Neuroscience Research 108 (2016) 60–66

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Alteration of amiloride-sensitive salt taste nerve responses in aldosterone/NaCl-induced hypertensive rats

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ARTICLE INFO

Article history:
Received 28 September 2015
Received in revised form 21 January 2016
Accepted 21 January 2016
Available online 29 January 2016

Keywords:
ENaC
RAAS
Salt-sensitive hypertension
Chorda tympani nerve response

ABSTRACT

Salt taste sensitivity is related to physiological condition, and declined in hypertensive patients. However, little is known about the mechanism underlying changes in salt taste sensitivity during the development of hypertension. This is largely due to lack of an appropriate animal model which shows the decline of salt taste sensitivity caused by hypertension. Previous studies have suggested that one of main causes of salt-sensitive hypertension is dysfunction of the renin–angiotensin–aldosterone system (RAAS). To examine the involvement of RAAS in modulation of salt taste sensitivity, we utilized aldosterone/NaCl-treated rats as a well-established model of salt-sensitive hypertension caused by RAAS dysfunction. Amount of sodium intake in aldosterone/NaCl-treated rats was higher than that in control rats. In addition to behavioral changes, the amiloride-sensitive salt taste nerve responses in aldosterone/NaCl-treated rats were remarkably lower by approximately 90% than those in the other groups. Moreover, αENaC mRNA expression in the epithelium of circumvallate papillae was significantly low in aldosterone/NaCl-treated rats. Thus, RAAS modulates salt taste system as is case in hypertensive patients. This report is to our knowledge the first to describe an animal model with decline of amiloride-sensitive salt taste nerve responses by RAAS dysfunction-mediated salt-sensitive hypertension.

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1. Introduction

Taste plays a critical role in detecting the nutrition of foods and preventing the ingestion of toxic substances. Mammalian perceives five basic tastes: sweet, sour, salty, bitter and umami. In the tongue, these tastants are mediated by separate classes of taste receptor cells (TRCs) in the taste buds. TRCs are innervated by taste nerves, and finely tuned to a single taste quality (Chandrashekar et al., 2006). Especially, salt taste ensures the proper dietary electrolyte balance, and perceived by two salt sensing pathways in the taste buds. One is mediated by amiloride-sensitive epithelial sodium channel (ENaC) that is selective for the sodium salt, and the other is amiloride-insensitive pathway to high concentrations of non-selective salt (Chandrashekar et al., 2010; Oka et al., 2013).

Salt taste is tightly related to physiological conditions in human. Amount of salt intake is correlated with increase of blood pressure (Simons-Morton and Obarzanek, 1997; Caudarella et al., 2009; He and MacGregor, 2010; Piovesana et al., 2012). Salt taste recognition threshold is elevated in hypertensive patients and those with chronic kidney disease (Fallis et al., 1962; Barylko-Pikielna et al., 1985; Kusaba et al., 2009). Smilarly, animal studies previously have shown that salt intake and preference are increased in rats with genetically and experimentally-induced hypertension (Ben-Ishay et al., 1976; Karen et al., 1977; Dean and Ingham, 1978; Formaker and Hill, 1990). In the case of experimentally-induced hypertension, the treatment of NaCl and deoxycorticosterone (DOCA) which is one of the mineralocorticoids induce hypertension, and enhance salt taste preference and intake. However, little is known about the mechanism of changes in salt taste sensitivity during the development of hypertension, because an animal model which shows the decline of salt taste sensitivity caused by hypertension has not been well established.

The renin–angiotensin–aldosterone system (RAAS) is a vasopressor hormone system that regulates blood pressure and water balance (Fujita, 2008). Renin, chymase and angiotensin I converting enzyme catalyze step in the production of angiotensin II (Ang II), which is a regulator of aldosterone (Aldo) production in the adrenal glands (Takai et al., 2010). Aldo which is one of the endogenous mineralocorticoid elevates sodium re-absorption in the distal nephron

Abbreviations: ENaC, epithelial sodium channel; RAAS, renin–angiotensin–aldosterone system; Aldo, aldosterone; Angiotensin II, Ang II; TRCs, taste receptor cells; CT, chorda tympani; SBP, systolic blood pressure.

