A naturally occurring point mutation in the rat aquaporin 5 gene, influencing its protein production by and secretion of water from salivary glands

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Aquaporins (AQPs) belong to a family of membrane-bound water channels, which transport water and other compounds (such as glycerol and urea) selectively. A cDNA for AQP5 was first cloned from salivary glands and is known as an exocrine gland-type water channel with a unique tissue expression (15). By Northern blot and in situ hybridization analyses, strong expression of AQP5 mRNA is found in many exocrine gland tissues such as the lacrimal gland, lungs, and trachea besides salivary glands (7, 15). AQP5 is thought to play a fundamental role in the water movement during the formation of saliva, tears, and other exocrine secretions (4). An AQP5 knockout experiment conducted in mice resulted in the production of saliva that was significantly hypertonic, viscous, and smaller in volume, directly indicating that AQP5 has an essential role in the secretion of saliva with normal characteristics (8). In the submandibular gland (SMG), AQP5 is localized at the plasma membrane of the acinar cells (9) and has a cAMP-dependent protein kinase target motif in its fourth loop (loop D), located in the intracellular domain (7, 15).

In a previous study (11), we found that the AQP5 expression level in the SMG was divergent among individual rats of the Sprague-Dawley (SD) strain, allowing us to classify them into two groups, i.e., high AQP5 producers and low AQP5 producers (they will be referred hereafter as “high producers” and “low producers,” respectively). The offspring of high producers expressed a significantly higher level of SMG AQP5 than those from low producers, suggesting that the different phenotype in the SMG AQP5 expression is a transmitted hereditary characteristic. The hybrid offspring between high and low producers showed either a high or an intermediate level of AQP5 expression, implying that the high level of AQP5 expression may be dominant. Because the result of our previous study suggested the existence of genetic diversity, in the present study, therefore, we sought to explore the cause of this diversity and to study the effects of such a difference on salivary secretion.

MATERIALS AND METHODS

Reagents. Complete EDTA-free protease inhibitor cocktail tablets, PCR digoxigenin (DIG) labeling mix, anti-DIG-Fab conjugated with alkaline phosphatase, and the disodium 3-{4-methoxyspiro-[1,2-dioxetane-3,2′-(5′-chloro)tricyclic-(3.3.1.14,7)-decan]-4-yl}-based chemiluminescent kit were obtained from Roche Diagnostics (Mannheim, Germany). Donkey anti-rabbit IgG horseshadish peroxidise and the enhanced chemical luminescence (ECL) detection kit were from Amersham Biosciences UK (Buckinghamshire, UK). Fuji RX X-ray film was a product of Fuji Film (Kanagawa, Japan). PMSF and aprotinin were procured from Wako Pure Chemicals (Osaka, Japan). The Bio-Rad protein assay kit was obtained from Bio-Rad Laboratories (Hercules, CA) and TR reagent was from Sigma Chemical (St. Louis, MO). SuperScript One-Step RT-PCR system came from Invitrogen (Carlsbad, CA). ExoSAP was obtained from USB (Cleveland, OH) and protease K was from Wako Pure Chemical Industries (Osaka, Japan). FITC-conjugated affinity purified goat anti-rabbit IgG (H+L) was from Jackson Immunoresearch (West Grove, PA). Tissue optimum cutting temperature compound was obtained from Sakura Finetechical (Tokyo, Japan), and aminopropylsilane (APS)-coated micro slide glasses and micro cover glasses were purchased from...
determined by a Bio-Rad protein assay kit using BSA as a standard (2). The supernatant thus obtained was centrifuged at 105,000 g for 15 min, at 42°C for 20 min, and then twice with 1% agarose gel containing 2% formaldehyde, and transferred onto a nitrocellulose filter according to Towbin et al. (20) for 1 h at 4°C to obtain the total membrane fraction. Protein concentrations were determined by a Bio-Rad protein assay kit using BSA as a standard (2).

