ORIGINAL ARTICLE

Relationship between aquaporin-5 expression and saliva flow in streptozotocin-induced diabetic mice?

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OBJECTIVE: To investigate the expression and distribution of AQP5 in submandibular acinar cells from sham- and streptozotocin (STZ)-treated mice in relation to the salivary flow.

METHODS: Mice were sham or STZ injected. Distribution of AQP5 subcellular expression in submandibular glands was determined by immunohistochemistry. AQP5 labelling indices (LI), reflecting AQP5 subcellular distribution, were determined in acinar cells. Western blotting was performed to determine the expression of AQP5 in submandibular glands. Blood glycaemia and osmolality and saliva flow rates were also determined.

RESULTS: AQP5 immunoreactivity was primarily located at the apical and apical-basolateral membranes of submandibular gland acinar cells from sham- and STZ-treated mice. No significant differences in AQP5 protein levels were observed between sham- and STZ-treated mice. Compared to sham-treated mice, STZ-treated mice had significant increased glycaemia, while no significant differences in blood osmolality were observed. Saliva flow rate was significantly decreased in STZ-treated mice as compared to sham-treated mice.

CONCLUSIONS: In STZ-treated mice, significant reduction in salivary flow rate was observed without any concomitant modification in AQP5 expression and localization.

Keywords: aquaporin-5; diabetic mice; hyperglycaemia; inflammatory infiltrates; salivary flow; submandibular glands

Introduction

The most frequent causes of xerostomia, consequent to salivary gland dysfunction, include ageing, anticholinergic drugs, autoimmune diseases such as Sjögren’s syndrome (SS) and diabetes (Moore et al, 2001; Visvanathan and Nix, 2010). Different mechanisms might account for salivary glandular dysfunction: salivary gland atrophy, autoantibodies against muscarinic M3 receptors, down-regulation of muscarinic receptors and inappropriate localization of aquaporins (Steinfeld et al, 2001; Tsubota et al, 2001; Dawson et al, 2006; Li et al, 2006; Hayashi, 2011).

Aquaporins (AQP5s) are small transmembrane proteins implicated in water transport (Agre, 2004). Several AQP5s have been identified in salivary glands (Delporte, 2009). Amongst those, AQP5 plays a critical role by contributing to salivary flow. Indeed, AQP5 knockout mice display a significant decrease in salivary flow in response to muscarinic stimulation, in contrast to AQP1 and AQP8 knockout mice displaying no significant diminution of salivary output (Ma et al, 1999; Krane et al, 2001; Yang et al, 2006). The implication of AQP5 in salivary gland pathology has been essentially addressed in SS, an autoimmune disease characterized by the lymphocytic infiltration of salivary and lachrymal glands essentially, entailing keratoconjunctivitis sicca and xerostomia (Fox, 2005). In salivary and lachrymal glands from patients suffering from SS and in salivary glands from non-obese diabetic mice, a mouse model for SS, abnormal expression and distribution of AQP5 have been reported (Steinfeld et al, 2001; Tsubota et al, 2001; Konttinen et al, 2005). The molecular mechanisms accounting for the abnormal expression and distribution of AQP5 still remains to be elucidated. However, it has been postulated that local inflammatory infiltrates could play a pivotal role in these phenomena (Soyfoo et al, 2007).

This study aimed at determining the expression and subcellular distribution of AQP5 in submandibular acinar cells in a mouse model of uncontrolled type 1 diabetes, induced by streptozotocin (STZ).

Material and methods

Animals

Male Naval Medical Research Institute non-inbred Swiss mice aged 10 weeks (NMRI; purchased from...
Janvier, Be Uden, the Netherlands) received an intraperitoneal administration of STZ \([140 \text{ mg kg}^{-1}]\) dissolved in 5 mM sodium citrate (pH 4.0); Sigma-Aldrich Corp St. Louis, MO, USA) (STZ-treated mice), or of 5 mM sodium citrate (pH 4.0) (sham-treated mice). Animals were used 8 days postintraperitoneal injection. All experiments were approved by the ethical committee for animal experiments of the Catholic University (Leuven, Belgium).

