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Glucocorticoid Enhances Na^+/K^+ ATPase mRNA Expression in Rat Olfactory Mucosa during Regeneration: A Possible Mechanism for Recovery from Olfactory Disturbance

Toshiro Nishimura, Shigekazu Teranishi, Atsuhiro Kawashima[1], Tadashi Ishimaru, Takaki Miwa and Mitsuru Furukawa

Department of Otorhinolaryngology and [1]Department of Pathology, Kanazawa University School of Medicine, Kanazawa, Japan

Correspondence to be sent to: Toshiro Nishimura, Department of Otorhinolaryngology, Kanazawa University School of Medicine, 13-1 Takaramachi, Kanazawa, Ishikawa, 920-8641, Japan. e-mail: nishimut@orl.m.kanazawa-u.ac.jp

Abstract

Systemic or topical application of glucocorticoid is the treatment of choice for olfactory disturbance. Recently, Na^+/K^+ ATPase and glucocorticoid receptor immunoreactivity in the olfactory mucosa was reported. To elucidate a glucocorticoid action on Na^+/K^+ ATPase production, an animal model was produced by an intra-nasal application of 5% $ZnSO_4$ solution to Wistar rats. Dexamethasone was injected i.p. (0.01 mg/100 g) for 14 days after the insult. Histologically, the regeneration process was completed on day 14 in both dexamethasone- and saline-injected control rats. We used a quantitative polymerase chain reaction (PCR) method to evaluate mRNA production of Na^+/K^+ ATPase and glucocorticoid receptor. In dexamethasone-injected rats, up-regulation of glucocorticoid receptor mRNA (95% more than control rats, $P = 0.00068$, unpaired *t*-test) and of Na^+/K^+ ATPase mRNA expression (76% more than control rats, $P = 0.0042$) was observed on day 14. The increased Na^+/K^+ ATPase expression in the regenerated olfactory mucosa is thought to be beneficial for an active uptake of K^+, which is released during excitation, around olfactory neurons and for the transepithelial absorption of Na^+ from olfactory mucus. Dexamethasone may thus contribute to the recovery of function after the morphological regeneration in part, at least, through its receptor by regulation of the ionic concentration in the olfactory mucosal microenvironment.

Introduction

Systemic or topical application of glucocorticoids is the treatment of choice for olfactory disturbance (Robinson *et al*., 1998; Fong *et al*., 1999). Although the glucocorticoids' mechanism of action remains unclear, it is supposed that they suppress the production of inflammatory cytokines and resolve inflammatory reaction in the olfactory mucosa. Glucocorticoids are thus thought to improve the airflow in the nasal cavity, allowing the odorant-containing airflow to reach the olfactory sensory mucosa (Robinson *et al*., 1998).

Recently, the presence and localization of glucocorticoid receptors (GR) (Kern *et al*., 1997a,b; Robinson *et al*., 1998) and Na^+/K^+ ATPase in olfactory mucosa of guinea pigs (Kern *et al*., 1991; Fong *et al*., 1999) have been reported. Within the olfactory epithelium, Na^+/K^+ ATPase immunoreactivity has been reported at the supranuclear region of sustentacular cells and/or dendrites of olfactory receptor neurons (Fong *et al*., 1999). Moreover, glucocorticoid immunoreactivity has been detected at the apical surface including the dendrites, knobs and cilia of olfactory receptor neurons and the supranuclear region of sustentacular cells (Robinson *et al*., 1998).

Na^+/K^+ ATPase is an ubiquitous transmembrane hetero-dimeric protein complex, consisting of a catalytic α and a glycosylated β subunit, that couples the hydrolysis of ATP to the vectorial transport of Na^+ outward and K^+ inward across the cell plasma membrane (Ewart *et al*., 1995; Verrey *et al*., 1996; Derfoul *et al*., 1998).

Na^+/K^+ ATPase expressed in olfactory receptor and sustentacular cells may participate in the maintenance and restoration of the receptor cells' resting potential, because Na^+/K^+ ATPase along the basolateral sustentacular cell membrane may buffer high extracellular K^+ concentrations released from adjacent olfactory neurons during excitation through the active uptake of K^+ (Kern *et al*., 1991).

It has also been suggested that Na^+/K^+ ATPase may maintain the ionic balance of olfactory mucus through transepithelial Na^+ absorption from the olfactory mucus (Fong *et al*., 1999). Na^+ in olfactory mucus is originally secreted from Bowman's gland and Na^+ is supposed to be removed by cotransport or exchange from olfactory mucus into sustentacular cells. Then Na^+ would be actively pumped from sustentacular cells into the interstitial space of

olfactory mucosa. Since the sodium concentration of olfactory mucus is reportedly lower than the mucus that covers the respiratory mucosa (Joshi *et al*., 1987), Na^+/K^+ ATPase is thought to play an important role in the maintenance of the proper ionic microenvironment necessary for olfactory transduction.

We produced animal models of olfactory mucosa injury, which were given i.p. glucocorticoid (dexamethasone) in order to clarify its effect on the olfactory mucosa during regeneration. The mRNA expressions of GR and Na^+/K^+ ATPase were assessed to determine whether they may indicate a direct action of glucocorticoid on the olfactory mucosa during regeneration following injury.