Preparation of membrane fraction and Western blotting. Total membrane fraction was prepared as described previously (13). Briefly, tissue specimens were homogenized in 9 vol (wt/vol) of ice-cold homogenization buffer [5 mM HEPES buffer (pH 7.5), 50 mM sodium phosphate, and 1 mM EDTA; pH 7.4], 50% formamide, 6.4% formaldehyde, and 5.3% sodium chloride, 10 mM EDTA, and 0.1% SDS by incubation at 55°C for 1 h isoamylalcohol (25:24:1) to precipitate the DNA. Exon 1 of the AQP5 gene was PCR amplified by using a set of primers, 5'-GACG-3' (antisense) under the thermal conditions consisting of denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 1.5 min. The RT-PCRs were conducted in a TaKaRa PCR Thermal Cycler MP model TP 3000. The primer sets used in the present study were the following: rAQP5–1 (sense; 5'-CCCACAGGCACATGAAAAA-3') and Clo-2A (antisense; 5'-TCAACACTTCTGGTTGCTG-3'), which consisted of 0.1 M NaCl, and 50 mM MgCl₂, and then treated with 100 times diluted Cspd in buffer III for 5 min. The excess liquid was blotted onto Whatmann 3 MM filter paper, and the membrane was then placed in a hybridization bag, preincubated at 37°C for 15 min in an air oven, and finally exposed to Fuji RX X-ray film.

Synthesis of DIG-labeled cDNA probes. The DIG-labeled cDNA probe was prepared by PCR using DIG labeling mix and AQP5 cDNA as a template. Namely, 2.5 µl of PCR DIG-Labeling Mix, 5 pmol of each primer, 2 units of Taq DNA polymerase, 50 pg of template AQP5 cDNA, and 1× PCR buffer (supplied with Ex Taq polymerase, Takara) were mixed in a total volume of 25 µl to make the reaction mixture. The sequences of sense and antisense primers, as well as the PCR thermal conditions used in this labeling experiment, were the same as those shown below (see RT-PCR and PCR cloning and DNA sequencing). cDNA was prepared from total RNA of high-producer SMG by RT-PCR as described in the same section below and purified by the Qiagen spin column method.

RT-PCR and PCR cloning. Full-length AQP5 cDNA was synthesized by RT-PCR using a SuperScript One-Step RT-PCR system; i.e., 0.5 µg of template RNA, 5 pmol of each primer, 0.5 µl of RT/Taq mix, and 12.5 µl of 2× buffer were mixed to make a 25-µl reaction mixture. The RT reaction was carried out at 45°C for 30 min followed by DNA amplification by PCR for 35 cycles, each consisting of denaturation at 94°C for 15 s, primer annealing at 55°C for 30 s, and extension at 72°C for 1.5 min. The RT-PCRs were conducted in a TaKaRa PCR Thermal Cycler MP model TP 3000. The primer sets used in the present study were the following: rAQP5–1 (sense; 5'-CCCCAAAGGCACATGAAAAA-3') and Clo-2A (antisense; 5'-TCAACACTTCTGGTTGCTG-3'), which consisted of 0.1 M NaCl, and 50 mM MgCl₂, and the sequence analysis was carried out as described below.

The above cDNA products were ligated into pGEM®-T Easy Vectors. The constructs were then introduced into competent E. coli. The bacterial colonies transfected with a plasmid having a 1.073-kb insert were grown in 2 ml of Super Broth medium containing 100 µg/ml ampicillin, and plasmid DNAs were extracted by the alkaline extraction method (21).

For analysis of the sequence around the Kozak area of AQP5 mRNA, total RNA from the SMG from high- and low-producer (15th and 10th generations, respectively) rats was prepared. The region from nt –36 to +204 of AQP5 cDNA was synthesized by RT-PCR using 5'-GCCACCTCCGGTGTCA-3' (sense; corresponding to nt –36 to –19) and 5'-GATGTTGCCCACTCTACAG-3' (antisense; corresponding to nt +185 to +204) primers under the same RT-PCR thermal conditions as described above. The reaction mixture was treated with ExoSAP to remove single-stranded DNA, and the cDNA synthesized was subjected to direct sequencing as described below.

Extraction of genomic DNA and PCR amplification. For analysis of the sequence of exon 1 of the AQP5 gene, genomic DNA from the SMG, liver, and lungs from the 8th generation of low-producer rats was extracted following the standard procedures. Briefly, 100-mg tissues were cut into small pieces and digested with 100 µg/ml proteinase K in 10 mM Tris-HCl (pH 8.0) buffer containing 150 mM NaCl, 10 mM EDTA, and 0.1% SDS by incubation at 55°C for 1 h with occasional mixing and then at 37°C overnight (16 h). After the solution had been extracted with neutral phenol, the aqueous phase was separated and mixed with a mixture of phenol, chloroform, and isomylalcohol (25:24:1) to precipitate the DNA. Exon 1 of the AQP5 gene was PCR amplified by using a set of primers, 5'-GCCACAT-AAAAGAGGTGT-3' (sense) and 5'-TGTGTTGTACCGCTTACGGC-3' (antisense) under the thermal conditions consisting of denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 1 min. The reaction mixture after amplification was treated with ExoSAP to remove single-stranded DNA, and the cDNA synthesized was subjected to direct sequencing as described below.

