Chronic Salt Loading Upregulates Expression of Adrenomedullin and Its Receptors in Adrenal Glands and Kidneys of the Rat

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Abstract—The vasodilator peptide adrenomedullin (AM) elicits diuresis and natriuresis and inhibits aldosterone secretion. The aim of this study was to better understand the role of AM in maintaining water and electrolyte balance during chronic salt loading. Male Wistar rats were divided into a high salt (HS) group that received a diet containing 8% sodium chloride (NaCl) and a normal salt group that received a diet containing 0.4% NaCl. Plasma AM concentrations as well as expression of AM mRNA in the adrenal gland and kidney were then measured after 3, 7, 14, and 28 days. After 28 days, sodium and water excretion were significantly higher in HS rats than in control, although blood pressure and fluid volume were not significantly affected. Moreover, although plasma AM remained unchanged for up to 14 days, it was increased 2.5-fold in HS rats after 28 days on a high salt diet, and there were corresponding 3-fold and 1.5-fold increases in the levels of AM mRNA in the adrenal gland and kidney, respectively. At the same time, expression of calcitonin receptor-like receptor mRNA was significantly upregulated in both kidney and adrenal gland, as was expression of receptor activity-modify protein 1 (RAMP1) and RAMP2 mRNA in the adrenals and expression of RAMP3 in kidneys. Taken together, these results suggest that AM plays a role in the regulation of water and electrolyte balance in animals chronically ingesting high levels of salt. (Hypertension. 2003;42:369-372.)

Key Words: adrenomedullin ■ diuresis ■ natriuresis ■ gene expression ■ sodium

Adrenomedullin (AM) is a potent vasodilatory peptide discovered in the extract of human pheochromocytoma based on its capacity to elevate rat platelet cAMP levels. AM is composed of 52 amino acids and shows slight structural homology with calcitonin gene-related peptide (CGRP) and amylin: it shares a disulfide bridge and C-terminal amide structure.1 In addition to its vasodilatory action, AM also exerts multiple biological effects by serving as a circulating and autocrine/paracrine hormone.2 In the kidney, for example, AM exerts diuretic and natriuretic effects, whereas in adrenal gland it inhibits angiotensin II–stimulated and potassium-stimulated secretion of aldosterone.3,4 AM thus appears to play a role in maintaining appropriate electrolyte and water balance.5

The receptor activity-modifying proteins (RAMP1, RAMP2, and RAMP3), which have a single transmembrane domain, are required for transport of calcitonin receptor-like receptor (CRLR), an orphan receptor with 7-transmembrane domains, to the plasma membrane and for definition of its agonist selectivity. RAMP1 shares ≈30% identity with RAMP2 and RAMP3. Coexpression of RAMP1 with CRLR produces a CGRP receptor, which can be blocked by CGRP(8–37), whereas coexpression of RAMP2 or RAMP3 with CRLR produces an AM receptor, which can be blocked by AM(22–52).6 Notably, our recent findings indicate that AM, too, can bind with RAMP1/CRLR, though it has 8-fold less affinity than does CGRP.7

It was previously shown that plasma AM levels are elevated in patients with hypertension, heart failure, or chronic renal failure.8–10 Moreover, in diseases such as obstructive nephropathy and heart failure, AM receptors are upregulated or downregulated to adapt to the pathophysiological conditions.11,12 There is, however, little information about the effect of a high salt diet on expression of AM or its receptors. Therefore, to better understand the function of AM in the maintenance of electrolyte and water balance, we examined the effects of salt intake on plasma AM concentrations and expression of AM and its receptors in adrenal glands and kidneys in the rat.

Methods

Animals and Experimental Protocol
Male Wistar rats purchased from Charles River Japan (Yokohama, Japan) at 6 weeks of age were maintained in a light- and temperature-
controlled room (25±1°C) with free access to drinking water. After 1 week, the rats were divided into two groups: a high salt (HS) group that received a diet containing 8% sodium chloride (NaCl) for 3 to 28 days and normal salt (NS) group that received a diet containing 0.4% NaCl over the same period. Each group consisted of 20 rats, with 4 each undergoing blood and tissue sampling before euthanasia on days 3, 7, and 14, and 8 rats on day 28. The rats were anesthetized with 50 mg/kg pentobarbital sodium, and 10 mL whole blood was drawn from inferior vena cava. The blood was promptly transferred into ice-cold tubes containing 10 mg Na$_2$EDTA and 700 µg aprotinin, centrifuging at 2000 g for 10 minutes at 4°C, and the plasma assayed for AM and aldosterone concentrations. After blood collection, adrenal glands and kidneys were resected and stored at −80°C until extraction of total RNA. In the animals studied out to 28 days, blood pressure and heart rate were also measured twice per week by the tail-cuff method (Softtron, BP-98A), urine samples were collected over days 26 to 28 with the use of a metabolic cage, and body weight was measured before euthanasia on day 28. Urine samples were assayed with the use of automatic biochemical analyzers for Na (Atwill EA06A) and creatinine (Cr) concentrations (Hitachi 7170).

