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Renal effects of a new member of adrenomedullin family, adrenomedullin2, in rats

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Abstract

A new member of the adrenomedullin family, adrenomedullin2, was identified in mammals. The effects of adrenomedullin2 on renal hemodynamics and urine formation were examined in rats. Intrarenal arterial infusion of adrenomedullin2 at rates of 30, 100 and 300 pmol/kg/min decreased blood pressure and increased heart rate in a dose-dependent fashion. Adrenomedullin2 infusion at 100 pmol/kg/min significantly increased renal blood flow and urine flow. At the higher infusion rate (300 pmol/kg/min), adrenomedullin2 significantly decreased urine flow. Continuous intrarenal infusion of adrenomedullin2 at 100 pmol/kg/min significantly increased renal blood flow from 6.7±0.5 to 8.8±0.5 ml/min and decreased renal vascular resistance from 16±1 to 11±1 mm Hg min/ml. Urine flow was significantly increased from 21.5±4.9 to 36.2±8.5 μl/min and urinary excretion of sodium was increased from 2.3±0.9 to 4.9±1.4 μEq/min. Blood pressure, heart rate and glomerular filtration rate did not change. Infusion of a similar dose of adrenomedullin also increased renal blood flow (6.8±0.4–8.8±0.6 ml/min), urine flow (25.4±3.2–42.8±9.4 μl/min) and urinary excretion of sodium (2.8±0.6–6.5±1.2 μEq/min), decreased renal vascular resistance (15±1–11±1 mm Hg min/ml) and did not alter glomerular filtration rate. Thus, the renal actions induced by adrenomedullin2 were similar to those of adrenomedullin. These data suggest that adrenomedullin2 may play an important role in the regulation of renal hemodynamics and urine formation.

Keywords: Adrenomedullin2; Renal hemodynamics; Vasodilation; diuresis; Natriuresis

1. Introduction

Adrenomedullin is a potent 52-amino acid vasodilator originally isolated from tissue extracts of human pheochromocytoma (Kitamura et al., 1993). Adrenomedullin has been detected in a variety of organs, such as the adrenal gland, kidney, heart, lung, spleen and brain (Ichiki et al., 1994; Sakata et al., 1994; Eto, 2001), and has also been found to be secreted from endothelial and vascular smooth muscle cells (Sugo et al., 1995; Chun et al., 1997; Isumi et al., 1998). Infusion of adrenomedullin to experimental animals lowers blood pressure and produces diuresis and natriuresis (Ebara et al., 1994; He et al., 1995; Jougasaki et al., 1995; Parkes, 1995; Majid et al., 1996; Willenbrock et al., 1999). Adrenomedullin is also implicated in various disease states. Plasma adrenomedullin levels are increased in patients with cardiac hypertrophy (Nishikimi et al., 1996), heart failure (Kato et al., 1996; Nakamura et al., 1997; Gao et al., 2002; Osajima et al., 2002), renal dysfunction (Cheung and Leung, 1997) and hyperglycemia (Hayashi et al., 1999). Adrenomedullin has been reported to act in an autocrine/paracrine fashion to

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prevent cardiovascular (Tsuruda et al., 1998; Terata et al., 2000; Dobrzynski et al., 2002) and renal damage (Owada et al., 1997; Nishimatsu et al., 2002). Thus, adrenomedullin is considered to be an important regulator of human homeostasis.

