Sympathetic and parasympathetic regulation of sodium transporters and water channels in rat submandibular gland

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Abstract

The present study was aimed 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 glandular expression of sodium transporters and water channels was determined by Western blot analysis. The expression of either α1 or β1 subunit of Na, K-ATPase was not significantly affected either by the sympathetic or by the parasympathetic denervation. The expression of subunits of epithelial sodium channels was significantly increased both in the denervated and contralateral glands either by the sympathetic or by the parasympathetic denervation. Neither the sympathetic nor the parasympathetic denervation significantly altered the expression of aquaporin-1 (AQP1). Nor was the expression of AQP4 affected significantly by the parasympathetic or the sympathetic denervation. On the contrary, the expression of AQP5 was significantly increased not only by the parasympathetic but also by the sympathetic denervation. These results suggest that sympathetic and parasympathetic nerves have tonic regulatory effects on the regulation of certain sodium transporters and AQP water channels in the salivary gland.

Key words
Sympathetic, Parasympathetic, Sodium transporters, Aquaporin water channels, Submandibular gland

INTRODUCTION

The salivary secretion is a two-stage process, in which the secretory endpieces, or acini, generate an isotonic plasma-like fluid that is then modified as it flows through the ductal system[1]. The acinar epithelial cells have high water permeability, whereas the ductal epithelial cells modify the primary secretion mainly through reabsorption of sodium and chloride without any significant reabsorption of water[2]. The emergent saliva is consequently hypotonic, particularly at low secretory rates.

Among the sodium transporters, Na, K-ATPase is distributed along the basolateralplasma membranes of acini and striated and excretory ducts[3,4]. Epithelial sodium channels (ENaC) are expressed in the striated duct of serous acini[5], constituting the rate-limiting step for sodium reabsorption in epithelial cells that line the duct of the salivary gland[6].

On the other hand, aquaporin (AQP) water channels including AQP1, AQP3, AQP4, AQP5 and AQP8 have been identified in the mammalian salivary gland[7-9]. The fluid secretion by the acinar cells is believed to involve the osmotic coupling of water flow to active electrolyte transport. Assuming that water flows across the membrane during the formation of primary saliva, the presence of AQP would help to compensate for the very small area of the luminal membrane compared with the basolateral membrane[10]. Although a role of autonomic nerves in the regulation of AQP channels has been suggested[11], it has not been examined in the salivary gland.

There is little evidence suggesting that hormones are released into the circulation to cause secretion of saliva

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under normal condition. On the contrary, it has been well-known that the control of secretory activity of the salivary gland is exclusively exerted by nerves, i.e., the parasympathetic contributes secretory, and the sympathetic vasoconstrictor nerve fibers. However, it has been often stated that the salivary glands receive secretory fibers from both divisions of the autonomic nervous system[12]. There is indeed substantial evidence showing that some glands receive sympathetic secretory and motor fibers[12,13]. Sympathetic stimulation induces a relatively low flow of saliva that is rich in protein, whereas parasympathetic stimulation induces a considerable flow of saliva that has low protein content[14].

There are changes in the inorganic constituents of the saliva following the denervation. The calcium concentration of the submandibular gland is increased after surgical removal of a superior cervical ganglion in rats[15]. Sodium and chloride ions are increased in the secretion of the denervated human parotid gland[16]. Nevertheless, the role of the autonomic nerves in regulating the salivary secretion and in modifying the secretion of inorganic constituents following the denervation at the molecular level has not been explored.

The present study was aimed 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 either the sympathetic or the parasympathetic nerves to the submandibular gland, and the expression of sodium transporters and water channels was determined.

MATERIALS AND METHODS

Parasympathetic and sympathetic denervation

Male Sprague-Dawley rats, weighing about 250 g, were used. Under anesthesia with ketamine (50 mg/kg, i.p.), the parasympathetic denervation was performed by severing the right chorda-lingual nerve at the level where the chorda tympani nerve merges to the lingual nerve. The sympathetic denervation was made by extirpation of the right superior cervical ganglion. The control group was treated similarly, while the nerves were left untouched. One week after the surgery, the denervated submandibular gland was taken. The whole experimental procedure conformed to the Institutional Guidelines for Experimental Animal Care and Use.

