of an unpaired *t*-test and linear regression analysis was used to assess the correlations. A P value less than 0.05 was considered significant.

3. Results

Fig. 1 shows the systolic blood pressure before and during the infusion of 1.0 μg/h human adrenomedullin or 0.9% saline in the 2K-1C and sham-operated groups. A significant ($P < 0.01$) reduction of the systolic blood pres-

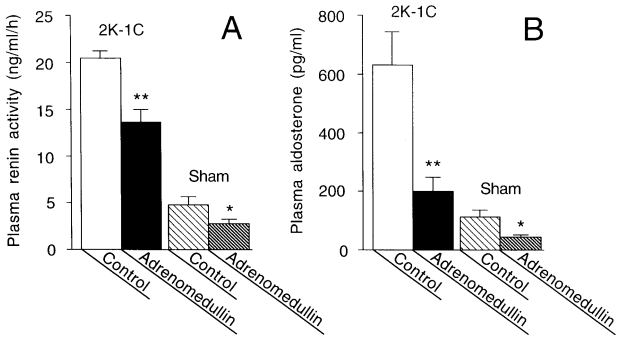

Fig. 2. Inhibitory effects of adrenomedullin on plasma renin activity (A) and aldosterone concentration (B) in the 2K-1C and sham-operated groups at day 14 of the infusion. * $P < 0.05$, ** $P < 0.01$, compared to controls.

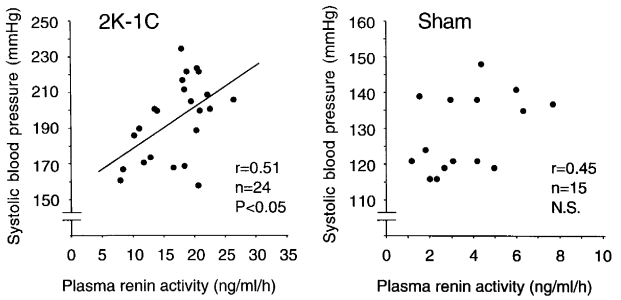

Fig. 3. Relationship between systolic blood pressure and plasma renin activity in 2K-1C and sham-operated rats. A significant correlation was seen between the two in the 2K-1C groups ($r = 0.51$, $P < 0.05$) but not in the sham groups. N.S., not significant.

sure was observed in the adrenomedullin-infused 2K-1C group at day 4 compared to the control 2K-1C, and the systolic blood pressure in the adrenomedullin group then remained significantly ($P < 0.01$) lower for the rest of the 14-day infusion period. The systolic blood pressure in the adrenomedullin-infused sham group was also found to be significantly ($P < 0.01$) lower than that in the control sham group infused with saline over the infusion period. No reflex increase in heart rate was recorded in either of the adrenomedullin-infused groups despite the reduction in blood pressure throughout the experimental period.

As shown in Fig. 2, both the plasma renin activity and aldosterone concentrations at day 14 of the infusion were significantly lower in the adrenomedullin-infused 2K-1C ($P < 0.01$) and sham-operated ($P < 0.05$) groups than in the respective control. A significant correlation was observed between systolic blood pressure and plasma renin activity in the 2K-1C groups ($r = 0.51$, $P < 0.05$) but not in the sham groups (Fig. 3).

The mean plasma human adrenomedullin concentration (fmol/ml) at day 14 of the infusion in each group was as follows: human adrenomedullin-infused 2K-1C group, 0.45 ± 0.05; human adrenomedullin-infused sham-operated group, 1.03 ± 0.36; control (saline-infused) groups, human adrenomedullin not detected. The plasma human adrenomedullin in the adrenomedullin-infused 2K-1C group was lower than that in the adrenomedullin-infused sham group, but the difference was not statistically significant.