Recently, five cDNAs encoding adrenomedullin-like peptides were identified from the pufferfish and named as adrenomedullin1 through adrenomedullin5 (Ogoshi et al., 2003). Among the members of the adrenomedullin family identified in teleost fish, the pufferfish adrenomedullin1, adrenomedullin4 and adrenomedullin5 genes were expressed in various tissues similar to mammalian adrenomedullin. However, any member of the adrenomedullin2 and adrenomedullin3 groups had not been identified in mammals. Following this discovery, Takei et al. (2004) identified cDNAs encoding a new member of the adrenomedullin family, adrenomedullin2 in mouse, rat and human. The similarity of the exon–intron structure and synteny of the neighboring genes showed that mammalian adrenomedullin2 is an ortholog of pufferfish adrenomedullin2 and a paralog of mammalian adrenomedullin. Adrenomedullin2 mRNA was expressed in the submaxillary gland, kidney, stomach and ovary of mice, but not in the adrenal gland or testis. Intravenous administration of the putative mature adrenomedullin2 exerted hypotensive, antidiuretic and antinatriuretic actions in mice. Thus, it can be considered that adrenomedullin2 plays an important role in the regulation of systemic hemodynamics and body fluid. However, any information regarding a possible role for adrenomedullin2 in the regulation of renal hemodynamics and function is not available, except for its hypotensive and antidiuretic actions. Thus, the present study was designed to determine the effects of synthesized human adrenomedullin2 on renal hemodynamics and urine formation in rats, and to compare with those of adrenomedullin.

2. Methods

2.1. Animal preparation

Experiments were performed with 9–11-week-old male Sprague–Dawley rats weighing 290–350 g (Japan SLC, Hamamatsu, Japan). The rats were housed in separate cages in a temperature-controlled room with a 12-h light and dark cycle. They were fed with a standard laboratory diet and water ad libitum. All surgical and experimental procedures were approved by the Animal Care and Use Committee, Kagawa University and conformed to the Guidelines for Animal Experimentation, Kagawa University.

Under sodium pentobarbital anesthesia, a polyethylene catheter (PE-50) was inserted into the abdominal aorta via the right femoral artery for measurement of blood pressure and collection of arterial blood. Another catheter (PE-50) was inserted into the inferior vena cava via the right femoral vein for continuous infusion of isotonic saline at a rate of 50 μl/min. The femoral arterial catheter was connected to a Statham pressure transducer (P-23ID). Heart rate was triggered by the blood pressure pulse wave form. The left kidney was exposed through a retroperitonieal flank incision. The renal artery was carefully isolated from the tissue connecting the renal hilum cephalic. A Doppler flow probe (HDP 10.20R; Crystal Biotech, MA, USA) was placed around the renal artery and renal blood flow was continuously monitored. A polyethylene catheter (PE-50) was inserted into the left ureter for urine collection. A 30-gauge needle was introduced into the left renal artery for administration of saline or drug solutions. After the completion of surgery, each rat was left alone for 60 min to allow stabilization of blood pressure, renal blood flow and urine flow. All chemicals were dissolved in saline solution.

2.2. Experimental protocols

Saline solution was infused into the renal artery at a rate of 67 μl/min and urine samples were collected during two consecutive 10-min control periods. At the midpoint of the second control period, 0.5 ml of systemic blood was collected from the arterial catheter. Blood and urine samples were analyzed for creatinine and electrolytes. After the second control period, adrenomedullin2 or adrenomedullin was infused into the renal artery based on the following protocols. Human adrenomedullin2 and adrenomedullin were purchased from the Peptide Institute.

2.2.1. Dose responses of renal hemodynamics and urine formation to adrenomedullin2

The dose responses of blood pressure, renal blood flow and urine flow to adrenomedullin2 were determined in 5 rats. After the second control period, adrenomedullin2 was infused into the renal artery at a rate of 30 pmol/kg/min for 20 min. After urine sample collection, the dose of adrenomedullin2 was sequentially increased from 30 to 100 and 300 pmol/kg/min at 20-min intervals and urine samples were collected at each intervention.

2.2.2. Effects of adrenomedullin2 or adrenomedullin on renal function

Based on the results of the previous protocol, we selected the medium dose of adrenomedullin2 (100 pmol/kg/min), which exhibited a small effect on blood pressure, because the changes in renal perfusion pressure significantly modified the renal function. The same dose (100 pmol/kg/min) was chosen for the adrenomedullin experiment. After the second control period, adrenomedullin2 (n=8) or adrenomedullin (n=8) was infused into the renal artery at a rate of 100 pmol/kg/min for 30 min. Urine and blood samples were collected during two 10-min clearance periods after a 5-min lag time for urine collection. After cessation of the adrenomedullin2 or adrenomedullin infusion, saline solution was infused for 40 min to assess the renal action

of these agents. Urine samples were collected for four consecutive 10-min clearance periods. A blood sample was collected at the last clearance period.