Materials and methods

Animal models

Sixty-one male Wistar rats (150–180 g) were obtained from Charles River Japan (Atsugi, Japan) and maintained on a 12 h light–dark cycle in a temperature-controlled room. All received similar amounts of a standard solid diet (CRF-1, Charles River Japan) and water *ad libitum* throughout the study. The experiment was in accordance with the protocol approved by the Animal Care and Use Committee of Kanazawa University (Kanazawa, Japan). Thirty-three rats were used for histologic (n = 3 for each observation) and 28 rats (n = 4 for each observation) for mRNA assessments.

Olfactory mucosa injury was produced by means of topical application of a 5% $ZnSO_4$ solution (Kimura *et al*., 1991). The animals were anesthetized with an i.p. pentobarbital sodium injection (1 mg/kg) and 0.2 ml of the $ZnSO_4$ solution was applied to the nasal cavity of each nostril with a plastic 20 gauge needle, after which the animals were kept immobile for 10 min.

A therapeutic dose of dexamethasone phosphate (Banyu Seiyaku, Tokyo, Japan) was injected i.p. (0.01 mg/100 g body wt) every day for 14 days, starting immediately after the olfactory mucosa insult. Control rats were injected with the same volume of physiological saline.

Sample preparations

For histological studies, rats were deeply anesthetized with pentobarbital sodium (30 mg/100 g) and transcardially perfused with ice-cold physiological saline followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The rats were then decapitated and the head post-fixed for 24 h in the same fixative, decalcified in 10% ethylene-diamine tetra-acetic acid (EDTA) solution for 14 days, dehydrated and embedded in paraffin. Finally, 4 μm sections of the head were made in a coronal direction.

For RNA extraction, the nasal mucosa was quickly microdissected and frozen in liquid nitrogen after the rats had been anesthetized. The specimens were stored at –70°C until the subsequent experiments.

Immunohistochemistry

Immunohistochemical studies used the avidin biotin peroxidase complex (ABC) method (Hsu *et al.*, 1981). After blocking of the endogenous peroxidase activity in a methanol solution containing 0.3% hydrogen peroxide, the sections were incubated in either 10% normal goat serum (for the polyclonal primary antibody) or horse serum (for the monoclonal antibody) for 30 min at room temperature to reduce non-specific antibody binding. The primary antibodies used were rabbit polyclonal antibody specific for the β_1 subunit of Na^+/K^+ ATPase (Upstate Biotechnology, Lake Placid, NY) at a 1:100 dilution, and mouse monoclonal antibody for glucocorticoid receptors (Oncogene Research Products, Cambridge, MA) at a 1:200 dilution. Specimens were incubated with a diluted primary antibody overnight at 4°C. Sections were then incubated with either biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) or biotinylated horse anti-mouse IgG (Vector Laboratories) at a 1:200 dilution for 30 min at room temperature. Reaction with the strept-avidin biotin peroxidase complex reagent (DAKO Japan, Tokyo, Japan) was performed at room temperature for 30 min. After color development, nuclear staining was performed with hematoxylin. For positive controls, rat kidneys were stained for Na^+/K^+ ATPase and glucocorticoid receptor. A negative control was included for each specimen by substituting a primary antibody for either normal goat serum or normal horse serum.

Real-time quantitative reverse transcription PCR procedure

Total RNA was extracted with the aid of RNeasy mini-kits (Qiagen, Tokyo, Japan) from 5 mg of olfactory mucosa from each rat. Tissue was manually homogenized with a disposable plastic micropestle in a microcentrifuge tube containing 350 μl of RLT lysis buffer. The homogenate was then passed through a QIAshredder microcolumn (Qiagen) by centrifugation. The resulting cell lysate was mixed with an equal volume of 70% ethanol in diethyl pyrocarbonate (DEPC)-treated water and loaded onto RNeasy spin columns and centrifuged. The columns were washed with 700 μl RW1 buffer and centrifuged for 15 s. A mixture of 70 μl of RDD buffer and 10 μl of 3 U/μl of deoxyribonuclease I solution (Qiagen) was then applied to the column for 15 min at 30°C. The column was centrifuged and serially washed with 700 μl of RW1 buffer and 500 μl of RPE buffer and centrifuged for 3 min. Total RNA was eluted by the addition of 50 μl of DEPC-treated water to the column followed by centrifugation. RNA concentration was determined with a spectrophotometer to measure absorbance at 260 nm.

One microgram of total RNA was reverse-transcribed with a First-Strand cDNA synthesis kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ) in a total reaction volume of 15 μl containing a bulk first-strand reaction

mix consisting of moloney murine leukemia virus reverse transcriptase, ribonuclease inhibitor, 0.08 mg/ml bovine serum albumin, 1.8 mM of dATP, 1.8 mM of dCTP, 1.8 mM of dTTP, 45 mM Tris (pH 8.3), 68 mM potassium chloride, 15 mM DTT, 9 mM magnesium chloride and 40 pmol of random hexadeoxynucleotides. The reaction was performed at 37°C for 60 min. A preliminary standard 40 cycle PCR, using the β-actin primer (Promega, Madison, WI) spanning one intron, was performed on 0.5 μl of each cDNA sample to confirm the quality of the sample to prevent contamination of genomic DNA (data not shown).