Chemical sympathectomy and parasympathectomy

Chemical sympathectomy was induced by injection of 6-hydroxydopamine (1 mg/kg) through the tail vein. The rats were used 3 days after the injection. Chemical parasympathectomy was achieved by daily subcutaneous injection of atropine (10 mg/kg/day) for 7 days.

Protein preparation and Western blot analysis

The denervated gland was isolated under anesthesia with ketamine. It was rapidly frozen and kept at 70℃ until assayed. The tissue was dissected and 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 spin (1,000×g, 10 min). Protein samples were loaded and electrophoretically size-separated with a discontinuous system consisting of 12.5% polyacrylamide resolving gel and 5% polyacrylamide stacking gel. They were then electrophoretically transferred to a nitrocellulose membrane at 20 V overnight. 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 for 1 h at room temperature with antibodies. The antibodies used were monoclonal mouse anti-α_1 and β_1 subunits 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), AQP4 (1:750, Alpha Diagnostic) and AQP5 (1:1,000, Alpha Diagnostic). 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

1. Expression of sodium transporters

The sympathetic denervation did not alter the expression of either α_1 or β_1 subunit of Na, K-ATPase in the ipsilateral and contralateral submandibular glands (Fig. 1 & 2). Similarly, the parasympathetic denervation did not

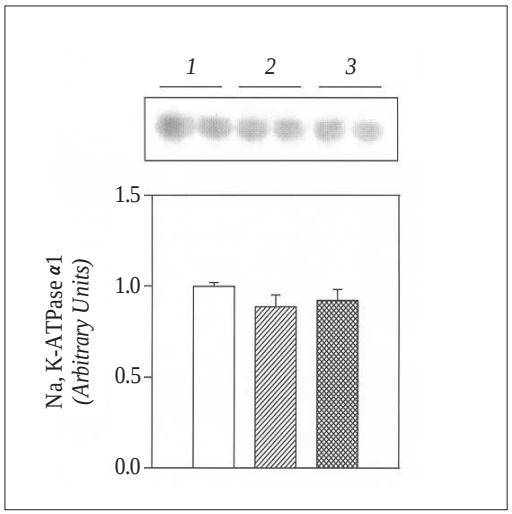

Fig. 1. Expression of α_1 subunits of Na, K-ATPase following the sympathetic-denervation in the submandibular gland. Representative immunoblots and densitometric data are shown. Lanes 1, 2, and 3 represent control, denervated, and contralateral glands, respectively. Symbols are: (□) control, (▨) denervated, and (▩) contralateral. Each column represents mean±SEM of 6 rats.

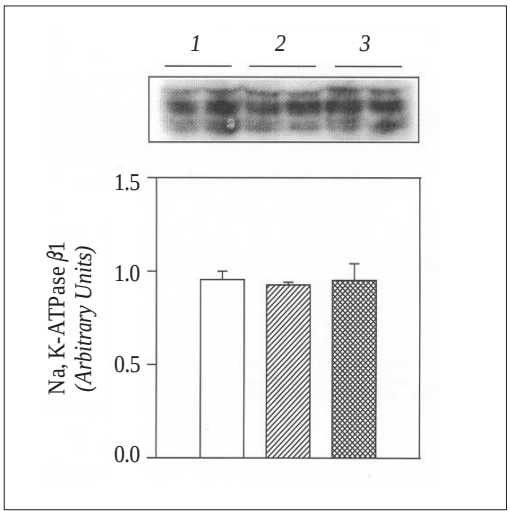

Fig. 2. Expression of β_1 subunits of Na, K-ATPase following the sympathetic-denervation in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats.