2.3. Analytical procedures

The plasma and urine concentrations of creatinine were determined using the method of Bonsnes and Taussky (1945). The urinary concentration of sodium was measured by flame photometry (Model 750; Hitachi, Japan). Renal vascular resistance was calculated as mean blood pressure/renal blood flow. Fractional excretion of sodium was calculated by standard methods as $UNaV/(PNa \cdot GFR) \cdot 100$, where UNaV and PNa represent the urinary excretion rate of sodium and the plasma concentration of sodium, respectively.

2.4. Statistical analysis

All values are expressed as mean±S.E. Data were analyzed by paired or unpaired Student's *t*-test or the two-way analysis of variance for repeated measurements with a posteriori comparison (Bonferroni), whenever appropriate. *P*-values less than 0.05 were accepted as statistically significant.

3. Results

3.1. Dose responses of renal hemodynamics and urine formation to adrenomedullin2

The responses of blood pressure, heart rate, renal blood flow and urine flow are shown in Fig. 1. Although adrenomedullin2 was infused into the renal artery, blood pressure was decreased and heart rate was increased dose-dependently. These changes were significant at the highest dose of adrenomedullin2. However, renal blood flow and urine flow were only significantly increased at the medium dose of adrenomedullin2 (from 6.7±0.4 to 8.8±0.3 ml/min and from 25.3±3.9 to 45.6±7.3 μl/min, respectively). In contrast, urine flow was significantly decreased at the highest dose of adrenomedullin2 (from 25.3±3.9 to 13.2±3.7 μl/min). Thus, antidiuresis was observed along with the reduction in blood pressure. Based on these findings, a dose of 100 pmol/kg/min was selected as a suitable dose of adrenomedullin2 for examining its renal actions.

3.2. Effects of adrenomedullin2 or adrenomedullin on renal function

The time courses of the blood pressure, heart rate, renal blood flow and renal vascular resistance responses to adrenomedullin2 and adrenomedullin are shown in Fig. 2. Intrarenal infusion of adrenomedullin2 or adrenomedullin at 100 pmol/kg/min resulted in a slight reduction in blood pressure during the infusion of these agents, but the changes were not statistically significant. Heart rate did not change during the infusion of adrenomedullin2, but slightly increased during adrenomedullin infusion. Renal blood flow was significantly increased from 6.7±0.5 to 8.8±0.5 ml/min during adrenomedullin2 infusion and returned to the control level within 20 min after the cessation of infusion. Adrenomedullin also increased renal blood flow from 6.8±0.4 to 8.9±0.6 ml/min, but renal