Real-time quantitative PCR (Gibson *et al*., 1996) was performed with an ABI Prism 7700 sequence detection system (Perkin-Elmer Applied Biosystems, Foster City, CA). The expression of 18S ribosomal RNA was used as the internal control (Bhatia *et al*., 1994). For the β_1 subunit of Na^+/K^+ ATPase and GR mRNA quantification, primers and probes (Table 1) were chosen with the aid of Primer Express (Perkin-Elmer Applied Biosystems). Primers were purchased from Takara-Syuzo (Kusatsu, Japan) and probes from Perkin-Elmer Japan (Urayasu, Japan). Amplification mixes (50 μl) contained the sample cDNA (~30 ng), 10 TaqMan buffer (5 μl), 200 mM dATP, dCTP, dGTP and 400 mM dUTP, 5 mM $MgCl_2$, 1.25 units of AmpliTaq Gold, 0.5 units of AmpErase uracil N-glycosylate (UNG), 300 nM of each primer and a 200 nM probe. Thermal cycling was performed for 2 min at 50°C and for 10 min at 95°C, and consisted of 40 cycles at 95°C for 15 s and at 65°C for 1 min.

Each assay included samples for a standard curve consisting of 1 μml of kidney cDNA solution (50 ng/μl) made from kidney total RNA serially diluted 1, 4, 16 and 64 times in duplicate, a non-template control and ~30 ng of the sample cDNA in quadruplicate.

All samples with a coefficient of variation >10% were re-tested. Authenticity of PCR products was confirmed by standard 4% agarose gel electrophoresis with ethidium bromide staining and direct sequencing of representative samples.

All measurements were done in triplicate and the mean values and standard deviations were calculated. The sample values were divided by the value of 18 S ribosomal RNA expression and the value of olfactory mucosa before injury was standardized at 1.0 to facilitate comparisons among samples.

Table 1 Primers and probes

Na^+/K^+ ATPase β_1 polypeptide (Accession No. NM 013113)	
Forward primer	(nucleotides 1246–1266) 5′-CAGTTCACCAACCTCACCTTG-3′
Reverse primer	(nucleotides 1298–1321) 5′-CACTGTACCCAATGTTCTCACCA-3′
Probe	(nucleotides 1270–1297) 5′-FAM-ACTGAAATCCGCATTGAGTGTAAGG CGT-TAMRA-3′
(Amplicon = 76 bp)	
Glucocorticoid receptor (Accession No. M14053)	
Forward primer	(nucleotides 1528–1548) 5′-TTCGAAGGAAAAACTGCCCAG-3′
Reverse primer	(nucleotides 1577–1597) 5′-CGAGCTTCAAGGTTCATTCCA-3′
Probe	(nucleotides 1550–1574) 5′-FAM-TGCCGCTATCGGAAATGTCTTCAGG-TAMRA-3′
(Amplicon = 70 bp)	

Data analysis

The statistical significance of differences between groups with numerically defined variables was established by means of ANOVA and the Student's *t*-test using a level of significance of $P < 0.05$ (StatView, SAS Institute Inc., NC).

Results

Na^+/K^+ ATPase and GCR immunohistochemistry in normal olfactory mucosa

Figure 1A shows that the most intense Na^+/K^+ ATPase immunoreactivity was observed at the supranuclear region of the sustentacular cells and the dendrites of olfactory receptor neurons. The supranuclear region of several sustentacular cells was stained very intensely and showed a wide staining pattern. Most of all, the dendrites of olfactory receptor neurons exhibited a clear linear staining pattern between sustentacular cells. Less intense activity was observed at the vascular wall and some of the acinar cells of Bowman's gland.

These results were in agreement with a previous report using guinea pigs (Fong *et al*., 1999), but that report showed more intense immunoreactivity in acinar and ductal cells of Bowman's gland. In our specimen, it was difficult to find ductal cells of Bowman's gland and the difference seemed to depend on the animal species used.

In positive control sections of rat kidney (Figure 1B), cells in collecting tubules were strongly positive. In negative control sections (Figure 1C), in which the primary antibody was omitted, there were no positive cells in the olfactory mucosa.

Figure 2 demonstrates that the immunoreactivity of GR was most intense at the surface layer of the olfactory mucosa. This area corresponds to the apical knobs, cilia and dendrites of olfactory receptor neurons and the supranuclear region of sustentacular cells. Almost all the sustentacular cells exhibited strong to moderate staining. Some linear pattern stains were observed between sustentacular cells and those were considered to be the dendrites of olfactory neurons. Although a previous report (Robinson *et al*., 1998) demonstrated an intense immunoreactivity in the

