Administration of adrenomedullin into subfornical organ inhibits Na⁺,K⁺-ATPase activity in single proximal renal tubule of rats

GAO Yuan*, LI Xing-Qi**
Department of Physiology, Zunyi Medical College Zhuhai Campus, Zhuhai 519041, China

Abstract: The present study was designed to investigate the effect of administration of adrenomedullin (ADM) into subfornical organ (SFO) on renal tubular Na⁺,K⁺-ATPase activity in rats. Rats under anesthesia were injected with ADM 0.1 μL (20 ng/μL) via an implanted cannula into SFO (n=6). Plasma ADM and serum endogenous digitalis-like factor (EDLF) levels were assayed with radioimmunoassay, and urine samples were collected via a cannula intubated in bladder. Urinary sodium concentration was assayed with flame spectrophotometry. Single proximal renal tubule segments were obtained by hand under stereomicroscope and its Na⁺,K⁺-ATPase activity was measured by liquid scintillation counting. In addition, single proximal renal tubule segments from normal rats (n=6) were incubated with serum from animals administered with ADM into SFO, and then the Na⁺,K⁺-ATPase activity was determined. The results showed that both urinary volume and sodium excretion amounted to the peak value at 30 min after ADM administration, and sustained a significant high level at 60 min (P<0.01). At 30 min after ADM administration, there was a significant increase in serum EDLF and a decrease in Na⁺,K⁺-ATPase activity of proximal tubule (P<0.01, respectively), but not in plasma ADM level. Na⁺,K⁺-ATPase activity was decreased significantly in single proximal renal tubule segments from normal rats incubated with serum from rats administered with ADM into SFO (P<0.01). These results suggest that the diuretic and natriuretic responses following administration of ADM into SFO are associated with the inhibition of renal tubule Na⁺,K⁺-ATPase activity. The inhibition of renal tubule Na⁺,K⁺-ATPase activity is related to the increase in the serum level of EDLF.

Key words: adrenomedullin; subfornical organ; Na⁺,K⁺-ATPase; proximal tubule; rat
Adrenomedullin (ADM), a peptide of 50 (in rat) or 52 (in human) amino acid residues with structural homology to calcitonin gene-related peptide (CGRP), was isolated originally from human pheochromocytoma by Kitamura and his colleagues in 1990s[1]. The peptide is produced in multiple tissues, most notably in the endothelial and smooth muscle components of the vasculature, adrenal medulla, anterior pituitary gland, and central nervous system[2-3]. Now it is well established that ADM functions as a circulating hormone and local paracrine mediator with multiple biological activities[4]. ADM exerts multiple physiological effects on a variety of tissues[5], and many of its actions are related to the homeostatic control of fluid and electrolyte, acting both peripherally and centrally[6]. In the central nervous system, intracerebroventricular administration of ADM in rats not only inhibited angiotensin II-induced drinking[6] and salt appetite[7] behavior, but also induced a diuretic and natriuretic response[8]. The exact central site, however, where ADM acts to initiate the effects, is not yet identified. In addition, the exact physiologic mechanism of diuretic and natriuretic action induced by administration of ADM in the central nervous system is still unclear.

Based on the results of in situ hybridization and Northern blot analysis, it has been shown that there is a considerable distribution of preproadrenomedullin mRNA[9] and the receptor-activity modifying protein (RAMP) mRNA[10] in the sub fornical organ (SFO), which is one of the circumventricular organs and plays an important role in maintaining fluid and electrolyte homeostasis[11]. SFO, considered as an important site for ADM action, is worth an investigation.

There are multiple mechanisms and factors to control the process of substance transport across renal tubule cells. As one of them, the Na⁺,K⁺-ATPase located on the basolateral membrane of renal tubule cells plays an important role in the majority of electrolyte transport across cell membrane[12]. It is of significance to investigate the effect of administration of ADM into SFO on renal tubule Na⁺,K⁺-ATPase activity and the level of serum endogenous digitalis-like factor (EDLF), an endogenous Na⁺,K⁺-ATPase activity inhibitor[13].

Therefore, the present study was designed to investigate the effect of administration of ADM into SFO on renal tubule Na⁺,K⁺-ATPase activity and to find the involved pathway and the consequence of such treatment in rats.

1 MATERIALS AND METHODS

1.1 Animals
Male Wistar rats (Grade II) weighing (210±10) g (provided by Experimental Animal Center of Guangdong Province, China) were housed in 12-hour light/12-hour dark cycle at 23 °C, and received standard laboratory chow with free access to water. After arrival, the rats were allowed to accustom to the new environment for one week.

