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The Role of Aquaporin Water Channels in Fluid Secretion by the Exocrine Pancreas

B. Burghardt[1], **S. Nielsen**[2], **M.C. Steward**[3]
[1]Molecular Oral Biology Research Group, Department of Oral Biology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
[2]Water and Salt Research Centre, University of Aarhus, DK-8000 Aarhus, Denmark
[3]Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK

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Abstract. The mammalian exocrine pancreas secretes a near-isosmotic fluid over a wide osmolarity range. The role of aquaporin (AQP) water channels in this process is now becoming clearer. AQP8 water channels, which were initially cloned from rat pancreas, are expressed at the apical membrane of pancreatic acinar cells and contribute to their osmotic permeability. However, the acinar cells secrete relatively little fluid and there is no obvious defect in pancreatic function in AQP8 knockout mice. Most of the fluid secreted by the pancreas is generated by ductal epithelial cells, which comprise only a small fraction of the gland mass. In the human pancreas, secretion occurs mainly in the intercalated ducts, where the epithelial cells express abundant AQP1 and AQP5 at the apical membrane and AQP1 alone at the basolateral membrane. In the rat and mouse, fluid secretion occurs mainly in the interlobular ducts where AQP1 and AQP5 are again co-localized at the apical membrane but appear to be expressed at relatively low levels. Nonetheless, the transepithelial osmotic permeability of rat interlobular ducts is sufficient to support near-isosmotic fluid secretion at observed rates. Furthermore, apical, but not basolateral, application of Hg^{2+} significantly reduces the transepithelial osmotic permeability, suggesting that apical AQP1 and AQP5 may contribute significantly to fluid secretion. The apparently normal fluid output of the pancreas in AQP1 knockout mice may reflect the presence of AQP5 at the apical membrane.

Key words: Aquaporins — Water channels — Epithelial transport — Exocrine secretion — Osmotic permeability — Isosmotic fluid transport — Paracellular pathway

Correspondence to: M. Steward; email: martin.steward@manchester.ac.uk

Introduction

The human exocrine pancreas secretes approximately two liters of fluid per day. This serves both as a vehicle for the powerful digestive enzymes produced by the pancreatic acinar cells, and also as a rich source of HCO_3^- ions, which help to regulate the pH of the duodenal contents. As a secretory epithelium, the pancreas is remarkable for its capacity to generate a virtually isotonic $NaHCO_3$ solution. Although much interest focuses on the secretory mechanisms responsible for HCO_3^- secretion (Steward, Ishiguro & Case, 2005), there are still unanswered questions relating to the pathways and driving forces responsible for water transport across the secretory epithelium. Our aims here are (i) to outline the basic characteristics of pancreatic fluid secretion, (ii) to describe the cellular distribution of aquaporin (AQP) water channels in the pancreas, and (iii) to evaluate their contribution to the secretory process using recent data from isolated duct preparations and knockout animal models.

Cellular Origin of the Secreted Fluid

Both functionally and histologically, the epithelial cells of the exocrine pancreas consist of two populations: (i) the acinar cells, which secrete digestive enzymes in response to the hormone cholecystokinin (CCK), and (ii) the ductal epithelial cells, which line the converging system of ducts that lead to the duodenum. Unlike the salivary glands, where the ductal system has a predominantly reabsorptive role, the ductal epithelium in the pancreas plays a major role in fluid secretion.

Although there are significant species differences, CCK generally evokes relatively little fluid secretion

from the pancreas (Case & Argent, 1993). What there is tends to be rich in Cl^- rather than HCO_3^- and is thought to derive from the acinar cells. Stimulation with secretin, on the other hand, evokes large volumes of HCO_3^--rich fluid and most evidence indicates that this is produced by the ductal epithelium. For example, in vivo treatments that lead to acinar cell atrophy greatly diminish the CCK-evoked fluid and protein output whilst having relatively little effect on the secretin-evoked production of HCO_3^--rich fluid (Smith, Sunter & Case, 1982). Micropuncture data also support the idea that the HCO_3^--rich fluid is secreted downstream from the acini (Mangos & McSherry, 1971; Lightwood & Reber, 1977). More recently, studies on isolated pancreatic duct segments have shown clearly that these are capable of fluid secretion in response to secretin and other secretagogues in the absence of acinar cells (Argent et al., 1986).