**Blood glycaemia and osmolality**

Blood glucose levels (mg dl\(^{-1}\)) were determined in blood from the tail vein with a glucometer (Accu-Chek Sensor Comfort, Roche Diagnostics, Mannheim, Germany). Blood osmolality (mOsm kg\(^{-1}\)) was determined using a cryoscopic osmometer (Osmomat 30; Gonotec GmbH, Berlin, Germany).

**Histology and immunohistochemistry**

Immediately after removal, salivary glands were fixed in 4% buffered formaldehyde, paraffin-embedded and sectioned (5 \(\mu\)m thick). Some salivary gland sections were stained with haematoxylin and eosin to assess inflammatory infiltrates and acinar destruction. Quantification of inflammatory infiltrates in the submandibular glands was performed using the focus score (FS) (a FS of 1 corresponds to 50 lymphocytes/4 mm\(^2\) of tissue). The FS was determined following the examination of 10 fields of each salivary gland specimen. A FS of 0 or \(\geq\) 1 was then assigned to each salivary gland examined. Immunohistochemical staining of AQP5 was performed as previously described using diaminobenzidine as chromogen (Soyfoo *et al.*, 2007).

**Quantification of AQP5 labelling in submandibular glands**

Ten submandibular gland acini from 10 fields of the salivary gland specimen, from six sham- and STZ-treated mice, were analysed for AQP5 subcellular localization. The acinar AQP5 immunoreactivity was classified into seven categories: (i) only at the apical membrane (A); (ii) at the apical membrane and the cytoplasm (AC); (iii) at the apical and basolateral membranes (ABC); (iv) only at the basolateral membranes (B); (v) at the basolateral membranes and the cytoplasm (BC); and (vi) only in the cytoplasm (C). The labelling index (LI) describes the percentage of acinar cells classified into one of the seven categories.

**Western blot analysis**

Western blot analysis of AQP5 expression was performed as previously described using 2.5 \(\mu\)g of crude plasma membranes from sham- and STZ-treated mice submandibular glands and anti-AQP5 antibody at a dilution of 1:10000 (Soyfoo *et al.*, 2007). The protein concentration was determined using the Bradford method.

**Measurement of saliva flow rates**

Mice were anaesthetized with ketamine (60 mg kg\(^{-1}\) body weight) and xylazine (8 mg kg\(^{-1}\) body weight). Saliva was then collected on a cotton swab for 20 min under pilocarpine stimulation (0.5 mg kg\(^{-1}\) body weight injected subcutaneously). The weight of the cotton swab was measured before and after saliva collection. Saliva weight was converted into saliva volume, assuming that 1 \(\mu\)g represents 1 \(\mu\)l. The amount of saliva (in \(\mu\)l) was normalized to gram of body weight per 20 min.

**Statistical analysis**

Data are expressed as mean \(\pm\) SEM. The statistical differences were evaluated using Mann–Whitney *U* test, followed by a Bonferroni correction or unpaired Student's *t*-test. All statistical analyses were carried out using GraphPad InStat version 5.0 (GraphPad Software, San Diego, CA, USA).

### Results

**Blood glycaemia and osmolality**

Sham-treated mice had glycaemia levels within the normal range. In contrast, the STZ-treated mice displayed significant increased levels of glycaemia \((P < 0.0001; \text{Figure } 1\text{a})\).

Blood osmolality from sham- and STZ-treated mice was not significantly different and within the normal range \((P = 0.854, \text{Figure } 1\text{b})\).

**Histological features and subcellular localization of AQP5 of submandibular glands from sham- and STZ-treated mice**

Absence of abnormal histological features, that is, lymphocytic infiltration and acini destruction, was observed in both sham- and STZ-treated mice (Figure 2).

In both sham- and STZ-treated mice, AQP5 was primarily localized at the apical membrane of acinar cells from submandibular glands of both sham- and STZ-treated mice (Figure 2). No alteration of AQP5 distribution was observed in submandibular acinar cells from STZ-treated mice compared to sham-treated mice. Indeed, labelling indices of AQP5 at the acinar apical membrane from sham- and STZ-treated mice were not significantly different and represented more than 90% of the AQP5 labelling \((P > 0.05; \text{Table } 1)\). Besides, labelling indices of AQP5 at both the acinar apical and basolateral membranes from sham- and STZ-treated mice were also not significantly different and accounted for 5–9% of the AQP5 labelling \((P > 0.05; \text{Table } 1)\).