1.2 Agents and solutions
ADM (adrenomedullin-1-50), Na₂ATP, EGTA, ouabain, HEPES, collagenase II and bovine serum albumin were all purchased from Sigma Co. [γ-32P]-ATP (activity>3 000 Ci/mmol, 50 μCi/μL) was from Beijing Fureit Bioengineering Co. (China). EDLF radioimmunoassay (RIA) kit was from Beijing North Institute of Biological Technology (China). ADM1-50 RIA Kit was from Beijing Sino-UK Institute of Biological Technology (China). Artificial cerebrospinal fluid (aCSF) contained (in mmol/L): NaCl 124, KCl 5, NaH₂PO₃ 3, CaCl₂ 3, MgSO₄ 2, NaHCO₃ 23, glucose 10, pH 7.4. ADM solution (20 ng/μL) was made in aCSF containing ADM1-50. All other reagents were from commercial sources. Preparations of renal perfusion solution, isolation solution A and B, as well as liquid scintillation solution had been described previously in detail[15].

1.3 Surgical procedure
After being anaesthetized with sodium pentobarbital, the rats were placed in a stereotaxic frame (Model 1C, Jiangwan, China) in a prone position with their head positioned vertically. A stainless steel guide cannula (500 μm outer diameter; 10 mm length) with an internal cannula was implanted into SFO (stereotaxic coordinates: 0.85–0.90 mm posterior to bregma, 0 mm lateral to midline, 4.85–5.35 mm below the surface of skull) according to the atlas of Paxinos and Watson[14].

1.4 Verification of injection position
At the end of the experiment, deeply anestheticized rats were injected with 2% eosin through the implanted cannula. The brain was removed and fixed in 4% paraformaldehyde overnight at 4 °C. The next day, coronal 50 μm-thick sections were cut through the brain on a vibratome and mounted on gelatinized slides. The sections were then stained with 2% cresyl violet to determine the injection site under microscope. Only the data from rats properly injected into the SFO, as shown in Fig. 1, were used for statistical analysis.

1.5 SFO microinjection and samples of urine, blood and renal tubules
The rats both in ADM group and control group were divided averagely at random into two parts respectively. In the first part, animal SFO was injected within 10 s with ADM solution 0.1 μL (20 ng/μL, n=6) or aCSF 0.1 μL (n=6), and urine samples were collected at 30 min interval via a cannula intubated in bladder for the measurement of urinary volume and urinary sodium concentration. In the
secondary part, 30 min after SFO administration with ADM solution 0.1 μL (n=6) or aCSF 0.1 μL (n=6), the blood samples were obtained for preparation of plasma and serum, and the kidneys were extirpated under anesthesia for preparation of single proximal tubule segments. In additional normal rats (n=6), kidneys were removed under anesthesia, and single proximal tubular segments were obtained for the incubation with the serum from animals in ADM or control group.

1.6 Isolation of single proximal tubules and determination of Na⁺,K⁺-ATPase activity

Procedure in the isolation of single proximal tubules and the determination of its Na⁺, K⁺-ATPase activity in rat had been described in detail previously[15,16], and was presented briefly here. The single proximal tubule segments were isolated by hand under stereomicroscope (XTJ-5400, Wuzhou Photics Instrument Factory, China) from collagenase II-treated renal cortical tissue. Some proximal tubule segments were identified morphologically under electron microscopy (CM-10, Philips Co.). The length of every single tubule segment obtained was measured using Digital Color Camera (HV2818, Unica Co.) under stereomicroscope. The images of isolated single proximal tubule under stereomicroscope and electron microscope had been shown in the paper published previously[19].

The single tubule segments were treated with hypoosmotic solution and with freeze-thaw successively before incubation with [γ-32P]-ATP. 32Pi hydrolyzed from [γ-32P]-ATP by total ATPase (both Na⁺,K⁺-ATPase and Mg²⁺-dependent ATPase) and by Mg²⁺-dependent ATPase in proximal tubules was assayed, respectively, with liquid scintillation counter (SN-6930A, Shanghai Hesuo Rihuan Photoelectric Instrument Co., Ltd., China). The levels of plasma ADM and serum EDLF were determined with SN-6100 Automatic RIA Gamma Counter (Shanghai Hesuo Rihuan Photoelectric Instrument Co., Ltd., China).

1.8 Statistical analysis

Data were presented as mean±SD. Statistical significance was assessed by Student’s t test and the statistical difference level was set at 0.05.

2 RESULTS

2.1 Changes in urinary volume and sodium excretion after administration of ADM into SFO

The changes in urinary volume and sodium excretion after administration of ADM into SFO were shown in Fig. 2. As compared with control group, urinary volume increased from (8.78±1.42) μL/min before ADM administration to peak value [(15.58±1.62) μL/min, P<0.01] at 30 min after administration, then decreased but sustained a considerable high level [(13.16±1.38) μL/min, P<0.01] at 60 min, and finally decreased near the value before injection at 90 min after administration [(8.54±1.43) μL/min] (P>0.05) (Fig. 2A).

Sodium excretion changed in the same tendency. Sodium excretion amounted to peak [(2.34±0.21) μmol/min, P<0.01] from (1.33±0.17) μmol/min (before administration) at 30 min after ADM administration, maintained at a high level (1.97±0.19 μmol/min, P<0.01) in 60 min, and then returned back to the level before administration [(1.42±0.16) μmol/min, P>0.05] at 90 min after administration (Fig. 2B).