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DNA sequencing. The purified plasmids (150–300 ng) described above were subjected to cycle sequencing by using Big Dye Terminator V3.1 and either one of two specific primers, 5'-TCTTGACTTTCCAGCTAGCC-3' (sense) or 5'-AAAGATCGGCTGGTTCAT-3' (antisense). The product was analyzed with an ABI Prism 3100 genetic analyzer, and the sequencing result was confirmed again according to the reported sequence of the AQP5 gene (15). Similarly, direct sequencing of the Kozak area of the AQP5 gene was carried out by using the same primers used for RT-PCR amplification (i.e., sense, 5'-GCAACCCTCCCGCTGCCA-3', and antisense, 5'-GATGTGGCATCACCCTCACAG-3'). For analysis of exon 1 of AQP5 genomic DNA, primers 5'-GGCACCATGAAAAAGGAGGT-3' (sense) and 5'-GTCCTTTGCCGAGGAGGT-3' (antisense) were employed for cycle sequencing.

Immunohistochemistry and hematoxylin-eosin staining. Fixative (3% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.4) was circulated through the entire rat body from the left ventricle, after which SMG was removed. The SMG tissue was cut at the size of 5 mm³ for immunohistochemistry and 1 mm³ for hematoxylin-eosin staining. Both specimens were fixed further at 4°C for 3 h, followed by a wash with PBS containing either 20% or 6.8% sucrose (for immunohistochemistry and hematoxylin-eosin staining, respectively) by a wash with PBS containing either 20% or 6.8% sucrose (for immunohistochemistry and hematoxylin-eosin staining, respectively) at 4°C overnight. The specimens for immunohistochemistry were then embedded in Tissue-Tec optimal cutting temperature compound and rapidly frozen in liquid nitrogen. AQP5 in the SMG tissue was examined as described previously (11, 14). Briefly, frozen sections of 5 μm thickness were cut and fixed further in ethanol at −20°C for 1 min. The sections were washed in PBS, blocked with 1.5% goat serum in PBS, and immunoreacted with 1,000 times diluted rabbit anti-AQP5 antiserum (primary antibody). After having been washed with PBS, the sections were reacted with 200 times diluted FITC-conjugated affinity purified goat anti-rabbit IgG (H+L) (second antibody) and washed with PBS. For control staining, some sections were incubated with the same concentration of antibody preabsorbed with the peptide used for immunogen. All sections were next incubated for 15 min at room temperature with PBS containing 0.1 μg/ml of propidium iodide and 20 μg/ml of RNase A and then washed with PBS to allow the nucleus to become stained. The stained specimens were examined under a fluorescent microscope equipped with a DXM 1200 digital camera (Nikon, Tokyo, Japan) with excitation at 450–490 nm (for FITC) and 510–560 nm (for propidium iodide). The fixed tissue pieces cut at 1 mm³ were embedded into Technovit 8100 following the manufacturer’s instructions. Sections at 2 μm thickness were cut and stained with hematoxylin and eosin following the standard procedure.

Measurement of salivary secretion. Secretion of saliva in high- and low-AQP5 producers was measured under both nonstimulated and stimulated conditions. For the former condition progenies of 3rd-generation rats were used, and spontaneously secreted saliva was collected every 5 min under urethane anesthesia; secretion rates were then compared between high and low producers. For measurement of salivary secretion, preweighed small cotton balls (weighing ~20 mg) were inserted into the animal’s mouth under the tongue. The balls were removed, and their weights were immediately weighed on a precision balance (cotton ball procedure; Refs. 16 and 17). The 11th-generation high- and 6th-generation low-producer rats were used to measure the salivary secretion under the stimulated condition. These rats were anesthetized with Nembutal (50 mg/kg body wt ip), and saliva production was stimulated by an injection of pilocarpine (1 mg/kg body wt ip). The saliva secreted before and after pilocarpine stimulation was collected by the cotton ball procedure as described above. The dose of pilocarpine was selected so as to induce the maximum response of salivary secretion in rats. In this experiment, daily water intake during 7 days and the body weight of both groups were also measured.