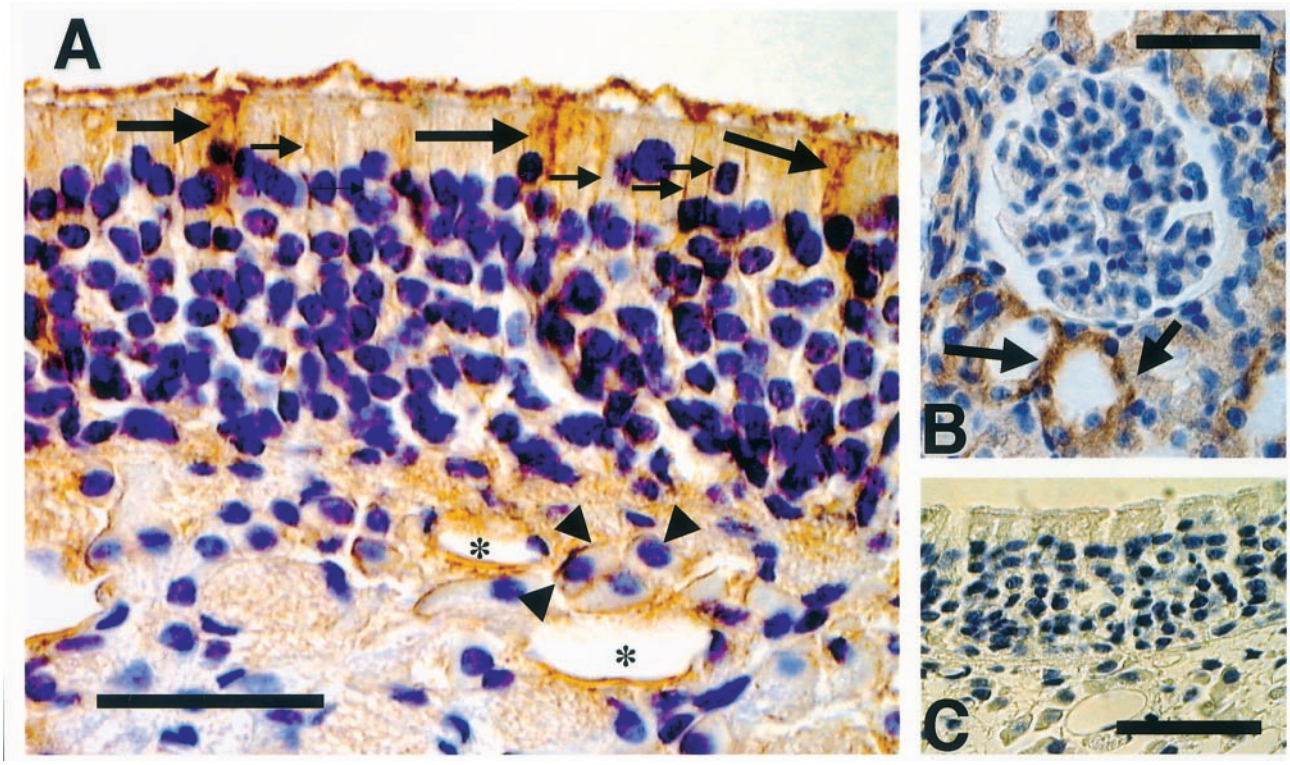

Figure 1 **(A)** Immunostaining of Na^+/K^+ ATPase in normal rat olfactory mucosa. The most intense Na^+/K^+ ATPase immunoreactivity was observed at the axons of olfactory receptor neurons, in which linear stains were observed (small arrows), and at the baso-lateral membrane of sustentacular cells (large arrows). Less intense activity was observed at the vascular wall and the acinar cells of Bowman's gland (arrowheads). (ABC method; original magnification 400; bars indicate 50 μm). **(B)** Positive control for Na^+/K^+ ATPase stain in normal kidney tissue where renal tubular cells are positive (arrows) (ABC method; original magnification 250; bars indicate 50 μm). **(C)** Negative control for Na^+/K^+ ATPase stain in normal olfactory mucosa with the primary antibody omitted. There were no positive cells (ABC method; original magnification 250; bars indicate 50 μm).

acinar cells of the Bowman's gland and nerve bundles of olfactory nerve processes, neither structure exhibited marked staining in our specimens. Again, the difference seemed to depend on the animal species and staining method used (the previous report used immunofluorescence method).

In positive control sections of rat kidney (Figure 2B), cells in renal tubules were strongly positive. In negative control sections (Figure 2C), in which the primary antibody was omitted, there were no positive cells in the olfactory mucosa.

Histological findings of regeneration of olfactory mucosa

Figure 3 shows that the olfactory mucosa was severely damaged after the application of a 5% zinc sulfate solution. In rats without dexamethasone injection, only a few cell layers of olfactory neurons remained on day 2. The number of layers had increased on days 5 and 8. On day 8, the sustentacular cells had regenerated and matured and eight or nine layers of regenerated olfactory neurons were observed on day 14.

In rats with dexamethasone injection, the sustentacular cells seemed more mature on day 8, but the number of layers of olfactory neurons was the same as that of rats without dexamethasone injection (six to seven layers). On day 14, eight or nine layers of the olfactory neurons were observed and the regeneration of the olfactory mucosa had morphologically been completed.

Na^+/K^+ ATPase and GR mRNA expression during regeneration of olfactory mucosa

Figure 4A demonstrates the kinetics of the reaction with change in fluorescent signal (ΔR_n) on the *y*-axis and the cycle of PCR on the *x*-axis, that was plotted with serial dilutions of kidney cDNA. The estimation of target sequence is related to the threshold cycle (the cycle at which statistically significant increase in ΔRn is first detected) and threshold is defined as the average standard deviation of normalized reporter emission intensity for the early cycles, multiplied by an adjustable factor (Gibson *et al.*, 1996).