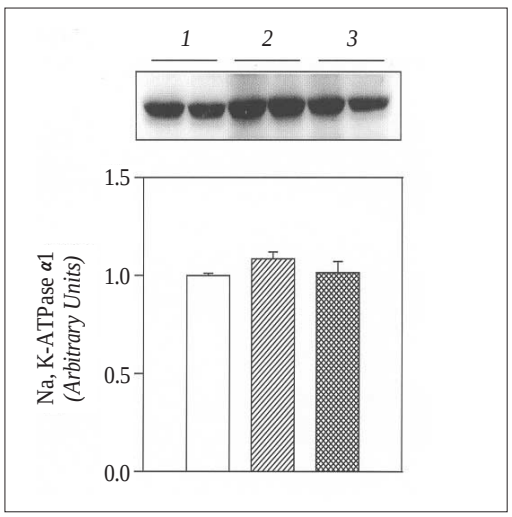

Fig. 3. Expression of α_1 subunits of Na, K-ATPase following the parasympathetic-denervation in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats.

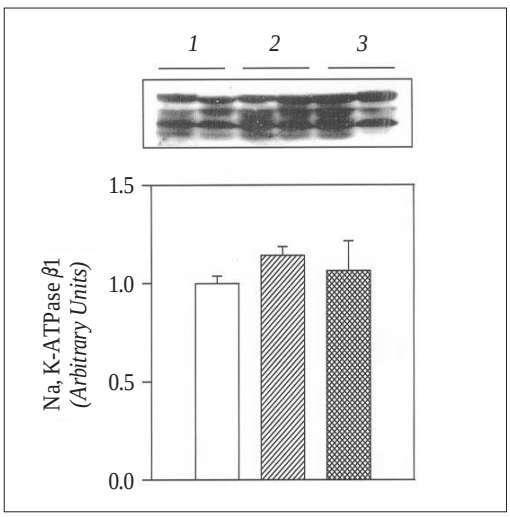

Fig. 4. Expression of β_1 subunits of Na, K-ATPase following the parasympathetic-denervation in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats.

significantly affect that of α_1 and β_1 subunits of Na, K-ATPase in the ipsilateral and contralateral submandibular glands (Fig. 3 & 4). The expression of α-subunits of ENaC was significantly increased in both ipsilateral and contralateral submandibular glands following the sympathetic denervation (Fig. 5). The parasympathetic denervation also increased the expression of α-subunits of ENaC in both ipsilateral and contralateral submandibular glands (Fig. 6).

2. Expression of AQP water channels

Neither sympathetic nor parasympathetic denervation significantly affected the expression of AQP1 in the ipsilateral or contralateral submandibular glands (Fig. 7 & 8). Nor was the expression of AQP4 affected either by

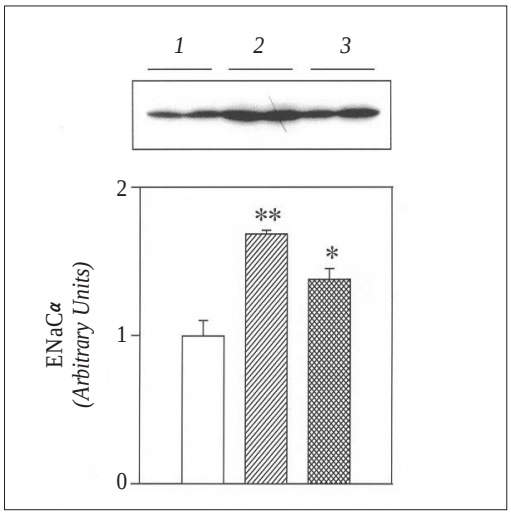

Fig. 5. Expression of α-subunits of ENaC following the sympathetic-denervation in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats. *p<0.05, **p<0.01; compared with control.