4. Discussion

Adrenomedullin is a novel vasoactive peptide discovered in tissue extracts of pheochromocytoma (Kitamura et al., 1993). The vasodilator properties of this peptide have been examined in in vivo and in vitro experiments (Ishiyama et al., 1993; Nuki et al., 1993), but the pharmacological dose of adrenomedullin was utilized in these experiments and the observation period was brief. For example, plasma adrenomedullin has been shown to rise to a level 100-fold higher than the physiological concentration after a bolus intravenous injection in rats (Ishiyama et al., 1995). In the present study, we infused a relatively low dose of adrenomedullin for two weeks in 2K-1C renovascular hypertensive rats, and observed a hypotensive effect of adrenomedullin in these hypertensive and sham-operated rats. The plasma concentrations of human adrenomedullin at day 14 in the adrenomedullin-infused 2K-1C and sham rats were found to be 0.45 ± 0.05 and 1.03 ± 0.36 fmol/ml, respectively, in a RIA that has no cross-reactivity with rat adrenomedullin. The plasma adrenomedullin level in rats has been reported to be 3.6 fmol/ml (Sakata et al., 1994), and the plasma adrenomedullin in 2K-1C rats is not significantly different from that in normotensive rats (Ishiyama et al., 1997). Thus, the present data suggest the significance of such a small increase of plasma adrenomedullin within the physiological limit for the regulation of blood pressure in 2K-1C as well as sham rats.

Adrenomedullin is thought to dilate blood vessels by increasing the cAMP level in the smooth muscle cells of the vascular wall (Kitamura et al., 1993). Consistent with this, Ishizaka et al. (1994) found that adrenomedullin binds to its specific receptors stimulating cAMP production in cultured rat vascular smooth muscle cells. On the other hand, Itahara et al. (1994) found that the hypotensive activity of adrenomedullin was diminished by pretreatment with *N*-nitro-L-arginine, a nitric oxide (NO) synthase inhibitor, a finding that suggests endothelium-dependent vasodilatation by adrenomedullin. We recently showed the hypotensive action of chronically administered adrenomedullin without an increase in urine volume or sodium excretion in spontaneously hypertensive rats, which suggested that adrenomedullin lowers blood pressure without plasma volume contraction (Khan et al., 1997). While we did not see how the reduced blood pressure returns to the control level after depletion of the pumps to pursue the aim of the present study, a direct effect of adrenomedullin on vascular tone, considering its potent vasodilator properties, seems to be one of the mechanisms for the blood pressure reduction observed in the present study.

With respect to the hypotensive action of adrenomedullin, an important result of this study is the inhibition of plasma renin activity, as shown by the significant relationship between systolic blood pressure and plasma renin activity in the 2K-1C groups. Vasoconstricting stimuli generally inhibit renin secretion, whereas most vasodilator hormones stimulate its release (Hackenthal and Taugner, 1986). In accord with this, adrenomedullin was found to increase plasma renin activity after intravenous injections in conscious rabbits (Fukuhara et al., 1995). In contrast, although plasma renin response was not examined at an early phase of the infusion, the chronic infusion of adrenomedullin significantly inhibited plasma renin activity in the present study. Adrenomedullin has been shown to attenuate the reflex-mediated sympathetic activation in

conscious rabbits (Fukuhara et al., 1995), which may partly explain the plasma renin activity reduction observed in the present study. Hirata et al. (1995) reported that the renal vasodilator effect of adrenomedullin is exerted via NO release from the vascular endothelial cells, which are in close proximity to the juxtaglomerular cells of the kidney. Since NO is known to increase intracellular cGMP, it is possible that adrenomedullin inhibits the renin release from juxtaglomerular cells by a cGMP-dependent process (Henrich et al., 1988). In any case, further research to specify the locus of action is necessary to elucidate the mechanism of the plasma renin activity reduction by adrenomedullin.

Adrenomedullin has been reported to have an inhibitory action on aldosterone secretion in rat adrenal zona glomerulosa cells (Yamaguchi et al., 1995). In the present study, adrenomedullin significantly reduced plasma aldosterone concentration, but whether this effect is secondary to an inhibited renin–angiotensin system or is a direct action of adrenomedullin on adrenal glomerulosa cells remains to be elucidated. Irrespective of the mechanisms, not only the suppressed plasma renin activity but also the decreased plasma aldosterone concentration may have contributed to the reduction of blood pressure observed in the present study.

In summary, the present results demonstrated that chronically infused adrenomedullin had a blood pressure-lowering effect accompanied by significant reductions of plasma renin activity and plasma aldosterone concentrations in 2K-1C hypertensive and sham-operated rats.

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