RESULTS

Expression of AQP5 in various tissues of high- and low-producer rats. By Western blot analysis using anti-AQP5 antiserum, the parotid gland, lacrimal gland, and lungs, in addition to the SMG of six male SD rats of the 5th generation (consisting of three high producers and three low producers) were analyzed for their AQP5 protein levels. The three rats (numbered 1-3) in the group of high producers expressed higher levels of AQP5 than those three rats (numbered 4-6) in the low-producer group. Compared with AQP5 in the SMG, that in the parotid gland, lacrimal gland, and lungs of the same rats gave completely parallel results (Fig. 1).

These tissues expressed a high level of AQP5 in high producers and a low one in low producers. Such results suggest that the difference initially found in the SMG is not restricted to this tissue but that such diversity is also true for other tissues. We considered two possibilities to explain this difference: 1) the transcription activity of the AQP5 gene is different between the two groups of rats or 2) the AQP5 structure is...
different, leading to a different amount of protein product conveyed to the cell membrane. In any case, such phenotype appeared to be genetically determined, being transmitted from generation to generation.

Detection of AQP5 mRNA in the SMG by Northern blotting.

To confirm the expression level of AQP5 mRNA, we performed Northern blotting using the DIG-labeled cDNA probe. Total RNA of the SMG from 5th-generation male SD rats (consisting of 3 high producers and 3 low producers) were analyzed for their AQP5 mRNA levels. By Northern blotting, a 1.6-kb band was specifically detected in all samples by using yellow boxes (b) compared with the identical domain in human AQP1 (c). Amino acid residues facing to the inside of the aqueous pore in the membrane are labeled with blue boxes (c), implying that the point mutation of rat AQP5 is located at the remote site from the aqueous pore in the membrane. The first nucleotide at the translation initiation site was taken as the first nucleotide for numbering. Data shown in c are taken from Ref. 10.

for translation efficiency, this area was also sequenced; however, the results revealed no alteration in this area (data not shown). The mutation found in the AQP5 gene resided in the third transmembrane domain of this membrane protein (Fig. 3b). The comparison of rat mutant AQP5 with normal human AQP1 implied that the third transmembrane domain and the subsequent sequence (loop C) are located at remote site from the aqueous pore of this water channel (Fig. 3, b and c). Thus it is speculated that the osmotic water flow may not be affected by the present mutation (see DISCUSSION).

Localization of AQP5 in the SMG of high and low producers. Sections of the SMG from low and high producers (15th and 18th generations, respectively) were stained for AQP5 by the immunofluorescence method. In both SMGs, AQP5 was limited area of apical membrane of very few acinar cells (Fig. 4). By exposing the same sections for longer time, we were able to observe the AQP5 expression in apical-lateral membrane, indicating the extremely weak expression of AQP5 in the mutant gland (Fig. 4d). In the gland of high producers or normal rats, we occasionally observed the irregularly shaped intercellular secretory canaliculi and the meshlike structure inside of the acinar cells (see DISCUSSION). All these positive stainings completely disappeared when the sections were incubated with the same concentration of antibody solution preabsorbed with the antigen peptide (Fig. 4c), clearly indicating that the positive staining was due to a specific reaction. No prominent morphological alteration occurred in the mutant gland (Fig. 4, e and f), despite extremely diminished expression of the AQP5 protein in this rat. All these data would account

Fig. 3. Location of a point mutation in the AQP5 cDNA of low producers from 5th- and 8th-generation rats and the deduced amino acid sequence of mutant AQP5 compared with human AQP1. a: Nucleotide sequences (nt 301–315) of high- and low-AQP5 producers and their deduced amino acid sequences. b: Amino acid sequence of rat AQP5. c: Amino acid sequence of human AQP1. The location of point mutation in the mutant AQP5 cDNA, leading to an amino acid replacement from glycine to aspartic acid, is indicated by a red box (a and b). Bold letters indicate transmembrane domains, whereas underlines indicate the NPA motif conserved within the family (b and c). Amino acid residues of the 3rd transmembrane domain, where the mutated amino acid is localized, are labeled with yellow boxes (b) compared with the identical domain in human AQP1 (c). Amino acid residues facing to the inside of the aqueous pore in the membrane are labeled with blue boxes (c), implying that the point mutation of rat AQP5 is located at the remote site from the aqueous pore in the membrane. The first nucleotide at the translation initiation site was taken as the first nucleotide for numbering. Data shown in c are taken from Ref. 10.

