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Autonomic Neural Regulation of Sodium Transporters and Water Channels in Rat Submandibular Gland

Sun Yeol Ryu, Hyun Jung, Ki Yung Kim, Miwon Kim[1], and JongUn Lee[2]

Department of Oromaxillofacial Surgery, Chonnam National University Dental School, [1]Department of Pediatric Nursing, Chonnam National University School of Nursing, [2]Department of Physiology, Chonnam National University Medical School, Gwangju 501-746, Korea

The present study was undertaken to explore the role of autonomic nerves in the regulation of sodium transporters and water channels in the salivary gland. Rats were denervated of their sympathetic or parasympathetic nerves to the submandibular gland. One week later, the expression of Na,K-ATPase, epithelial sodium channels (ENaC), and aquaporins (AQP) was examined in the denervated and contralateral glands. The sympathetic denervation slightly but significantly decreased the expression of α1 subunit of Na,K-ATPase, whereas the parasympathetic denervation increased it. The expression of α-subunit of ENaC was significantly increased in both the denervated and contralateral glands either by the sympathetic or parasympathetic denervation. The sympathetic denervation significantly increased the expression of AQP5 in both the denervated and contralateral glands, whereas the parasympathetic denervation decreased it. It is suggested that the autonomic nerves have a tonic effect on the regulation of sodium transporters and AQP water channels in the salivary gland.

Key Words: Sympathetic, Parasympathetic, Na,K-ATPase, Epithelial sodium channels, Aquaporin water channels, Submandibular gland

INTRODUCTION

The salivary secretion is a two-stage process: generation of isotonic plasma-like fluid by the acini and modification of the primary saliva as it flows through the ductal system. The acinar epithelial cells have high water permeability, while the ductal epithelial cells reabsorb sodium and chloride without any significant reabsorption of water (Young & Van Lennep, 1979). The emergent saliva is consequently hypotonic, particularly at low secretory rates.

Na,K-ATPase is distributed along the basolateral membrane of acini and striated and excretory ducts (Speight & Chisholm, 1984; Peagler & Redman, 1999). Epithelial sodium channels (ENaC) are expressed in the striated duct of serous acini, constituting the rate-limiting step for sodium reabsorption (Canessa et al, 1994; Duc et al, 1994). The increased output of sodium and chloride in the secondary saliva after denervation indicates a neural control over reabsorption of electrolytes in the glandular duct (Levin & Khaikina, 1987).

Aquaporin (AQP) water channels have been identified in the mammalian salivary gland (Koyama et al, 1996; Gresz et al, 2001; Hoque et al, 2002). The secretion by the acinar cells involves osmotic coupling of water flow to active electrolyte transport. The presence of AQP would help compensate for the very small area of the luminal membrane compared with the basolateral membrane (Poulsen & Bundgaard, 2002). In rat parotid glands, the apical trafficking of AQP5 has been shown to be mediated through adrenoceptors and muscarinic receptors (Ishikawa et al, 1998; Ishikawa et al, 1999). We have recently demonstrated a tonic excitatory effect of the sympathetic nerves on the expression of AQP channels in the kidney (Lee et al, 2006). However, the role of autonomic nerves in the regulation of sodium transporters and AQP channels in the salivary gland has not been determined.

The present study was undertaken to explore the role of sympathetic and parasympathetic nerves in the regulation of sodium transporters and water channels in the salivary gland. Rats were denervated of their sympathetic and parasympathetic nerves to the submandibular gland, and the expression of sodium transporters and water channels was determined in the denervated and contralateral glands.

METHODS

Sympathetic and parasympathetic denervation

Male Sprague-Dawley rats, weighing about 250 g, were used. The whole experimental procedure conformed to the Institutional Guidelines for Experimental Animal Care and

Corresponding to: JongUn Lee, Department of Physiology, Chonnam National University Medical School, 5 Hak-dong, Gwangju 501-746, Korea. (Tel) 82-62-220-4262, (Fax) 82-62-232-1242, (E-mail) julee@jnu.ac.kr

ABBREVIATIONS: ENaC, epithelial sodium channels; AQP, aquaporins.