A plot of the threshold cycle at the various kidney control cDNA dilutions (black dots) is shown in Figure 1B that demonstrates a linear relationship that was noted over the range tested (0.78–50 ng of kidney cDNA) with a correlation efficient of 0.999. Unknown samples from olfactory mucosa were also plotted for the estimation of mRNA expression.

In Figure 5A, Na^+/K^+ ATPase and GR mRNA expression was measured on days 0, 3, 7 and 14 until the damaged

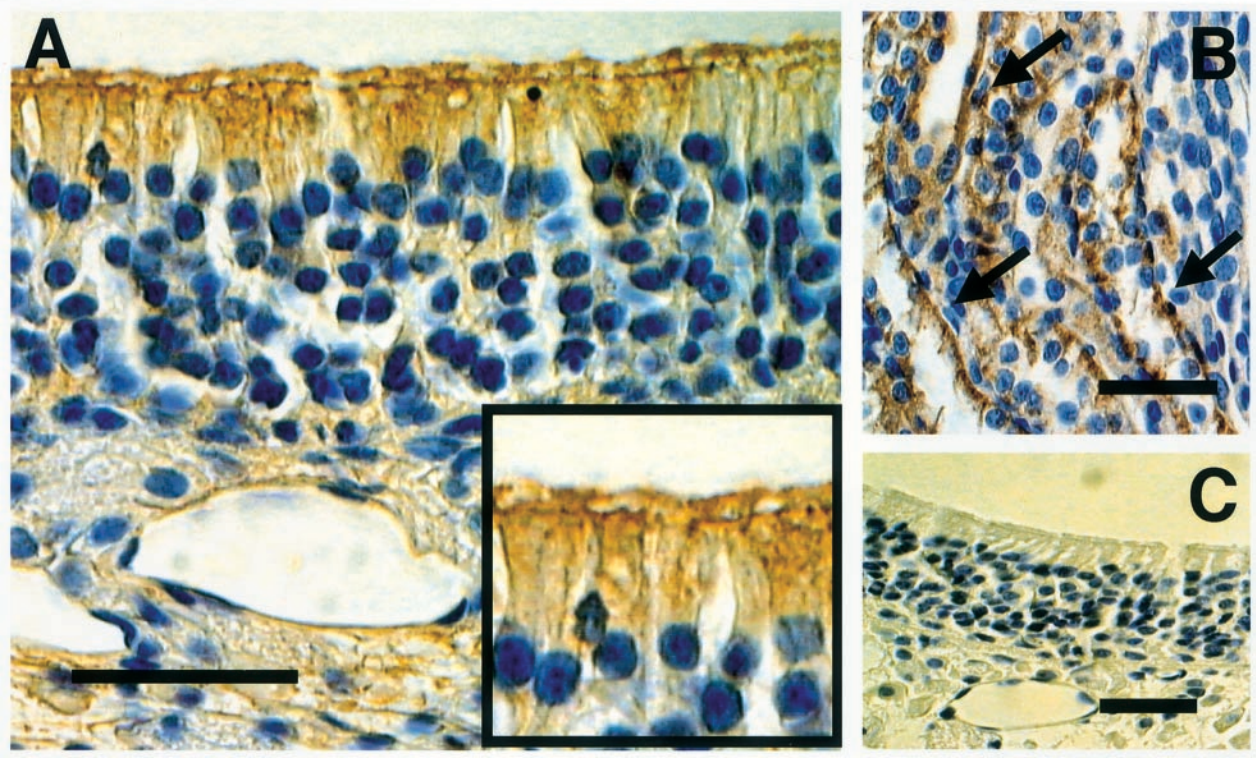

Figure 2 **(A)** The immunoreactivity of glucocorticoid receptors (GR) was most intense at the surface layer of the olfactory mucosa, including the cytoplasm of sustentacular cells and dendrites of olfactory receptor neurons (inset) (ABC method; original magnification 400; bar indicate 50 μm). **(B)** Positive control for GR stain in normal kidney tissue where renal tubular cells are positive (arrows) (ABC method; original magnification 250; bars indicate 50 μm). **(C)** Negative control for GR stain in normal olfactory mucosa, with the primary antibody omitted. There were no positive cells (ABC method; original magnification 250; bars indicate 50 μm).

olfactory mucosa had morphologically been completely regenerated. Figure 5A shows that Na^+/K^+ ATPase mRNA expression in the olfactory mucosa gradually increased after the 5% zinc sulfate solution insult. In glucocorticoid-treated rats, there was a statistically significant increase of 76% in Na^+/K^+ ATPase expression compared to that in control rats on day 14 ($P = 0.0042$, unpaired t-test).

Figure 5B demonstrates that GR mRNA expression of the olfactory mucosa was similar to Na^+/K^+ ATPase mRNA expression and this expression gradually increased after the insult. On day 14, GR expression of glucocorticoid injected rats was 95% higher than that of control rats ($P = 0.00068$, unpaired t-test).

