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Aquaporin Water Channels in Exocrine Glands

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Exocrine glands secrete large volumes of fluid in response to neural or hormonal stimulation. Micropuncture studies have shown that in most cases the acini generate a near-isotonic primary fluid that is subsequently modified as it flows through the ductal system of the gland. Fluid secretion is dependent on metabolism and appears not to be due to pressure-filtration. It can even continue in the presence of relatively large opposing hydrostatic pressure gradients. The simplest interpretation of these findings is that (a) water flow is coupled osmotically to the active secretion of electrolytes by the acinar cells, and (b) the water permeability of the epithelium is sufficiently large for the water flow to be driven by a relatively small osmotic gradient.

Until recently, very little was known about the cellular pathway of water transport in exocrine glands. Unlike absorptive epithelial cells, acinar cells may have a very small apical (luminal) membrane area which inevitably restricts the transcellular flow of water. In addition, measurements of the water permeability of salivary acinar cell membranes suggest that the transcellular permeability might not be sufficient to account for the observed secretory flow rates (1). Indirect observations have raised the possibility that water flow might instead occur via a paracellular pathway through the tight junctions between the cells (2).

Interest in this problem was renewed in 1992 when the water channel protein CHIP28 was discovered in the erythrocyte membrane (3). This was subsequently renamed aquaporin-1 (AQP1) and found to be just one of a family of at least ten related mammalian water channel proteins, each with a characteristic distribution amongst the various fluid-transporting epithelia and endothelia of the body (4, 5).

The aquaporins all share a common basic structure, consisting of a single polypeptide chain of about 30 kDa with six membrane-spanning domains and connecting loops (6, 7). The water-selective pore is thought to be created by interactions between hydrophobic regions of two of the loops (8). Some aquaporins are exclusively permeable to water while others are also permeable to glycerol and/or urea. Unfortunately, physiological studies are hampered by the lack of selective aquaporin blockers. The only effective blockers are the mercurial sulphydryl reagents, and not all of the aquaporins are blocked by these.

Not surprisingly, aquaporins have now been identified in a number of mammalian exocrine glands and are thought to play a significant role in fluid secretion. We focus here on the expression, localisation and function of aquaporins in the salivary glands and exocrine pancreas.

Salivary glands

Given the two-stage mechanism of salivary secretion, AQP expression might be anticipated in the acini, where the isotonic primary fluid is generated, but not in the ductal system where a low transepithelial water permeability allows the production of a hypotonic saliva.

Although AQP1 is expressed in rat parotid and submandibular glands, immunohistochemistry has shown that it is located in the capillary endothelial cells rather than in the secretory epithelial cells (9) and is therefore not directly involved in fluid secretion.

Another member of the aquaporin family, AQP5, which was cloned first from rat submandibular gland, is found in the salivary and lacrimal glands, and also in the secretory epithelia of the lungs and eye (10). In the salivary glands it is located in the apical and canalicular membranes of the acini (11-13) where its presence may compensate for the very small area of this membrane domain. There is evidence from immunoelectron microscopy that AQP5 is also localised in the apical membranes of the intercalated duct cells, suggesting that these cells may also provide a pathway for osmotic water flow into the secreted fluid.

A functional role for AQP5 in salivary secretion has been indicated by western blot studies showing that, in

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the rat parotid, the channels undergo translocation from intracellular membranes to the apical membrane of the acinar cells following cholinergic stimulation (14). However, the most direct evidence for the functional significance of AQP5 in salivary secretion comes from studies of AQP5 knock-out mice in which pilocarpine-induced saliva production is reduced by 60% and the saliva is markedly hypertonic (15).

It is not yet clear whether aquaporins are also present in the basolateral membranes of salivary acinar cells. Although AQP3 and AQP4 are found in this location in other secretory epithelia, neither has been detected in the major salivary glands, apart from one report of AQP4 expression in the excretory duct of an unspecified rat salivary gland (16). On the other hand, AQP8 has recently been detected by RT-PCR (17), and in situ hybridisation studies indicate that AQP8 may be expressed in the acinar cells (18) so this would be an obvious candidate for the basolateral membrane.

Very little is known about the pattern of expression of aquaporins in human salivary glands although there is no reason to suppose that it will be markedly different from the situation in rodents. However, RT-PCR and northern blot studies of human minor salivary glands (labial and buccal glands) have shown that these glands express not only AQP1 and AQP5 but also AQP3. Preliminary immunohistochemical data suggest that AQP1 is present in both the capillaries and in the myoepithelial cells (19), while AQP3 and AQP5 are present in the basolateral and apical membranes respectively of the mucous acinar cells.

Exocrine pancreas

The exocrine part of the pancreas differs from salivary glands in that both the acini and the ductal epithelial cells are capable of secreting a near-isotonic fluid in response to appropriate stimulation. Allowing for both luminal and basolateral locations, there are therefore at least four possible sites where aquaporin expression might be expected.

High-stringency northern analysis of RNA extracted from whole rat pancreas shows a strong signal for AQP8. This is to be expected since AQP8 was originally cloned from pancreas and liver (18). A weaker signal is detected for AQP1, but nothing for AQP3, AQP4 or AQP5. Immunohistochemistry shows that AQP1 is again confined to the microvasculature, but AQP8 is clearly associated with the luminal membranes of the acinar cells and may therefore play a role in acinar fluid secretion. Furthermore, immunoelectron microscopy has revealed labelling of intracellular vesicles in the sub-apical region of these cells suggesting a regulated trafficking of AQP8 analogous to that of AQP5 in salivary glands.

Since the ducts represent such a small fraction of the pancreas, ductal aquaporins are much less likely to be detected by northern or RT-PCR analysis of RNA extracted from the whole pancreas. Studies are therefore in progress to identify the ductal aquaporins by RT-PCR of RNA from interlobular duct segments isolated by collagenase digestion and microdissection. The fact that none of the antibodies for the known aquaporins show positive staining of the ductal system in the rat pancreas suggests either that novel aquaporins await discovery in these cells or that aquaporins are not required for ductal fluid secretion.

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