**Western blot quantification of AQP5 expression in submandibular glands from sham- and STZ-treated mice**

Protein immunoblots of crude plasma membranes from submandibular glands of sham- and STZ-treated mice were probed with anti-AQP5. Western blot analysis revealed the presence of a \(\pm\) 28 kDa band corresponding to AQP5 (Figure 3a). Band density, also called the measured volume, represents the sum of all pixel intensities composing the band spot and depends on both the number of pixels composing the spot and the intensity of these pixels. AQP5 expression in submandibular glands from STZ-treated mice was not significantly
different from that from sham-treated mice ($P = 0.100$; Figure 3b).

**Discussion**

In the present study, the histological features of submandibular gland from both sham- and STZ-treated mice were normal as no inflammatory infiltrates and acinar destruction could be detected. Besides, both subcellular distribution and expression of AQP5 were not altered in STZ-treated mice compared to sham-treated mice. However, STZ-treated mice presented a significant 75% decrease in salivary flow as compared to sham-treated mice.

The normal AQP5 distribution in submandibular acinar cells observed in both sham- and STZ-treated mice is in agreement with studies suggesting a potential link between inflammation and abnormal AQP5 distribution (Soyfoo *et al*, 2007). Indeed, both sham- and STZ-treated mice did not present any inflammatory infiltrates in their submandibular glands. Furthermore, our data are in agreement with those showing similar
apical AQP5 distribution in rat parotid acinar cells from both control and STZ-treated rats (Wang et al., 2011). Our data also showed similar AQP5 protein expression in both membranes of submandibular glands from sham- and STZ-treated mice, in contrast to the results reporting a decreased AQP5 protein expression in membranes from parotid glands from STZ-treated rats, compared to control rats (Wang et al., 2011).

The involvement of AQP5 in salivary flow has been clearly established using knockout mice (Ma et al., 1999; Krane et al., 2001). However, the significant saliva flow decrease observed in STZ-treated mice, as compared to sham-treated mice, cannot be related to a modification in AQP5 distribution. Indeed, submandibular acinar cells from both sham- and STZ-treated mice exhibited similar patterns of AQP5 distribution. Therefore, other mechanisms could account for the observed decreased salivary flow in STZ-treated mice. Decreased saliva flow could be partly attributed to hyperglycaemia measured in STZ-treated mice because those animals did not receive insulin. Hyperglycaemia has indeed been shown to lead to modified osmotic gradients, thereby limiting salivary flow (Moore et al., 2001). In our STZ-treated mice, hyperglycaemia was not concomitant with a modification of blood osmolarity. This is likely due to the loss of sodium into the urines. Independently of hyperglycaemia, other mechanisms such as muscarinic and adrenergic receptor dysfunction could account for the observed diminished salivary flow in the STZ-treated mice (Kimura et al., 1996). Under normal conditions, AQP5 translocation to the apical membrane is triggered by the activation of adrenergic and muscarinic receptors (Ishikawa et al., 2004). However, abnormal AQP5 translocation upon muscarinic agonist administration was observed in rat parotid acinar cells from STZ-treated rats as compared to control rats (Wang et al., 2011). Further studies are required to determine whether the modification of osmotic gradient concomitant to hyperglycaemia, or hyperglycaemia itself, alters salivary flow in STZ-treated mice and accounts for the dysfunction of adrenergic and muscarinic receptor.

In conclusion, we showed that STZ-treated mice displayed decreased saliva flow despite both normal AQP5 distribution and expression. The possible involvement of hyperglycaemia and/or dysfunction of adrenergic and muscarinic receptors in the altered saliva flow of STZ-treated mice remains to be investigated.

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Author contributions

M.S.S performed the experiments; acquired, analysed and interpreted the data; and drafted the article. N.B. performed the experiments and acquired data. I.D. performed experiments, acquired acquisition and revised the article. C.D. designed the experiments; acquired, analysed and interpreted data; and revised the article.

References