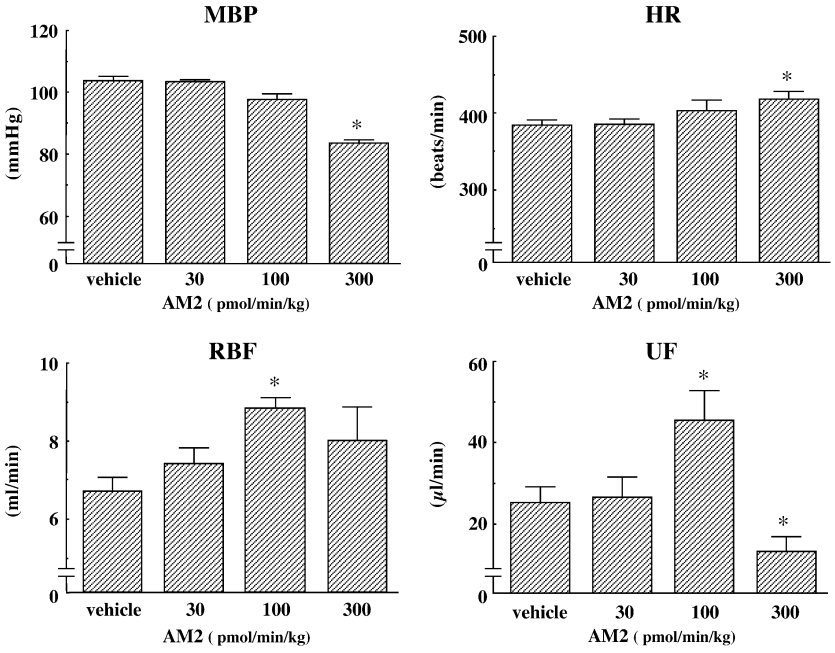

Fig. 1. Dose responses of mean blood pressure (MBP), heart rate (HR), renal blood flow (RBF) and urine flow (UF) to intrarenal infusion of adrenomedullin2 (AM2). *P<0.05 vs. vehicle.

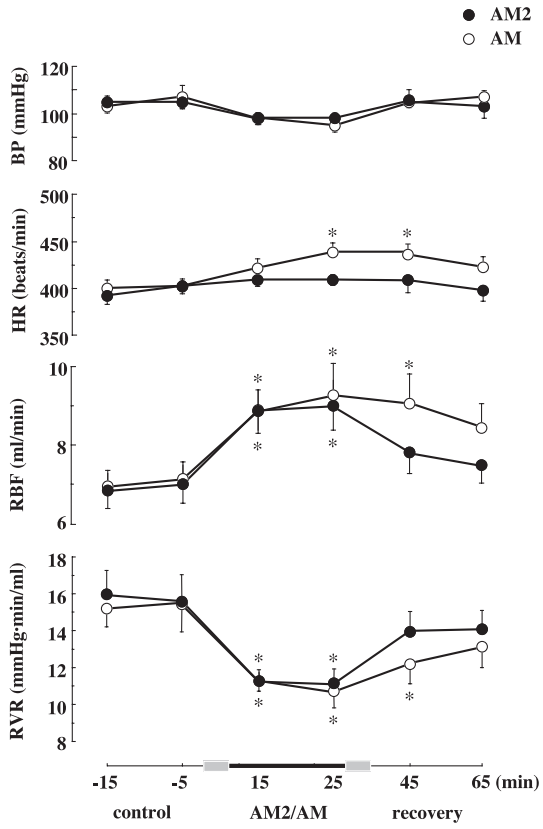

Fig. 2. Time courses of MBP, HR, RBF and renal vascular resistance (RVR) responses to AM2 (closed circles, 100 pmol/kg/min) and adrenomedullin (AM, open circles, 100 pmol/kg/min). *P<0.05 vs. control.

blood flow was maintained at a higher level even after the cessation of infusion. Adrenomedullin2 and adrenomedullin decreased renal vascular resistance by 30±3% and 34±4%, respectively, indicating their renal vasodilatory action.

Fig. 3 shows the effects of adrenomedullin2 and adrenomedullin on the urine formation. Neither adrenomedullin2 nor adrenomedullin had any effect on glomerular filtration rate. Adrenomedullin2 significantly increased the urine flow and urinary excretion of sodium from 21.5±4.9 and 2.3±0.9 to 36.2±8.5 μl/min and 4.9±1.4 μEq/min, respectively. However, urine flow and UNaV returned to their respective control levels 20 min after the cessation of adrenomedullin2 infusion. As a result, fractional excretion of sodium was significantly increased during adrenomedullin2 infusion, indicating the inhibition of sodium reabsorption. adrenomedullin also increased the urine flow and urinary excretion of sodium, and the degrees of these responses were identical of those to adrenomedullin2. However, higher levels of urine flow and UNaV were maintained even at 40 min after the cessation of adrenomedullin infusion.