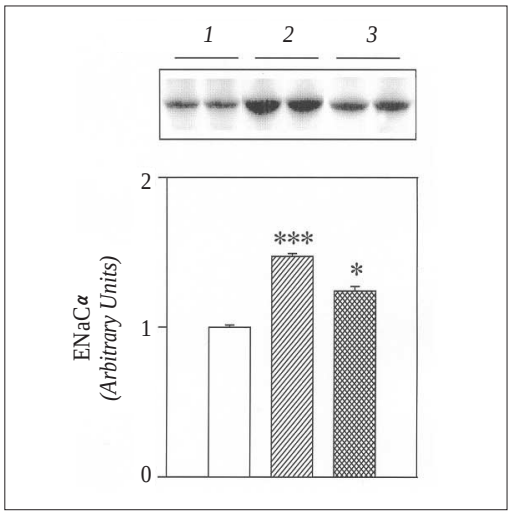

Fig. 6. Expression of α-subunits of ENaC following the parasympathetic-denervation in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats. *p<0.05, ***p<0.001; compared with control.

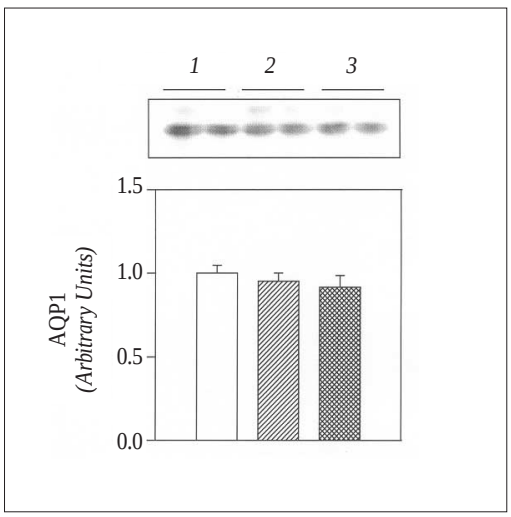

Fig. 7. Expression of AQP1 following the sympathetic-denervation in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats.

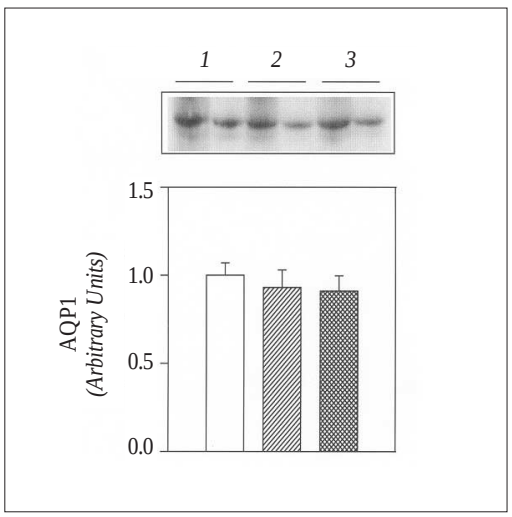

Fig. 8. Expression of AQP1 following the parasympathetic-denervation in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats.

sympathetic or parasympathetic denervation in the denervated or contralateral glands (data not shown). On the contrary, the sympathetic denervation increased the expression of AQP5 both in the denervated and contralateral glands (Fig. 9). The expression of AQP5 was similarly increased both in the denervated and contralateral submandibular glands following the parasympathetic denervation (Fig. 10). However, chemical sympathectomy or parasympathectomy did not significantly alter the expression of AQP5 (Fig. 11 & 12).

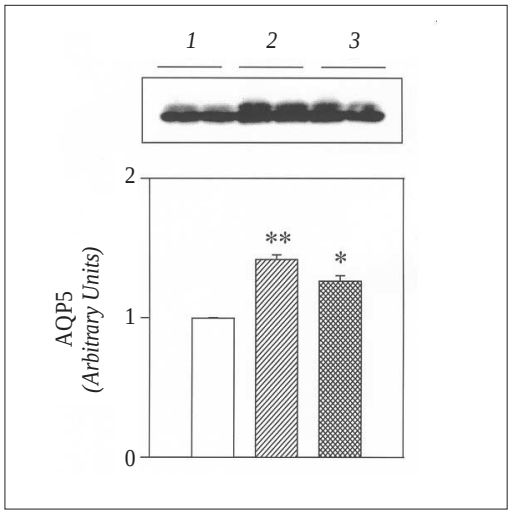

Fig. 9. Expression of AQP5 following the sympathetic-denervation in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats. *p<0.05, **p<0.01; compared with control.