Discussion

Our study indicates that glucocorticoid could have a direct effect on Na^+/K^+ ATPase expression in rat olfactory mucosa, although glucocorticoid was of little benefit for the morphological regeneration of the olfactory mucosa after the injury.

In renal and cardiac cells, aldosterone but not dexamethasone activates Na^+/K^+ ATPase gene expression, while the reverse is observed in colonic surface cells. This suggests that both mineralocorticoid and glucocorticoid receptors (MR and GR) are involved in the regulation of the enzyme (Orlowski *et al.*, 1990; Ikeda *et al.*, 1991; Derfoul *et al.*, 1998; Zemanoveá *et al.*, 1998). Our result offers support for the notion that glucocorticoid (dexamethasone) can induce Na^+/K^+ ATPase gene expression in the olfactory mucosa.

The immunoreactivities of regenerated olfactory mucosa in dexamethasone-injected rats and control rats were not directly compared in this study. Because there is a possibility of the post-transcriptional down-regulation of both Na^+/K^+ ATPase and GR mRNA (Orlowski *et al.*, 1990; Ikeda *et al.*, 1991; Derfoul *et al.*, 1998), this possibility should be further examined from the viewpoint of protein production using the Western blot technique.

In our experiment, the most significant increase in both GR and Na^+/K^+ ATPase mRNA production occurred after day 7, when the sustentacular cells had completed their regeneration. In the early regeneration period, an increase in the number of cell is thought to be mainly responsible for to the total increase of mRNA production. But in the later regeneration period after day 7, an increase in the mRNA production of each cell due to dexamethasone administration is considered to be the main contributory factor in the overall increase in mRNA production in the olfactory mucosa.

Glucocorticoids enter most cells by diffusion and bind to glucocorticoid receptors. The receptor–hormone complex