All experiments were performed according to the regulations of the Animal Research Committee of Miyazaki Medical College. This investigation conformed to the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (1996).

Radiomimuonoassays

Plasma AM concentrations were determined by radioimmunoassay, with the use of an AM IRMA kit (Shionogi Co). Plasma aldosterone concentrations were determined through the use of an SPAC-S Aldosterone kit (Daich Radioisotope Laboratories). These assays were performed according to the manufacturer’s instructions.

Real-Time Quantitative Polymerase Chain Reaction

Total RNA was isolated as previously described. Briefly, total RNA was extracted from rat tissues with the use of the acid guanidinium thiocyanate-phenol chloroform method with TRIzol Reagent (Invitrogen), according to the manufacturer’s protocol. The quality of extracted RNA was confirmed by detection of intact 18S and 28S bands on ethidium bromide-stained agarose gels. Samples (2 µg) of total RNA were then reverse-transcribed with SuperScript reverse transcriptase (Invitrogen), and the resultant cDNA samples were subjected to real-time quantitative PCR (Prism 7700 Sequence Detector, Applied Biosystems) by using previously described oligonucleotide primers and probes. The amplification protocol entailed 40 cycles of denaturation at 95°C for 15 seconds, with annealing at 60°C for 60 seconds. The level of mRNA was normalized relative to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

Calculations and Statistics

Results are expressed as mean±SEM. Analysis of variance and Student t tests were used when appropriate to assess the significance of differences between groups. Simple regression analysis was performed to identify potential associations among variables. Values of P<0.05 were considered significant.

Results

Effect of Salt Intake on Blood Pressure and Electrolyte Balance

The Table shows the parameters for rats maintained on a high salt (HS) or normal salt (NS) diet for 28 days. The increased excretion of sodium and water in the urine is indicative of renal adaptation to the high salt diet. Note that salt loading for 28 days had no effect on blood pressure, with a highly slower heart rate for the HS group. In comparison between the HS and NS groups at the other time points before day 28, no significant differences were observed in blood pressure and heart rate throughout the experimental period (data not shown).

Plasma AM and Aldosterone Concentrations

We measured plasma AM and aldosterone concentrations at 4 time points during the experimental period (Figure 1). Although they remained unchanged for up to 14 days, AM levels in HS rats were increased by as much as 2.5-fold over control after 28 days on a high salt diet (Figure 1A). As expected, however, plasma aldosterone levels in the HS group were significantly lower than that in the NS group at all time points examined (Figure 1B). Data analyses within the 28-day group revealed that plasma AM levels were significantly correlated with urinary sodium excretion (r=0.85, P<0.01) and with plasma aldosterone levels (r=−0.71,

![Figure 1](https://example.com/figure1.png)
P<0.01), whereas plasma aldosterone levels were also correlated with urinary sodium excretion ($r = -0.73, P<0.01$).

Expression of AM mRNA in Adrenal Glands and Kidneys

To further investigate the involvement of AM in mitigating the volume expansion and increases in blood pressure that one might expect to see with salt loading, expression of AM mRNA in the kidneys and adrenal glands was examined with the use of real-time quantitative PCR. As shown in Figure 2A, levels of AM mRNA in the adrenal glands of HS rats remained unchanged for 14 days but were increased 3.3-fold after 28 days. Similarly, in the kidneys of HS rats, AM mRNA was increased 1.6-fold after 14 days and remained significantly elevated at 28 days (Figure 2B). We examined relations between the plasma AM levels and AM mRNA expressions at each time point, and the plasma AM levels were found to be significantly related with adrenal AM mRNA on 28 days of salt loading ($r = 0.76, P<0.01$), whereas the relations were insignificant on the earlier time points and in the kidneys.

Expression of AM Receptor

Expression of mRNA coding for AM receptor components was measured in the adrenal glands and kidneys after the 28-day experimental period. Expression of CRLR mRNA was increased $\approx 2.3$-fold in both of the adrenal glands and kidneys of the HS group.