4. Discussion

In the present study, we showed that infusion of a novel adrenomedullin2 into the rat renal artery at a rate of 100 pmol/kg/min increased renal blood flow without any change in blood pressure and heart rate, indicating that adrenomedullin2 has a vasodilatory effect on renal blood

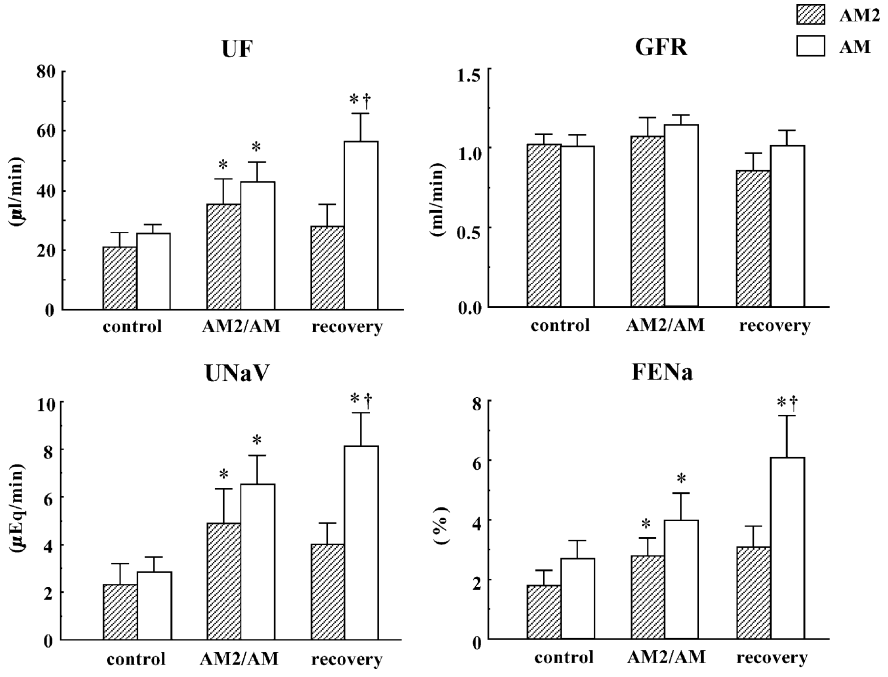

Fig. 3. Renal responses to intrarenal infusion of AM2 (hatched bars, 100 pmol/kg/min) and AM (open bars, 100 pmol/kg/min). GFR, glomerular filtration rate; UNaV, urinary excretion of sodium; FENa, fractional excretion of sodium. *P<0.05 vs. control. †P<0.05 vs. AM2.

vessels. In addition, adrenomedullin2 increased urine flow and urinary excretion of sodium without changing glomerular filtration rate, indicating that adrenomedullin2 inhibits the tubular reabsorption of sodium. However, these renal actions of adrenomedullin2 disappeared 10–20 min after the cessation of adrenomedullin2 infusion. Adrenomedullin infused into the renal artery at a rate of 100 pmol/kg/min also increased renal blood flow and urine flow. These changes were almost the same as those induced by adrenomedullin2. However, these parameters were maintained at higher levels even 40 min after the cessation of adrenomedullin infusion. Thus, the renal actions of adrenomedullin2 and adrenomedullin were similar, but the duration of the renal action of adrenomedullin2 appears to be short compared to that of adrenomedullin.

Recently, adrenomedullin-like peptides were found in the pufferfish and named adrenomedullin1 through adrenomedullin5 (Ogoshi et al., 2003). Among the members of the adrenomedullin family in teleost fish, the pufferfish adrenomedullin1, adrenomedullin4 and adrenomedullin5 genes were expressed in various tissues similar to mammalian adrenomedullin. However, no member of the adrenomedullin2 and adrenomedullin3 groups had been identified in mammals. Takei et al. (2004) identified cDNAs encoding a new member of the adrenomedullin family, adrenomedullin2, in mouse, rat and human. Adrenomedullin2 mRNA was expressed in various tissues in mice, especially in the kidney, but not in adrenal gland. Thus, it is interesting that the tissue distribution of adrenomedullin2 is different from that of adrenomedullin. However, physiological concentrations of adrenomedullin2 in plasma or local tissues have not been determined yet. Nevertheless, high expression of adrenomedullin2 mRNA was detected in the kidney, implying the possible role of adrenomedullin2 in the local regulation of renal function. In fact, Takei et al. (2004) reported that intravenous administration of adrenomedullin2 exerted hypotensive, antidiuretic and antinatriuretic actions in mice, and speculated that adrenomedullin2 might play an important role in the regulation of systemic hemodynamics and body fluid. It is well known that adrenomedullin has a potent vasodilatory and hypotensive action, and induces diuresis and natriuresis. Thus, it is meaningful to compare the renal effects of adrenomedullin2 with those of adrenomedullin for understanding the physiological role of adrenomedullin2.