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http://dx.doi.org/10.1016/j.neures.2016.01.007
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and increases blood pressure. In regard to association of RAAS with development of hypertension, RAAS dysfunction is thought to be one cause of salt-sensitive hypertension (Fujita, 2008; Gekle and Grossman, 2009; Tomaschitz et al., 2010). Evidently, the long-term Aldo and NaCl treatment induces salt-sensitive hypertension caused by RAAS dysfunction in rats (Nishiyama et al., 2004).

In addition, vasopressor hormones have been suggested to modulate salt taste sensitivity. Single systemic administration of Aldo elevates the amiloride-sensitive nerve responses to NaCl by upregulating the apical localization of ENaC in the taste buds (Lin et al., 1999). Conversely, single Ang II administration attenuates the amiloride-sensitive taste nerve responses to NaCl (Shigemura et al., 2013). Although these findings suggest that acute effect of Aldo or Ang II treatment which is not enough to induce hypertension is to modulate salt taste sensitivity, there is no knowledge of the long-term effect of vasopressor hormones treatment on salt taste sensitivity. Because hypertensive patients showed low sensitivity to salt tastant (Fallis et al., 1962; Barylko-Pikielna et al., 1985), we hypothesized that the long-term RAAS dysfunction caused not only salt-sensitive hypertension, but also decline of salt taste sensitivity. In this study, we examined the effect of the long-term RAAS dysfunction on the salt taste system using Aldo/NaCl-treated rats as a well-established animal model of salt-sensitive hypertension.

2. Material and methods

2.1. Animal preparation and procedures

All experimental procedures were performed in accordance with the guidelines for the care and use of animals established by the Kao Corporation. Four-week-old male Sprague-Dawley rats (SLC), weighing 130–150 g at the beginning of the experiments, were randomly grouped into 3 groups according to the type of treatment by weight and blood pressure. Distilled water was given to group 1, and a 1% NaCl solution (w/v) was given to groups 2 and 3 as drinking water. Aldo was encapsulated into an osmotic minipump (model 2006; Alza Co.) designed to deliver a dose of 0.75 μg/h, and the pump was implanted subcutaneously under isoflurane anesthesia (Dainippon Sumitomo Pharma Co.) at the dorsum of the neck in group 3. The vehicle (9% ethanol/87% propylene glycol/4% H_2O) was administered to groups 1 and 2. The dose of Aldo, 18 μg/day per rat, was determined on the basis of results from previous studies in rats (Rocha et al., 1999; Blasi et al., 2003). These treatments were continued for 5 weeks. Rats were maintained under 12 h light/12 h dark conditions and fed CE-2 chow (including 0.3% NaCl, Clea Company) *ad libitum*.

Systolic blood pressure (SBP) was measured in conscious rats by tail-cuff plethysmography (BP-98A; Softron Co.) once a week. To calculate sodium intake, food and fluid intake were measured for 24 h, and urine samples for 24 h were collected for measuring urinary sodium excretion, Aldo and creatinine in a metabolic chamber 4 weeks after the beginning of NaCl- and/or Aldo treatment. The chorda tympani (CT) response was measured, and plasma, both kidneys, heart and epithelium of circumvallate/non-gustatory papillae samples were harvested at week 5. Both kidneys and heart were weighted, and weight of both kidneys was averaged. For RNA extraction, the left kidney was immersed in RNAlater (QIAGEN) and stored at −20 °C. Urinary sodium ion level was measured using Compact Sodium Ion Meter (B-722 LAQUAtwin, HORIBA). Urine (at week4) and plasma (at week 5) creatinine and urine Aldo (at week 4) were quantified by using a QuantiChrom Creatinine Assay Kit (Bioassay systems) and Aldo EIA Kit (Cayman), respectively. Creatinine clearance was calculated as urine creatinine excretion at week 4 divided by plasma creatinine at week 5 (Kierulf-Lassen et al., 2015).