Use. Under anesthesia with ketamine (50 mg/kg, i.p.), the sympathetic denervation was made by extirpation of the right superior cervical ganglion. The parasympathetic denervation was performed by severing the right chordalingual nerve at the level where the chorda tympani nerve merges to the lingual nerve. Control rats were treated the same way, except that their nerves were left untouched. One week after the surgery, the denervated and contralateral submandibular glands were taken under anesthesia with ketamine.

Chemical sympathectomy and parasympathectomy

Chemical sympathectomy was carried out by injection of 6-hydroxydopamine (1 mg/kg) through the tail vein. The right and left submandibular glands were taken 3 days after the injection. Chemical parasympathectomy was achieved by daily subcutaneous injection of atropine (10 mg/kg/day) for 7 days, and the right and left submandibular glands were taken.

Protein preparation and Western blot analysis

The excised glands were rapidly frozen and kept at −70°C until assayed. They were homogenized at 3,000 rpm in a solution containing 250 mM sucrose, 1 mM ethylenediaminetetraacetate, 0.1 mM phenylmethylsulfonyl fluoride and 10 mM Tris-HCl buffer at pH 7.6. Large tissue debris and nuclear fragments were removed by low speed centrifugation (1,000×g, 10 min). Protein samples were loaded and electrophoretically size-separated with a discontinuous electrophoresis system consisting of 12.5% polyacrylamide resolving gel and 5% polyacrylamide stacking gel. An equal loading of proteins was assessed by staining the initial gel with Coomassie blue. They were then electrophoretically transferred overnight to a nitrocellulose membrane at 20 V. The membrane was washed in Tris-based saline buffer (pH 7.4) containing 0.1% Tween-20 (TBST) and blocked for 1 h with 5% nonfat milk in TBST. It was then incubated with antibodies for 1 h at room temperature.

The α-subunit predominates over β-subunit of Na,K-ATPase in rat salivary glands (Collins et al, 1987; Graves et al, 1989). Therefore, we used monoclonal mouse anti-α1 subunit of Na,K-ATPase (1 : 2,500, Upstate Biotechnology; Lake Placid, NY, USA). Polyclonal rabbit anti α-subunits of ENaC (1 : 500, Alpha Diagnostic; San Antonio, TX, USA), and affinity-purified anti-rabbit polyclonal antibodies against AQP1 (1 : 1,000, Alomone Labs; Jerusalem, Israel) and AQP5 (1 : 1,000, Alpha Diagnostic) were used. The membranes were then incubated for 2 h with a horseradish peroxidase-labeled goat anti-rabbit IgG (1 : 1,200) in 2% nonfat milk in TBST. The bound antibody was detected by enhanced chemiluminescence (Amersham; Little Chalfont, Buckinghamshire, UK) on hyperfilm. The relative protein levels were determined by analyzing the signals of autoradiograms using the transmitter scanning videodensitometer.

Drugs and statistical analysis

Drugs were purchased from Sigma Chemical Company (St. Louis, MO, USA), unless stated otherwise. Results are expressed as mean±SEM. The statistical significance of differences between the groups was determined using unpaired t-test.

RESULTS

Expression of Na,K-ATPase and ENaC

The sympathetic denervation slightly decreased, whereas the parasympathetic denervation increased, the expression of α1 subunit of Na,K-ATPase in the denervated gland (Fig. 1). The expression of α-subunit of ENaC was significantly increased in both the denervated and contralateral glands, following the sympathetic or parasympathetic denervation (Fig. 2).