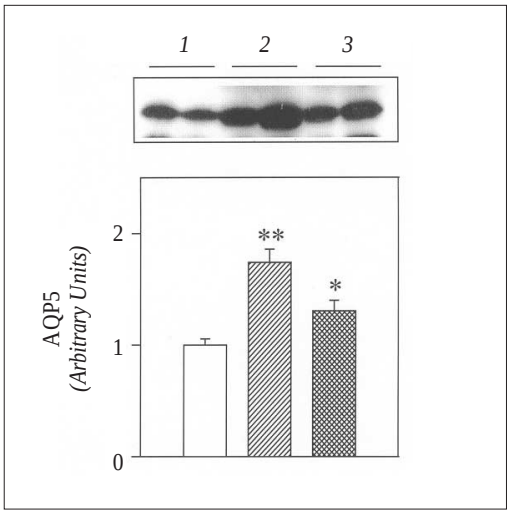

Fig. 10. Expression of AQP5 following the parasympathetic-denervation in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats. *p<0.05, **p<0.01; compared with control.

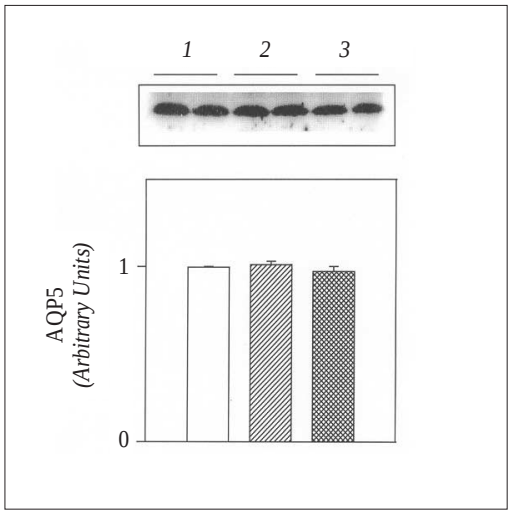

Fig. 11. Expression of AQP5 following the 6-hydroxydopamine-treatment in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats.

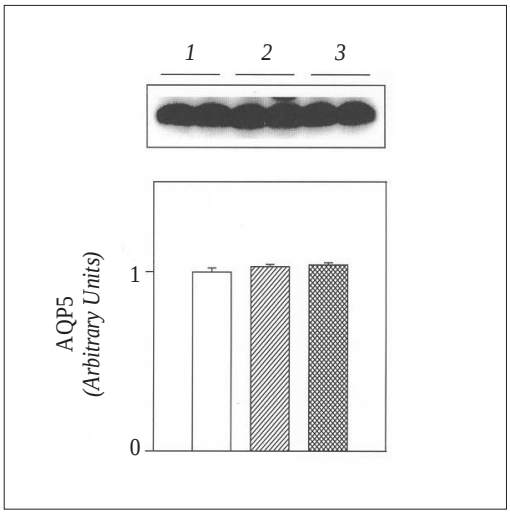

Fig. 12. Expression of AQP5 following the atropine-treatment in the submandibular gland. Legends as in Fig. 1. Each column represents mean±SEM of 6 rats.

DISCUSSION

Among the inorganic constituents of the saliva, sodium appearing in the primary saliva at a concentration similar to that in the extracellular fluid is resorbed in the duct system. It has been long known that the salivary secretion is exclusively regulated by the autonomic nervous system. A lack of neural innervation may thus result in a failure to control over reabsorption of electrolytes in the glandular duct[16]. Therefore, the present study examined whether there are alterations in the regulation of certain sodium transporters and water channels following the glandular denervation.