Although abundantly expressed in adrenal glands, expression of RAMP3 mRNA was unaffected by salt intake (Figure 3A). By contrast, expressions of RAMP1 and RAMP2 were upregulated by 2.8- and 2.6-fold, respectively, in the HS group. In the kidneys, RAMP2 and RAMP3 were abundantly expressed, and RAMP3 was upregulated by 1.7-fold in the HS group (Figure 3B).

Discussion

In the present study, we found that maintaining rats on a high salt diet for 28 days led to significant increases in plasma AM and upregulation of AM transcription and transcription of its receptors in kidney and adrenal gland. In previous studies, AM was detected in the mesangial and microvascular areas of rat glomeruli, and video microscopic analysis showed that it dilated both the afferent and efferent arterioles in a cAMP-dependent manner. Intrarenal infusion of AM had marked diuretic and natriuretic effects and increased the glomerular filtration rate. Plasma AM was found to be elevated in patients with impaired renal function, and an intimate correlation was noted between levels of plasma AM and those of norepinephrine and atrial natriuretic peptide. Taken together, these findings strongly suggest that AM contributes to the modulation of kidney function that counterregulates volume expansion in the face of a high salt diet. Consistent with that interpretation was our finding of a significant correlation between the plasma AM levels and urinary sodium excretion. In fact, salt loading for 28 days had no significant effect on blood pressure or volume expansion indicated by the absence of a rise in body weight.

One earlier study in which rats were salt-loaded for 10 days failed to detect an increase in transcription of AM or AM receptors in kidney. This is consistent with our finding that expression remained unchanged for at least 7 days of salt loading. At present it remains unclear why, despite a high salt diet, induction of AM and its receptor is delayed for 2 weeks or more.

Figure 2. Relative AM mRNA levels measured at indicated times in adrenal glands (A) and kidneys (B) from HS and NS rats. Levels of AM mRNA levels were normalized relative to those of GAPDH mRNA. Data are expressed as mean±SEM of 4 (days 3, 7, and 14) and 8 (day 28) animals. *P<0.05 vs NS group.

Figure 3. Relative levels of mRNA coding for AM receptor components in adrenal glands (A) and kidneys (B) of rats maintained for 28 days on HS or NS diet. Levels of target mRNA levels were normalized relative to those of GAPDH mRNA. Data are expressed as mean±SEM of 8 animals. *P<0.05, **P<0.01 vs NS group.
As mentioned above, we also found that salt loading led to increased transcription of AM and its receptors in the adrenal gland. Previous studies have shown that AM acts through a nitric oxide–dependent mechanism to inhibit potassium-stimulated aldosterone secretion from zona glomerulosa cells of the rat adrenal gland20 and that infusion of AM reduces plasma aldosterone levels in sheep.21 In addition to the local effects of adrenal AM, our finding that the plasma AM levels are negatively correlated with plasma aldosterone levels suggests the elevated plasma AM seen after 28 days of salt loading may also have contributed to the inhibition of aldosterone secretion.

The receptor subtype(s) that may have mediated the effect of AM on aldosterone secretion remains uncertain; however, both AM(22–52) and CGRP(8–37) have been shown to concentration-dependently reverse AM-induced inhibition of potassium-stimulated aldosterone secretion.20 This suggests that AM acts not only through CRLR/RAMP2 or 3 but also through CRLR/RAMP1. In view of the finding that levels of both RAMP1 and RAMP2 mRNA were increased 3-fold in the adrenal glands of HS rats, we suggest that both RAMP1/CRLR and RAMP2/CRLR mediate the adrenal effects of AM. Further analysis of the differential regulation of RAMPs in kidney and adrenal gland should shed additional light on this question as well as the respective roles of the three RAMP isoforms in salt loading.

Perspectives

Although a number of genetic and behavioral factors are known to be involved in the development of salt-sensitive hypertension, it is generally accepted that the retention of sodium, fluid, or both is a key feature. The increased blood volume that results from fluid retention leads to increases in cardiac output and contributes to increased peripheral resistance.22–24 Thus, any factor that counteracts these effects would serve to attenuate the rise in blood pressure that would otherwise occur. The findings of the present study suggest that AM contributes to the renal adaptation necessary to stabilize blood pressure and electrolyte balance in the face of high salt intake. To further clarify the role of AM, we now ask the question: Does depletion of AM, or a blunted or absent response to AM, lead to the development of salt-sensitive hypertension? To begin to address this question, we are currently characterizing the effects of a neutralizing anti-AM antibody.

References

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