Identification of a single nucleotide mutation1 in the AQP5 cDNA of low producers. The sequence analysis of AQP5 cDNA from low producers showed the existence of a point mutation (G to A) at nucleotide number 308, resulting in replacement of Glycine with Aspartic acid (Fig. 3a). The presence of such a mutation in the AQP5 cDNA was confirmed by analyzing the genomic DNA (exon 1 of the AQP5 gene; data not shown). Since the sequence immediately before the translation initiation site (the Kozak area; Ref. 5) is important

1 Nucleotide numbering: the first nucleotide, adenine at the translation initiation site (ATG), was denoted as nucleotide number 1 in this study.
for the difference in the rate of saliva secretion, which was also confirmed in this study (see the next section).

Salivary secretion of high and low producers of SD rats. The rate of salivary secretion under urethane anesthesia in the resting condition differed between high and low producers (Fig. 5), being 7.5 times higher in the high producer than in the low producer. This fact suggests that variation in AQP5 expression resulted in variation in the rate of salivary secretion. The SMG weight was the same in both groups (Fig. 5).

The rate of salivary secretion during the initial 5 min after pilocarpine stimulation also differed between the high and low producers examined in this study (Fig. 6), although there was no statistical difference in the gland weight between the two groups. Since a high level of AQP5 is expressed in the apical membrane, this variation in the AQP5 expression may well account for the variation in the rate of salivary secretion. We also noticed that the daily water intake by the low producers was significantly higher than that by the high producers.

In a time-course study, the pattern of salivary secretion was different between high and low producers (Fig. 7). The initial phase that appeared in high producers around 12 min was lacking in the low producers; i.e., there was an obvious delay in secretion in low producers compared with high producers.

Fig. 4. Immunohistochemical localization of AQP5 in the SMG of high and low producers. Cryostat sections of the SMG from high and low producers were incubated with anti-rat AQP5 antiserum. In the high producer (a), the fluorescence image of FITC indicating the presence of AQP5 is seen on the entire acinar cell membrane, which includes apical, lateral (small arrow), and basal (large arrows) aspects. The arrowhead in a indicates the irregularly shaped intercellular secretory canaliculi, whereas the cells beside the asterisk showed the meshlike structure, which appeared occasionally in the acinar cells throughout the section. In the low producer (b), the strong fluorescence signal is seen at only limited areas of the apical membrane in very few cells (small arrows). The rest of apical and lateral membranes expressed AQP5 very weakly (small arrows) as revealed by the same section exposed for longer time (d). The section from a high producer reacted with the anti-AQP5 antibody preabsorbed with its antigen peptide did not show positive reactions (c). Hematoxylin-eosin staining of the section of tissue embedded in Technovit 8100 showed very similar morphology between high and low producers (e and f). All photographs were taken with ×20 magnification objective lens. A, acini; sD, striated ducts; gD, granular duct. Bars = 20 µm.

Fig. 5. Salivary secretion under the resting condition and SMG weight in low and high producers among 3rd-generation progenies. Results were analyzed by Mann-Whitney’s U-test. *P < 0.05, significantly different from the high-producer group; #, not significantly different from the high-producer group.
DISCUSSION

We have known that AQP5 is strongly expressed in the salivary glands as well as other exocrine gland tissues. On the basis of a previous study (11), we reported for the first time the existence of genetic diversity in AQP5 expression. In the present study, we found that such diversity was not restricted to the SMG but was seen also in the parotid gland, lacrimal gland, and lungs (Fig. 1).

To determine whether such difference might be based on a difference in transcription activity, we examined the mRNA level for AQP5 in the SMG of the two groups of rats. Northern blot analysis demonstrated the presence of a 1.6-kb mRNA for AQP5 in the SMG, parotid gland, lacrimal gland, and lungs (1, 6, 15). Even though the protein level of AQP5 in the 5th-generation high producers was significantly higher than that in the same generation of low producers, the Northern blot analysis showed that the AQP5 mRNA level in the two groups was almost same (Fig. 2), indicating that the decreased expression of AQP5 protein in the membrane fraction is not a consequence of a change in the mRNA level. The sequence immediately before the AUG translation initiation codon (nt -18 to -1), which is known as the Kozak sequence (5), was also the same between the two groups. However, by analyzing the sequence of cDNA and genomic DNA (exon 1) for AQP5, we found a point mutation in the third transmembrane domain that led to the replacement of Gly with Asp (Fig. 3). Probably this mutation may not affect the water flow (osmotic water permeability coefficient value) of the molecule since the mutated amino acid is located at the third transmembrane domain, which is distant from the aqueous pore of the AQP5 molecule. An experiment that explores this point is now being conducted using the Xenopus laevis oocyte.