2.2 Inhibition of Na⁺,K⁺-ATPase activity in single proximal tubule after administration of ADM into SFO

As shown in Fig. 3A, the level of Na⁺, K⁺-ATPase activity in single proximal tubule at 30 min after administration of ADM into SFO [(1 136±154) pmol/min per hour] was significant lower than that in control group [(1 950±188) pmol/min per hour, P<0.01].

2.3 Rising in level of serum EDLF but not in that of plasma ADM after administration of ADM into SFO

At 30 min after administration of ADM into SFO, there was a significant difference in level of serum EDLF between the
animals administered with ADM into SFO ([72.12±5.16] pg/mL) and those with aCSF ([41.78±4.13] pg/mL, P<0.01) as shown in Fig. 4A, but there was no significant difference in plasma ADM level between the animals administered with ADM into SFO ([38.13±10.12] pg/mL) and those with aCSF ([37.85±9.31] pg/mL, P>0.05), as shown in Fig. 4B.

2.4 Inhibition of Na⁺,K⁺-ATPase activity in normal single proximal tubule incubated with serum from rats administered with ADM into SFO

As shown in Fig. 3B, the level of Na⁺,K⁺-ATPase activity [(1 267±149) pmol/mm per hour] in normal single proximal tubular segments incubated with serum from rats administered with ADM into SFO for 30 min, was significantly lower than that incubated with serum from the animals SFO treated with aCSF [(1 972±179) pmol/mm per hour, P<0.01].

3 DISCUSSION

Israel et al. reported that ADM administered intracerebroventricularly in conscious rats had induced a dose-related diuretic and natriuretic response[8]. The exact acting site by ADM in central nervous system is still unclear. There are many types of preproadrenomedullin mRNA[9] and corresponding RAMP mRNA[10] in SFO, and SFO is an important center integrating fluid and electrolyte homeostasis[11]. It is, therefore, essential for understanding the effect of ADM into SFO on renal response. In the present study, both urinary volume and sodium excretion got to the peak value at 30 min after SFO administration of ADM, and both the diuretic and natriuretic responses were lasting for 60 min. The results strongly suggest that SFO may be, at least, one of the acting sites where ADM acts to initiate diuretic and natriuretic response.

Excretion process of water and sodium via kidneys is controlled by multiple mechanisms and factors. Most remarkably, Na⁺,K⁺-ATPase, or sodium pump, located in basolateral membrane of renal tubule epithelia, is the major determinant factor for cytoplasmic Na⁺. Therefore, it plays an important role in regulating cell volume, cytoplasmic pH and Ca²⁺ levels through the Na⁺/H⁺ and Na⁺/Ca²⁺ exchangers, respectively, and in driving a variety of secondary transport processes such as Na⁺-dependent glucose and amino acid transport[17]. Renal tubule Na⁺,K⁺-ATPase activity in response to SFO administration of ADM, accordingly, is worthy of an investigation. The results of the present study showed that, at 30 min after SFO administration of ADM, diuretic and natriuretic response was accompanied by a significant inhibition of Na⁺,K⁺-ATPase activity in single proximal tubule. It is supposed that the
actions of diuresis and natriuresis in response to SFO administration of ADM are associated, at least in part, with the inhibition of Na⁺,K⁺-ATPase activity in renal tubule.

So far little is known about the pathways via which Na⁺,K⁺-ATPase activity in renal tubule is inhibited following administration of ADM into SFO. It had been believed that ADM was a potent natriuretic agent which acted peripherally not only via increasing renal blood flow[18] but also via direct tubular actions[19]. There was not, however, a significant change of peripheral circular ADM level in rats administered with ADM into SFO in the present study. It seems that the inhibition of Na⁺,K⁺-ATPase activity in renal tubule and diuretic and natriuretic responses may result in the actions of ADM on SFO, but not in the peripheral actions of ADM.

EDLF, an endogenous Na⁺,K⁺-ATPase inhibitor, existing in blood and urine, as well as in various tissues, in particular in the hypothalamus is believed to facilitate natriuresis through inhibition of the Na⁺,K⁺-ATPase in renal tubule basolateral membranes[10,20]. The role of EDLF in the case of administration of ADM into SFO on Na⁺,K⁺-ATPase activity in renal tubule, therefore, needs investigation. According to the results of the present investigation, there was not only a marked inhibition of Na⁺,K⁺-ATPase activity in proximal tubule, but also a significant rising of serum EDLF level. In addition, It was observed that Na⁺,K⁺-ATPase activity in single proximal tubular segments from normal rats incubated with serum from additional rats, which had been administered with ADM into SFO for 30 min, decreased significantly. It appears that action of ADM on SFO may result in inhibition of renal tubule Na⁺,K⁺-ATPase activity. The diuretic and natriuretic responses following administration of ADM into SFO are associated with the inhibition of renal tubule Na⁺,K⁺-ATPase activity. There is a relationship between the inhibition of renal tubule Na⁺,K⁺-ATPase activity and the increase in serum level of EDLF. Exact conclusion is not confirmed until multiple investigations in detail are performed, although the initial results in the paper provide some valuable evidence.

REFERENCES