It has been shown that the sympathetic stimulation caused no detectable changes in Na, K-ATPase immunoreactivity of the cells lining striated and excretory ducts, whereas the parasympathetic stimulation gradually increased the immunostaining of submandibular demilune cells in cats[17]. In the present study, however, neither the parasympathetic nor the sympathetic denervation significantly altered the expression of α_1 and β_1 subunits of Na, K-ATPase. The mechanism whereby parasympathetic stimulation evokes a flow of submandibular saliva may not involve an increase of Na, K-ATPase activity at the base of the gland's demilune cells.

On the contrary, an increased expression of α-subunits of ENaC was demonstrated either following the chorda or the sympathetic denervation. This finding suggests that both divisions of the autonomic nervous system have tonic inhibitory effects on the expression of ENaC. The expression of ENaC may be inhibited to reduce the reabsorption of sodium in the ductal system during a stimulated secretion. Conversely, the increased expression of ENaC may result in an enhancement of sodium reabsorption and hence decreases of sodium contents in the saliva flowing from the denervated gland.

It has been recognized that the flow rate of the affected submandibular gland decreases after chorda division, although chronic otopathy itself does not lead to a disturbance of either the sense of taste or the function of the glandin humans[18]. Furthermore, when twenty patients were studied, the unilateral chorda tympani section was without effect on submandibular flow in seven patients and only reduced submandibular flow by approximately 54% in the remaining 13 patients[19]. There were no significant changes in the concentrations of most important extracellular ions (sodium, potassium, chloride and phosphorus) in the contralateral gland, whereas there was a statistically significant decrease in the concentration of potassium as intracellular cation and of phosphorus as extracellular anion in the gland of which chorda tympani nerve was interrupted[20]. Taken together, the chorda tympani section alone may not be enough to affect the stimulated salivary flow. Alternatively, this finding may indicate that both divisions of the autonomic nervous system have the same secretory effects.

Altered concentrations of inorganic constituents may also be related to an altered regulation of water channels. Being localized in the capillary endothelium[21], AQP1 may be involved in movement of water between plasma and interstitial fluid during the formation of primary saliva. In the present study, however, the expression of AQP1 was not significantly altered either following the sympathetic or the chorda denervation. It is suggested that the autonomic nerves have no direct tonic effects on the expression of AQP1.

The expression of AQP4 has been detected in the excretory duct of rat salivary glands[22]. The present study also demonstrated no significant changes in the expression of AQP4 following the parasympathetic or sympathetic denervation. In fact, the ductal epithelial cells have little effects on the reabsorption of water[2]. The role of AQP4 in the excretory duct, if any, may be minimal and undiscovered in a physiological state.

AQP5 is localized in the luminal membrane of the serous acinar cells of rat submandibular and parotid glands[23,24]. It is believed to provide the main pathway for osmotic water flow from the acinar cells to the lumen in the formation of the primary saliva[8]. An altered expression of AQP5 has been shown in Sjøgren's syndrome[25,26]. In the present study, the expression of AQP5 was increased either by the parasympathetic or sympathetic denervation. AQP5-null mice exhibit defective secretion of saliva[27,28]. Therefore, the altered expression of AQP5 after the autonomic denervation may account for an altered formation of saliva in the denervated gland.

It has been stated that the salivary glands receive secretory fibers from both divisions of the autonomic nervous system, parasympathetic stimulation causing a lively secretion of watery saliva and sympathetic stimulation a slow flow of viscid saliva[12]. Although the existence of sympathetic secretory fibers has often been a matter of dispute, the similarity of the responses between the sympathetic and the parasympathetic denervation may indicate that both divisions convey secretory fibers.

In summary, sympathetic and parasympathetic nerves

have tonic effects on the expression of certain sodium transporters and AQP water channels in the salivary gland. The altered regulation of these transporters and channels may be functionally related to alterations in the volume and electrolyte composition of the saliva following the denervation.

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