On the other hand, AQP5 expression at lateral and basal plasma membrane was extremely diminished in the SMG of the mutant rat (low producers) compared with high producers (Fig. 4). In the mutant rat, only limited area of the apical membrane in very few acinar cells showed positive reaction. The appearance of irregularly shaped intercellular secretory canaliculi (omega-shaped structure) implies the AQP5 localization in the intracellular vesicles or secretory granules and their trafficking toward the plasma membrane. The meshlike structure sometimes appeared inside of the acinar cells, which implies that they may be the granular membrane or the structure conveying the AQP5 vesicles (e.g., AQP5 in the SMG, parotid gland, lacrimal gland, and lungs (1, 6, 15). Even though the protein level of AQP5 in the 5th-generation high producers was significantly higher than that in the same generation of low producers, the Northern blot analysis showed that the AQP5 mRNA level in the two groups was almost same (Fig. 2), indicating that the decreased expression of AQP5 protein in the membrane fraction is not a consequence of a change in the mRNA level. The sequence immediately before the AUG translation initiation codon (nt -18 to -1), which is known as the Kozak sequence (5), was also the same between the two groups. However, by analyzing the sequence of cDNA and genomic DNA (exon 1) for AQP5, we found a point mutation in the third transmembrane domain that led to the replacement of Gly with Asp (Fig. 3). Probably this mutation may not affect the water flow (osmotic water permeability coefficient value) of the molecule since the mutated amino acid is located at the third transmembrane domain, which is distant from the aqueous pore of the AQP5 molecule. An experiment that explores this point is now being conducted using the Xenopus laevis oocyte.

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microtubules). Thus, in the SMG, the membrane of intracellular vesicles or secretory granules appeared to bear AQP5. On the other hand, in the low producer (mutant rats), the aspect of AQP5 localization in the intracellular structure and cell membrane was not clear because of the extreme reduction of its expression, although very weak or poor AQP5 expression was evident in the apical and lateral areas.

The mutation may have affected the expression of AQP5 protein in the plasma membrane, probably because either the insertion of AQP5 into the vesicle membrane would be diminished or trafficking of AQP5 toward the apical membrane would be ineffective. In our preliminary experiments using MDCKII cells, expression of mutant AQP5 in the cell membrane was decreased compared with that of normal AQP5 (M. R. Karabas, unpublished observations). Therefore, the mutation appears to have caused the decreased expression of AQP5 in the plasma membrane (Fig. 4), resulting in decreased water secretion from the salivary gland.

The basolateral membrane of acinar cells in the salivary glands is believed to have high water permeability, and it has been implied that AQP5 in the luminal area serves as a rate-limiting step in the water transport across the acinar cells (3). We noticed that the rate of spontaneous salivary secretion was different between high and low producers, although there was no difference in the gland weight (Fig. 5). Under Nembutal anesthesia and pilocarpine stimulation, the rate of salivary secretion of saliva.

In the salivary gland, the doctrine of the presence of two pathways for water transport in acinar cells, i.e., transepithelial and paracellular (12), is generally accepted; and the study by Ma et al. (8) clearly showed that AQP5 is responsible for the water transport in the transepithelial pathway. Our data well agree with the data of their AQP5 knockout experiment. The results of our time-course study suggest that transepithelial transport activity is very low in low producers. The lack of the initial secretion in the low producers agrees with the earlier finding that the transepithelial transport pathway is involved in the initial phase of salivary secretion (18). We found that the daily water intake in low producers was higher than that in high producers. This result suggests that the decreased salivary secretion may have induced thirst or dryness appreciably in the oral cavity and triggered an increase in water drinking in the low producers.

The AQP5 mutant rat showed a low level of AQP5 expression in the salivary gland cell membrane, as well as a low level of salivary secretion, suggesting the existence of a strong linkage between this AQP5 mutation and some type of xerostomia. Because this AQP5 mutation was found in rats, a study should be undertaken to learn whether it also occurs in humans.

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