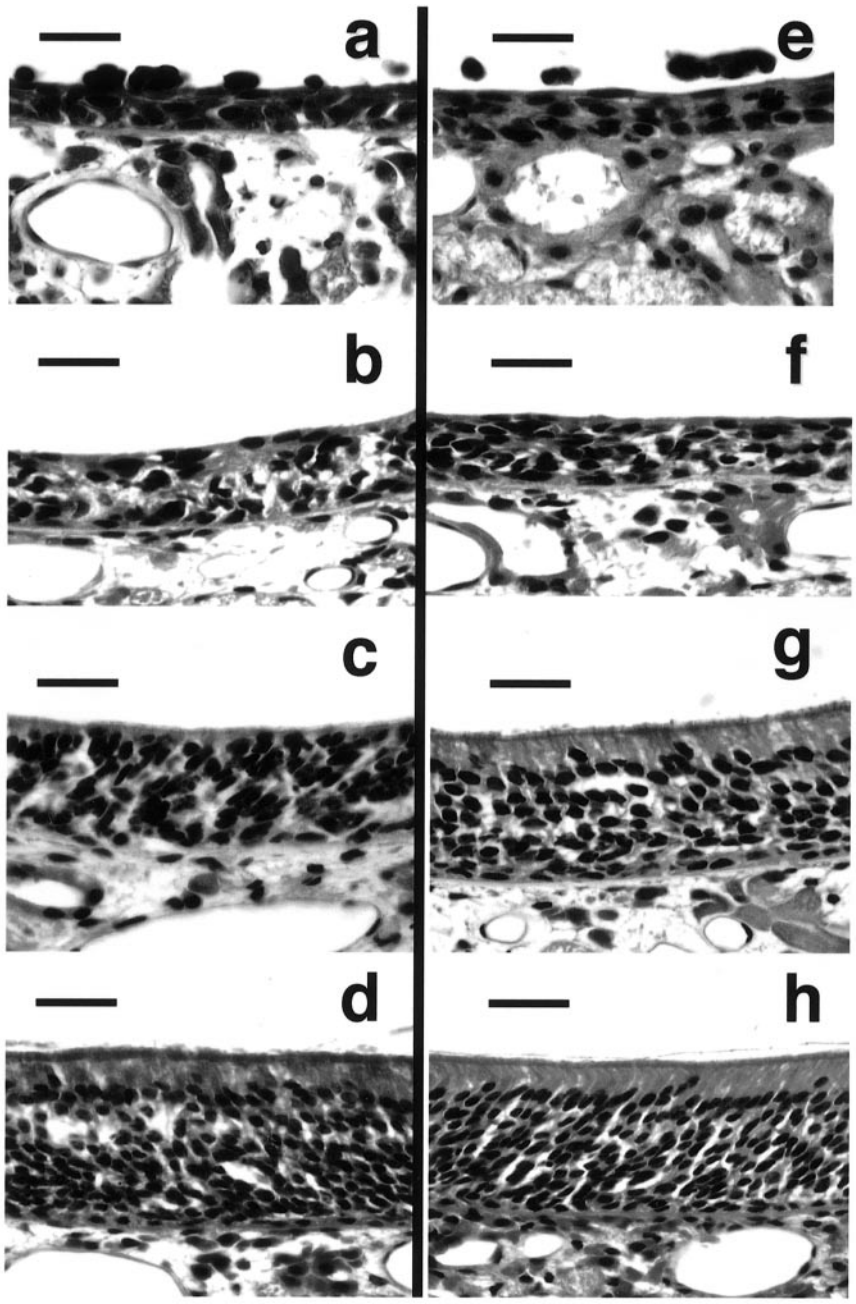

Figure 3 Photomicrograph of the damaged olfactory mucosa of saline-injected rats **(a–d)** and dexamethasone-injected control rats **(e–h)** stained with hematoxylin and eosin (original magnification 400; bars indicate 50 μm). There was no obvious morphological evidence of delay in olfactory mucosa regeneration in the dexamethasone-treated rats compared to control rats. (a, e) The olfactory mucosa was severely damaged, so that only a few layers of olfactory receptor neurons remained on day 2 after the 5% zinc sulfate solution insult. (b, f) On day 5, four or five layers of olfactory receptor neurons were observed, but there was no obvious presence of sustentacular cells. (c, g) On day 8, the sustentacular cells had started to regenerate and seemed more mature in the dexamethasone-injected rats. However, the number of layers of olfactory neurons of the dexamethasone-injected rats was the same as that of the control rats (6 or 7 layers). (d, h) On day 14, 8 or 9 layers of olfactory neurons were observed in both rats. The morphological regeneration of the olfactory mucosa completed in both group.

then becomes dimerized and the dimers bind to glucocorticoid response elements (GRE) in target DNA enhancer sequences. This usually results in gene activation (Bookstein *et al.*, 1992). A functional GRE at position –650 of the Na^+/K^+ ATPase β_1 gene promotor for both glucocorticoids and mineralocorticoids has been reported (Derfoul *et al.*, 1998).

Glucocorticoids usually down-regulate GR in an intact organ (Bookstein *et al.*, 1992), but their regulatory activities in various organs during regeneration remain to be solved.

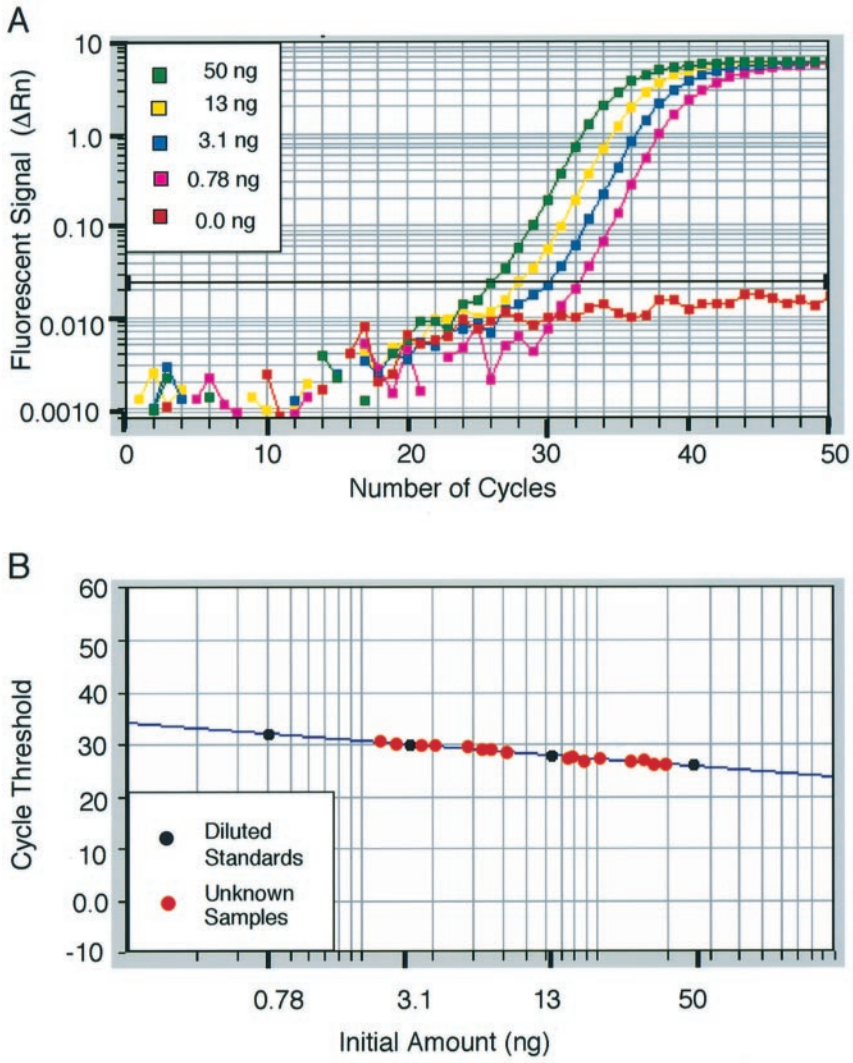

Figure 4 **(A)** An example of an amplification plot for a standard curve construction of Na^+/K^+ ATPase mRNA expression. Serial dilution of 1 μl of kidney cDNA solution (50 ng/μl), originally made from kidney total RNA, was used as a positive control. The kidney cDNA solution was diluted as 1 (green dots, 50 ng of cDNA), 4 (yellow dots, 13 ng), 16 (blue dots, 3.1 ng) and 64 (purple dots, 0.78 ng). The red curve represents a negative control without kidney cDNA that did not reach threshold cycle after 50 cycles of amplification. Only one of each duplicate standard sample per dilution is depicted for clarity. **(B)** An example of a standard curve for Na^+/K^+ ATPase amplification. The four black dots indicate kidney standards that were obtained from the amplification plot illustrated in Figure 5A. The 16 red dots represent samples obtained from the olfactory mucosa and plotted on the standard curve. The ratio of the expression of Na^+/K^+ ATPase from unknown samples to that of kidney cDNA was calculated.