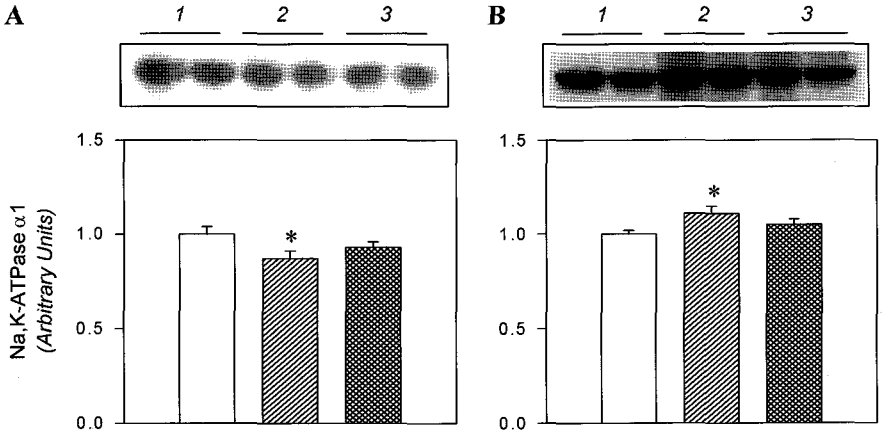

Fig. 1. Expression of α1-subunits of Na,K-ATPase in the denervated and contralateral submandibular glands following the sympathetic (A) and parasympathetic denervation (B). Representative immunoblots and densitometric data are shown. Lanes 1, 2, and 3 represent control, denervated, and contralateral glands, respectively. Symbols are: (□) control; (▨) denervated; and (▩) contralateral. Each column represents mean±SEM of 6 rats. *$p<0.05$, compared with control.

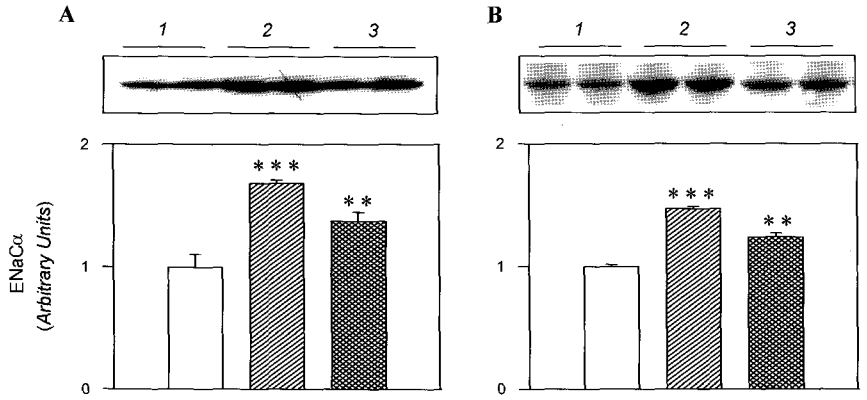

Fig. 2. Expression of α-subunits of ENaC in the denervated and contralateral submandibular glands following the sympathetic (A) and parasympathetic denervation (B). Legends are the same as in Fig. 1. Each column represents mean±SEM of 6 rats. **p<0.01, ***p<0.001; compared with control.

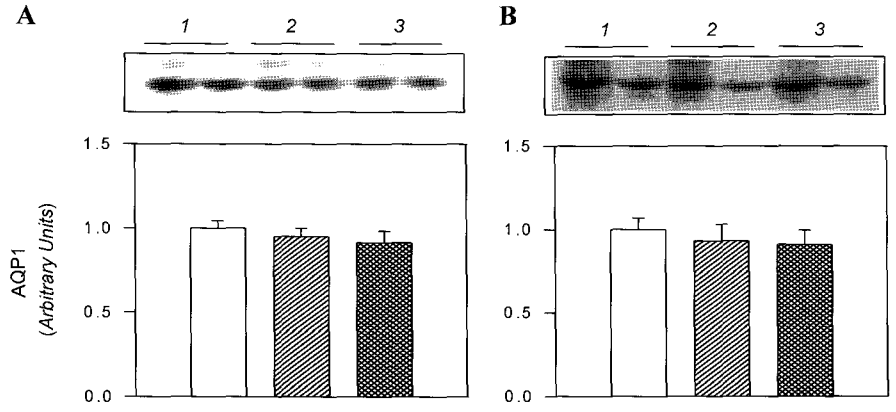

Fig. 3. Expression of AQP1 in the denervated and contralateral submandibular glands following the sympathetic (A) and parasympathetic denervation (B). Legends are the same as in Fig. 1. Each column represents mean±SEM of 6 rats.

