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Expression of Volume-Activated Anion Channels in Exocrine Acinar Cells

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Introduction

Volume-sensitive anion channels ($I_{Cl.swell}$) are expressed in most mammalian cells (1). The molecular identity of $I_{Cl.swell}$ is not known, however, several candidate proteins have been proposed including: p-glycoprotein, pI_{Cln}, ClC-2 and ClC-3 (2). The properties of ClC-3 make it one of the most likely candidate proteins, e.g. it has a structure which is very similar to that of known Cl^- channels (ClC-0 and ClC-1), and it produces an outward-rectifying Cl^- conductance when expressed in *Xenopus* oocytes or mammalian cell lines (3).

Lacrimal gland acinar cells are unusual because volume regulation is not thought to involve $I_{Cl.swell}$. Instead, cell swelling causes an increase in intracellular Ca^{2+} which is sufficient to active the Ca^{2+}-activated Cl^- channels allowing Cl^- efflux (4, 5). Lacrimal acinar cells may therefore provide a useful natural "knock-out" in which to study the role of ClC-3, i.e. they can be used to test the hypothesis that ClC-3 is not expressed in cells which do not exhibit $I_{Cl.swell}$. The present study has therefore examined the expression of ClC-3 and $I_{Cl.swell}$, in lacrimal gland acinar cells, using molecular biological and electrophysiological methods respectively.

RT-PCR for ClC3

Experiments were performed on mRNA isolated from rat lacrimal gland, submandibular salivary gland and brain (positive control). A single 259 bp PCR product was obtained with the ClC-3 primers and cDNA from the salivary gland and brain tissue. No product, however, was obtained from the lacrimal gland cDNA. Southern analysis confirmed that a ClC-3 product was obtained from salivary gland and brain, but not from the lacrimal gland.

Western analysis for ClC-3

Expression of ClC-3 protein was examined by western analysis using an antibody raised against rat ClC-3 (Alomone). A single protein which cross-reacted with the ClC-3 antibody was observed in brain and salivary gland. The immuno-reactive protein had a molecular weight of approximately 80 kDa, which is close to the predicted size of the ClC-3 protein (84.5 KDa) (6). The antibody did not cross react, however, with any protein in the lacrimal gland.

$I_{Cl.swell}$ in rat lacrimal and submandibular acinar cells

Experiments were performed using conventional whole-cell methods. K^+-free solutions were employed to eliminate any contribution from K^+-channels to the whole-cell conductance. The electrode solution contained 5 mM BAPTA to inhibit the Ca^{2+}-activated Cl^- channels. Cells were bathed either in an isotonic (306 mOsmol.Kg H_2O) or hypotonic (213 mOsmol.Kg H_2O) bath solution. Cell volume changes were monitored using a video-imaging method (5).

1) Submandibular acinar cells: On exposure to the hypotonic solution submandibular cells swelled to a maximum relative volume of 1.29 ± 0.05 (n=5) in 3 min. Cell swelling was accompanied by the development of an outward-rectifying conductance with properties which were similar to $I_{Cl.swell}$. The increase in the conductance lagged slightly behind the changes in cell volume, so that an increase in current was first observed after 91 ± 8 sec (n=7) and the maximum current attained in 298 ± 31 sec (n=7). The maximum current observed at Vm = +100 mV was 52.9 ± 3.7 pA/pF in submandibular cells.

2) Lacrimal acinar cells: The lacrimal gland cells swelled to a maximum volume (1.30 ± 0.03, n=5) within 3 min of exposure to the hypotonic solution. After a substantial latent period (306 ± 24 sec, n=7; significantly different to submandibular cells; $p < 0.05$), an increase in the whole-cell conductance was observed. The current had properties typical $I_{Cl.swell}$. Maximum activation occur-

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red at 1,110±53 sec ($p<0.05$), and the maximum current at Vm = +100 mV was 48.0±4.9 pA/pF (not significantly different from the submandibular cells; $p>0.1$).

Conclusions

Expression of mRNA encoding ClC-3 and ClC-3 protein was detected in rat submandibular gland by RT-PCR and western analysis. Rat lacrimal gland cells, however, expressed neither mRNA encoding for ClC-3 nor the ClC-3 protein. $I_{Cl.swell}$ was observed in both rat lacrimal gland and submandibular salivary gland acinar cells. The conductance was of a similar size in both cells, however, it was much slower to activate in the lacrimal cells. The data suggest that ClC-3 is not an absolute requirement for the expression of volume-sensitive Cl^- channels.

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