In rats, glucocorticoid receptor expression was found to be elevated during liver regeneration and dexamethasone induced hepatic tyrosine aminotransferase and tryptophan oxygenase in the regenerating rat liver after partial hepatectomy (Karabélyos *et al.*, 1999). Our results represent supportive evidence that olfactory mucosa shows a similar up-regulation of GR during the regeneration period.

The significance of an increased expression of Na^+/K^+ ATPase in the olfactory mucosa during regeneration is twofold. One is that this increase maintains and restores the receptor cells' resting potential and the other is that it maintains the ionic balance of the olfactory mucus through transepithelial Na^+ absorption (Kern *et al.*, 1991; Fong *et al.*, 1999). These speculations are mainly based on indirect evidence of morphological studies. Since no direct evidence of electrophysiological data has been reported, direct measurements of the resting potential of the olfactory receptor neurons and the concentrations of sodium and potassium ions in the olfactory mucus during regeneration are required.

In the olfactory mucosa, both GR and Na^+/K^+ ATPase were up-regulated during regeneration, and glucocorticoid further enhanced these expressions. Our results suggest that the clinical implication of glucocorticoid administration is that it induces Na^+/K^+ ATPase expression during regeneration and may contribute to recovery from olfactory

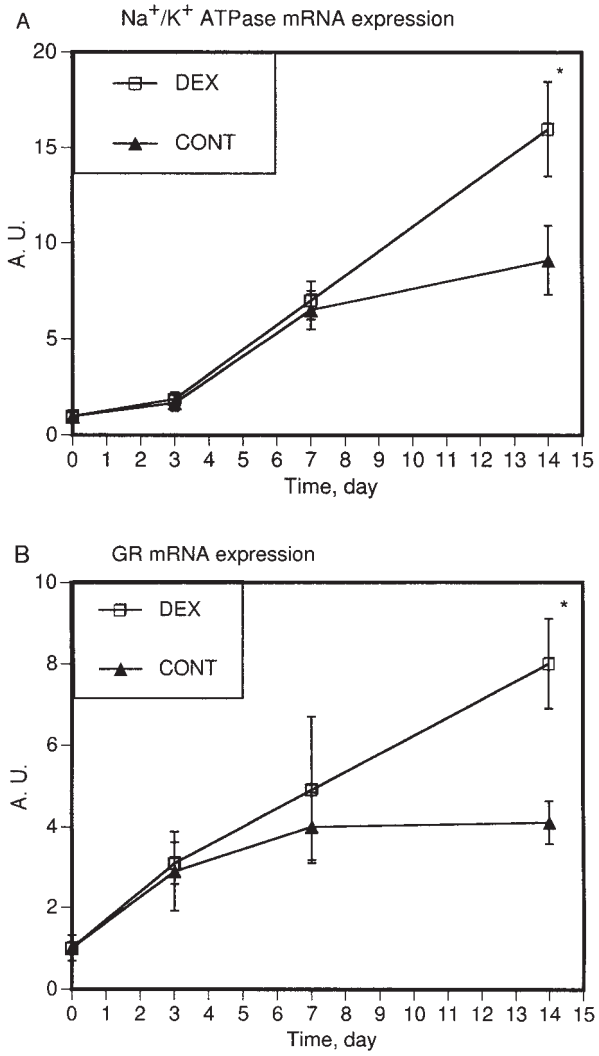

Figure 5 For each figure, the mRNA expression was calculated with the formula (quantitative PCR value of target gene)/(quantitative PCR value of 18S RNA expression). The expression of olfactory mucosa mRNA on day 0 was then standardized to 1.0 (n = 4 for each observation; DEX, dexamethasone injected rats; CONT, control rats; values represent means ± SD). **(A)** Na^+/K^+ ATPase mRNA expression in rat olfactory mucosa after 5% zinc sulfate solution treatment. Na^+/K^+ ATPase mRNA expression in the olfactory mucosa gradually increased after the insult. On day 14, there was a statistically significant increase of 76% in Na^+/K^+ ATPase expression of dexamethasone-treated rats compared to non-injected control rats (A.U., arbitrary unit; $*P = 0.0042$, unpaired t-test). **(B)** Glucocorticoid receptor (GR) mRNA expression of olfactory mucosa. GR mRNA expression of the olfactory mucosa was similar to mRNA expression of Na^+/K^+ ATPase, and this expression gradually increased after the insult, with GR expression of dexamethasone injected rats being 95% higher than that of control rats on day 14. (A.U., arbitrary unit, $*P = 0.00098$; unpaired t-test).

disturbance in part, at least, through its receptor by means of regulation of the ionic concentration in the olfactory mucosal microenvironment.

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