2.2. Isolation of epithelium of circumvallate and non-gustatory papillae from the tongue

Isolation of epithelium of the tongue was described previously in detail (Ozdener et al., 2006). The tongue was dissected proximal to circumvallate papillae and immediately placed into an isolation solution [26 mM $NaHCO_3$, 2.5 mM NaH_2PO_4, 20 mM glucose, 65 mM NaCl, 20 mM KCl, and 1 mM ethylenediaminetetraacetic acid (EDTA)] for 5–10 min on ice. The preparation was then removed from ice and approximately 1 ml of the isolation buffer mixed with 1.5 mg/ml pronase E (Sigma) and 1 mg/ml elastase (Sigma) was uniformly injected with a 25-gauge Norm-Ject syringe under and around the epithelium of circumvallate papillae of dissected tongue. After 20 min of incubation in isolation buffer at room temperature, the epithelium was gently peeled from the underlying muscle layer, and cut the epithelium of circumvallate papillae under a dissecting microscope (Leica S6 E). Lingual epithelium was also collected as non-gustatory papillae control tissues. Isolated tissues were put into RNlater (QIAGEN) and stored −20 °C for gene expression analysis.

2.3. Analysis of the amounts of ENaC subunit mRNAs

Total RNA was isolated by using RNeasy Mini Kit (QIAGEN). Concentration and purity were assessed with NanoDrop-1000 (Peqlab). RNA was reverse-transcribed and amplified by using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The amount of ENaC mRNA in the kidney was quantitatively analyzed by using TaqMan gene expression assays, TaqMan Master Mix, and the 7500 Fast Real-time PCR system (Applied Biosystems). The TaqMan gene expression assays Rn00580652_m1 (αENaC), Rn00561892_m1 (βENaC), Rn00566891_m1 (γENaC), and Rn01775763_g1 (Glyceraldehyde-3-phosphate dehydrogenase, GAPDH) were utilized. Data are shown as the ratios to the values in rats treated with vehicle/water after normalization to the amount of GAPDH mRNA in individual samples (Chabrashvili et al., 2002; Nishiyama et al., 2004).

2.4. Chorda tympani (CT) nerve recordings

Chorda tympani (CT) nerve responses were measured as described previously (Osada et al., 2003). CT nerves carry taste information from the anterior tongue to the brain. In preparation for recording from the CT nerve, each rat was deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg/kg body, Dainippon Sumitomo Pharm.) and urethane (150 mg/kg body weight, Sigma), and the trachea was cannulated to facilitate breathing. Supplemental doses of anesthetic agents were administered when the rat responded to foot pad pinching. Body temperature was maintained with the aid of thermal pads. Access to the nerve was obtained *via* a lateral approach to the junction of the CT nerve. The nerve was exposed and cut, and the neural activity of the whole nerve was recorded with a handmade-platinum/iridium electrode. The activity was amplified differentially against a neutral electrode attached to an exposed muscle, passed through an integrator, and displayed with a pen recorder (Parmacia). In each test, 4 ml of stimulating solution maintained at 28 °C was applied to the anterior tongue through a supply tube over a 5-s period, and a nerve recording was measured for 60 s. The tongue was rinsed with deionized water after stimulation for 2 min, and 2 min was allowed to elapse between stimulations. The CT nerve responses to 150 mM NaCl with or without 100 μM amiloride-HCl (Sigma) were measured. In addition, 0.3 M NH_4Cl (Sigma) was applied as an internal control (Shigemura et al., 2013). The order of stimulations was generally 0.3 M NH_4Cl, 150 mM NaCl and 150 mM NaCl with 100 μM amiloride. In some cases, 0.3 M NH_4Cl or 150 mM NaCl was

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