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CLONING AND ROLE IN AMILORIDE-SENSITIVE CHLORIDE SECRETION OF AN APICAL A1 RECEPTOR IN A6 EPITHELIA.

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Chloride secretion in the apical bath of A6 epithelia was recorded under short circuit conditions in the absence of apical Na^+. Treatment of the apical surface with adenosine (ADO, 3 μM) evoked a transient current increase within 60 s of 8.53±0.57 μA/cm^2. The peak current declined to a steady plateau of 2.49±0.24 μA/cm^2 (n=9). The currents were inhibited by NPPB (100 μM) and abolished after removal of Cl^- from the basolateral bath and thus identified as Cl^- secretion. A similar Cl^- secretion was observed by apical addition of a specific A1 agonist (N6-cyclopentyladenosine, CPA, 1 μM). Peak and plateau currents obtained with CPA were 3.19±0.30 and 1.56±0.15 μA/cm^2 (n=19), respectively. The idea of an involvement of an A1 receptor was supported by the complete inhibitory effect of an A1 antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 0.1 μM). Because intracellular Ca^{2+} concentration is hardly affected by ADO or CPA stimulation, Cl^- secretion does not seem to occur through Ca^{2+} activated Cl^- channels, but G-protein-coupled Cl^- channels could be involved. Amiloride (AMI) exerted a dose-dependent inhibitory effect on ADO and CPA activation of the Cl^- currents. With CPA=1 μM the AMI concentration for half maximal inhibition (K_A) was 1.26 μM. Increasing CPA to 2 μM augmented K_A to 2.45 μM. The shift of K_A indicates a competition of AMI with CPA at the adenosine-binding site. Further characterization of the A1 receptor was achieved by cloning, sequencing and functional expression in *Xenopus laevis* oocytes. A cDNA encoding the Xenopus A1 adenosine receptor (xA1) was obtained by RT-PCR starting from total RNA from A6 cells. Oligonucleotide primers were designed based on the cDNA sequence for the xA1 (GenBank/EMBL/DDBJ database: accession number AJ249842). The cloned receptor was functionally tested in *Xenopus laevis* oocytes by co-injection of xA1 cRNA and cRNA′s encoding mGIRK1 and mGIRK2 which form a bona fide G-protein-coupled inward rectifying K^+ channel (Kobayashi et al. 2002). Injected and control oocytes were exposed to high K^+ solutions (90 mM) and inward currents (I_K) were recorded at −70 mV. Basal I_K values were 210±64 nA (n=9). 100 nM ADO elicited a 3.5-fold stimulation of I_K. The addition of 50 μM AMI to the bath reduced the ADO-stimulated I_K component by 57%. These observations are consistent with a competitive inhibition between ADO and AMI at the xA1 receptor and indicate that the A1 receptor can activate G-protein-coupled ion channels.

Kobayashi et al. 2002. Br. J. Pharmacol. 135, 313-322.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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CELL SPECIFIC LOCALISATION OF AQUAPORIN-5 IN THE HUMAN ECCRINE SWEAT GLAND SECRETORY COIL

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Aquaporins, membrane water channels, provide a possible explanation for the route of osmotically driven water passage across the plasma membranes of many cell types. The aquaporin-5 (AQP-5) subtype has been localised in mouse, rat (Nejsum et al., 2002) and horse (Bovell et al., 2004) sweat glands. The human eccrine sweat gland differs from the other sweat glands by having both secretory and reabsorptive portions. The secretory coil has two major cell types defined as the dark and light cells, with the latter thought to have a major involvement in the production of sweat fluid. Although AQP-5 has been shown to be present in the reabsorptive duct portion (Nejsum et al., 2002), its presence in the secretory coil cells has not been completely elucidated. Using immunohistochemical techniques we have investigated the presence of AQP-5 within the secretory coil and reabsorptive duct of the human eccrine sweat gland.

Skin biopsies were obtained with informed patient consent and with local medical ethical committee approval. Samples were fixed, processed and embedded in paraffin wax using standard techniques. Staining was performed using a rabbit anti-AQP-5 antibody (1:500, DPC Biermann, Germany) with an avidin-biotin procedure for immunohistochemistry and an FITC labelled secondary antibody for immunofluorescence.

Human glands were found to have AQP-5-like immunoreactivity localised to the membranes of what appeared to be the light secretory cells of the eccrine secretory coil. The light cells but not the dark cells of the coil have been shown to express carbonic anhydrase II (CAII) (Brigmann et al., 1983) making CAII a good marker for light cells. Immunoreactivity showed that AQP-5 and CAII stained the same cells. Additionally, the reabsorptive duct was found to have AQP-5-like immunoreactivity in the apical membranes.

The localisation of AQP-5-like immunoreactivity at the membranes of the light cells of the eccrine secretory coil, provides evidence that these channels offer a possible route of water transport and are consequently implicated in the production of sweat. The reabsorptive duct is thought to be relatively impermeable to water although restricted movement may occur at the apical membrane (Burry et al., 1997). The localisation of AQP-5 at the apical membrane of the reabsorptive duct provides an explanation for this limited water movement and confirms the previous work of Nejsum and colleagues (2002).

Bovell et al., 2004 J. Physiol, 42P, In press

Brigmann et al., 1983 Am.J.Pathol, 112, 250-257

Burry et al., 1997 Wiener.Klinische.Wochenschrift, 109, 542

Nejsum et al., 2002 Proc.Natl.Acad.Sci.U.S.A, 99(1), 511-6

Thanks to the patients of the Princess Royal Maternity Hospital

Where applicable, the experiments described here conform with Physiological Society ethical requirements.