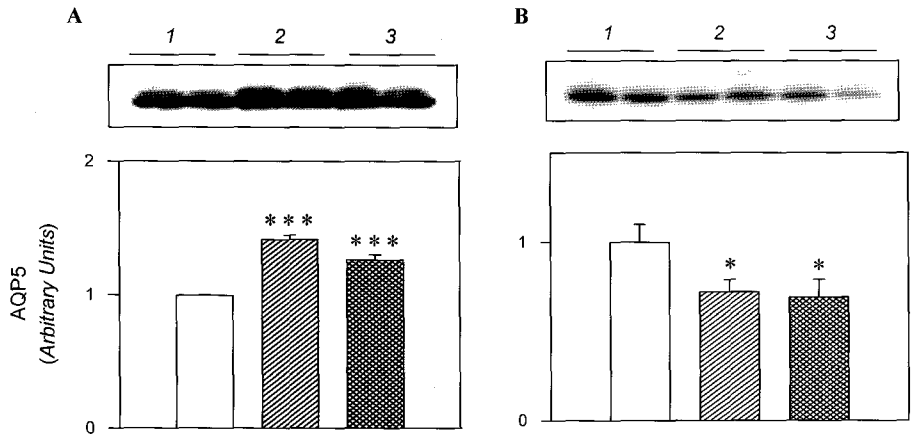

Fig. 4. Expression of AQP5 in the denervated and contralateral submandibular glands following the sympathetic (A) and parasympathetic denervation (B). Legends are the same as in Fig. 1. Each column represents mean±SEM of 6 rats. *p<0.05, ***p<0.001; compared with control.

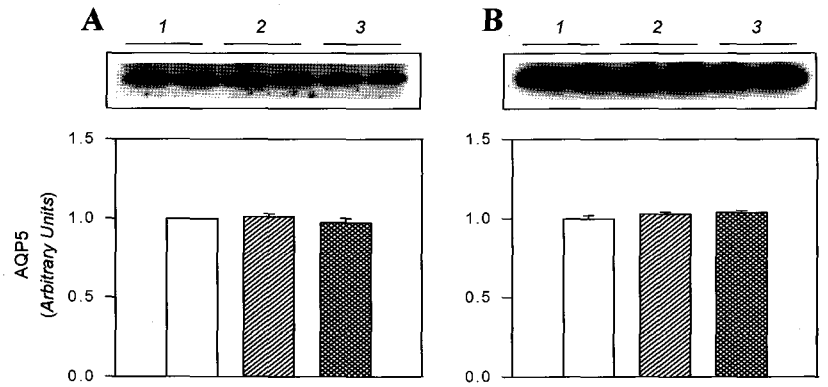

Fig. 5. Expression of AQP5 in the left and right submandibular glands following treatment with 6-hydroxydopamine (A) and atropine (B). Lanes 1, 2, and 3 represent control, left, and right glands, respectively. Symbols are: (□) control; (▨) left; and (▩) right. Each column represents mean±SEM of 4 rats.

Expression of AQP water channels

The sympathetic or parasympathetic denervation did not significantly affect the expression of AQP1 either in the denervated or in the contralateral glands (Fig. 3). The expression of AQP5 was increased by the sympathetic denervation in both the denervated and contralateral glands, whereas it was significantly decreased by the parasympathetic denervation in both glands (Fig. 4). Atropine or 6-hydroxydopamine did not affect the expression of AQP5 (Fig. 5).