It is well known that adrenomedullin is a potent vasorelaxant and induces a diuresis and natriuresis in various experimental animals (Ebara et al., 1994; He et al., 1995; Jougasaki et al., 1995; Parkes, 1995; Majid et al., 1996; Willenbrock et al., 1999) and human (Nagaya et al., 2000; Eto, 2001; McGregor et al., 2001), and that these actions of adrenomedullin are mediated via adenylate cyclase coupled with cyclic AMP or nitric oxide-cyclic GMP through its specific receptors (Miura et al., 1995; Shimekake et al., 1995; Edwards et al., 1996; Willenbrock et al., 1999; Terata et al., 2000). The present study has shown that adrenomedullin2 also exerts a potent renal vasodilatory action. This action was appeared immediately after the start of the adrenomedullin2 infusion and disappeared within 20 min after the cessation of infusion. On the other hand, the renal vasodilation induced by adrenomedullin continued even after the cessation of adrenomedullin infusion. Such long-lasting effects of adrenomedullin were previously reported by Vari et al. (1996). In addition, both adrenomedullin2 and adrenomedullin increased renal blood flow, but not glomerular filtration rate, indicating that these agents dilate both afferent and efferent arterioles to same degree. Thus, the vasodilatory potency of adrenomedullin2 was similar to that of adrenomedullin, but the action duration of adrenomedullin2 was relatively short compared to that of adrenomedullin.

Infusion of the medium dose (100 pmol/kg/min) of adrenomedullin2 induced significant diuretic and natriuretic actions. Renal blood flow was increased, while glomerular filtration rate was not. As a result, the filtration fraction decreased. These data suggest that adrenomedullin2 directly or indirectly inhibits the tubular reabsorption of sodium and water. A reduction in the filtration fraction should decrease the protein concentration and colloid osmotic pressure in the peritubular capillaries, which may induce the inhibition of proximal tubular reabsorption. However, based on our data, it remains to be elucidated whether the diuretic and natriuretic actions of adrenomedullin2 result from direct tubular action of adrenomedullin2, since there is currently no information about the receptor and signal transduction pathway for adrenomedullin2. However, the higher dose of adrenomedullin2 (300 pmol/kg/min) decreased the urine flow along with a reduction in blood pressure. Takei et al. (2004) reported that intravenous administration of adrenomedullin2 induced antidiuresis with a significant reduction in blood pressure, similar to our observation of antidiuresis at the higher dose of adrenomedullin2. Thus, adrenomedullin2 should have diuretic and natriuretic activities, but it can be considered that a reduction in blood pressure might mask its diuretic action. Adrenomedullin also exerted a similar diuretic action to that of adrenomedullin2, but the diuretic action of adrenomedullin continued after the cessation of adrenomedullin infusion. Thus, adrenomedullin2 exerted similar diuretic and natriuretic actions to adrenomedullin.

In summary, intrarenal arterial infusion of a new member of the adrenomedullin family, adrenomedullin2, at a rate of 100 pmol/kg/min in rats resulted in significant increases in renal blood flow, urine flow and urinary excretion of sodium. The renal actions induced by adrenomedullin2 were similar to those of adrenomedullin. Thus, theses findings suggest that adrenomedullin2 may play an important role in the regulation of renal hemodynamics and urine formation.

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