DISCUSSION

The immunohistochemistry of Na,K-ATPase revealed its localization in the basolateral membrane of acinar and striated duct cells in the rat salivary gland (Speight & Chisholm, 1984). The present study demonstrated that the sympathetic denervation slightly decreased, whereas the parasympathetic denervation augmented, the expression of Na,K-ATPase in the denervated gland. This finding is partly contradictory to the previous observation which showed that the parasympathetic stimulation promotes a gradual increase of Na,K-ATPase immunoreactivity of submandibular demilune cells, and the sympathetic stimulation causes no detectable change of the cells lining striated and excretory ducts in cats (Winston et al, 1990).

However, it has been shown that the sympathomimetics inhibit potassium release from rat parotid slices in association with an elevated Na,K-ATPase activity (Miyamoto & Ohshika, 1985). An interaction between sympathetic and parasympathetic nerves in the control of salivary secretion has been demonstrated in anaesthetized dogs: the sympathetic stimulation evokes a significant attenuation of vasodilator and secretory responses to the parasympathetic stimulation (McCloskey & Potter, 2000). In the myocardium, a high level of parasympathetic tone alone does not affect the activity of Na,K-ATPase, while muscarinic stimulation can reciprocally modulate Na,K-ATPase activity if sympathetic tone is high (Gao et al, 1997). The sympathetic stimulatory effect may be apparent only when the parasympathetic nerves have been sectioned, and vice versa.

The expression of α-subunits of ENaC was increased following the sympathetic or parasympathetic denervation. This finding suggests that both sympathetic and parasympathetic nerves play a tonic inhibitory role in the regulation of ENaC. Among the inorganic constituents of the saliva, sodium in the primary saliva is resorbed in the ductal system. Therefore, a lack of neural innervation may result in a failure to control over reabsorption of electrolytes in the glandular duct (Levin & Khaikina, 1987). The increased expression of ENaC may stimulate the reabsorption of sodium, hence decreasing the sodium content in the saliva flowing from the denervated gland. Conversely, the inhibitory effect of autonomic nerves on the expression of ENaC may be augmented during either sympathetically- or parasympathetically-stimulated salivary secretion, resulting in a reduced reabsorption of sodium in the ductal system.

Concentrations of inorganic constituents may also be related to the activity of AQP channels. Being localized in the capillary endothelium (Li et al, 1994; Akamatsu et al, 2003), AQP1 may be involved in the movement of water between plasma and interstitial fluid during formation of primary saliva. In the present study, however, the expression of AQP1 was not affected following the sympathetic or parasympathetic denervation. The autonomic nerves appear not to significantly contribute to the regulation of AQP1.

On the other hand, AQP5 is localized in the luminal membrane of the serous acinar cells of rat submandibular and parotid glands (He et al, 1997; Nielsen et al, 1997). An altered expression of AQP5 has been shown in Sjϕgren's syndrome (Beroukas et al, 2001; Steinfeld et al, 2001). Furthermore, AQP5-null mice exhibit defective secretion of saliva (Ma et al, 1999; Krane et al, 2001). In the present study, the expression of AQP5 was increased by the sympathetic denervation and decreased by the parasympathetic denervation. It has been known that the sympathetic stimulation induces a relatively low flow of saliva, whereas parasympathetic stimulation induces a considerable flow of saliva (Garrett et al, 1991). Taken together, the sympathetic nerve may play a tonic inhibitory role and the parasympathetic nerve an excitatory role in the formation of primary saliva.

Interestingly, however, chemical sympathectomy or parasympathectomy did not affect the expression of AQP5. Following a unilateral denervation, the imbalance of neural influence between the affected and contralateral glands may activate the antagonistic pair of autonomic nerves through glandulo-glandular reflex. For instance, when the sympathetic nerve is denervated unilaterally, the parasympathetic nerves may be activated in the ipsilateral and contralateral glands. On the contrary, following a chemical sympathectomy, both glands are equally affected, thereby arising no glandulo-glandular reflex.

In conclusion, it is suggested that the autonomic nerves have a tonic effect on the regulation of sodium transporters and AQP water channels in the salivary gland, contributing to the determination of volume and electrolyte composition of the saliva.

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