Characteristics of the Secretory Mechanism

Pioneering studies on the isolated, perfused cat pancreas showed that the juice secreted by this gland was almost exactly isosmotic with the perfusate over a wide range of osmolarities (Case, Harper & Scratcherd, 1968). This was true regardless of whether NaCl or sucrose was added to the perfusate; but when sucrose was added, the increase in the osmolarity of the secretion was found to be due to an increase in salt concentration rather than sucrose. This is exactly what had been observed in earlier experiments on the fluid-absorbing epithelium of the gall-bladder (Diamond, 1964) and such results had led to the concept of 'isotonic' water transport. Osmotic coupling theories were developed in an attempt to explain how epithelia could transport salt and water in approximately isosmotic proportions in the absence of an osmotic driving force, and how they could even transport water *against* an opposing osmotic or hydrostatic gradient. (Curran & MacIntosh, 1962; Whitlock & Wheeler, 1964; Diamond & Bossert, 1967).

The consensus view was, and probably still is, that salt and water fluxes are coupled osmotically by a small hypertonicity maintained as a result of salt transport into a restricted space somewhere within the epithelium — probably the lateral intercellular space. With sufficiently high osmotic permeabilities at the apical and basolateral membranes, relatively small osmotic gradients would be sufficient to drive the observed water flow through the transcellular pathway (Spring, 1983). Other data, however, suggested that water crosses the epithelium via a paracellular pathway through the tight junctions, and that the driving force might not even be osmosis (Hill, 1980; Hill & Shachar-Hill, 1993).

Because of the geometry of the secretory epithelium in exocrine glands, it is difficult to control the composition of the fluid bathing the apical surface of the secretory cells. Consequently we do not know whether the epithelia of the pancreas can transport water against opposing osmotic or hydrostatic gradients. However, there have been various attempts to define the paracellular permeability of the pancreas — both by measuring the secretion of radioactively labeled non-electrolytes of different sizes (Jansen, De Pont & Bonting, 1979) and by examining the extent to which the bulk addition of non-electrolytes reduces the secretory rate (Dewhurst et al., 1978; Bonting et al., 1980). The conclusions are that the pancreas is moderately leaky to small extracellular markers such as mannitol, sorbitol and erythritol — the rabbit pancreas much more so than the cat pancreas. There is also a small but finite permeability to larger markers such as sucrose and inulin, but it is thought that these might pass through the cells in vesicles by a process of transcytosis (Melese & Rothman, 1983).

Interestingly, the permeability of the pancreas to extracellular markers appears to increase following stimulation with secretin (Jansen et al., 1979). As the secretory flow rate increases, so too does the concentration of non-electrolyte tracers in the secreted fluid — the opposite of what would be expected if the non-electrolytes entered the fluid by simple diffusion. Such a phenomenon might be indicative of solvent drag, implying that there is water flow through the paracellular pathway. On the other hand, it could simply reflect a substantial increase in diffusive non-electrolyte permeability. Resolving this question will require more detailed information about the relationship between the non-electrolyte fluxes and the secretory water flow.

Given the apparently tight osmotic coupling between salt transport and water flow in the pancreas, it was widely anticipated that the secretory epithelial cells might express aquaporin (AQP) water channels. Much of the recent work on water transport in the pancreas has therefore aimed to determine the cellular localization of aquaporins within the gland and to evaluate their contribution to epithelial water permeability and fluid secretion in vivo.

Water Channel Expression in Pancreatic Acinar Cells

Of the eleven known aquaporins in mammals (King, Kozono & Agre, 2004), several are associated with exocrine glands. In particular, AQP5 is present at the apical membrane of the acinar cells in salivary, lacrimal and airway submucosal glands, and AQP3 and AQP4 are often found at the basolateral membrane. This pattern of distribution is consistent with expectation because the acinar cells are thought to be the main site of isotonic fluid secretion in these tissues.

Without the enhancement of water permeability due to AQP5, the very small apical surface area of a salivary acinar cell, for example, might pose a serious limitation to its ability to secrete a near-isosmotic fluid. Support for this idea comes from studies of AQP5 knockout mice in which saliva production was found to be reduced in volume and increased in osmolarity (Ma et al., 1999). Similar changes have been seen in the output of airway submucosal glands in these animals (Song & Verkman, 2001), consistent with a reduced apical membrane water permeability. However, in both cases, the absence of AQP5 also reduced total electrolyte output, suggesting that AQP5 may have other roles in salivary and submucosal gland secretion (Hill, Shachar-Hill & Shachar-Hill, 2004). Furthermore, in other exocrine systems, notably lacrimal glands (Moore et al., 2000) and sweat glands (Song, Sonawane & Verkman, 2002), deletion of AQP5 has no significant effect on the volume or osmolarity of the secretion, raising further questions about the precise role of aquaporins in the secretory mechanism.

Aquaporin 8

Although initial studies did not report any expression of AQP3, −4 or −5 in the pancreas, a new family member, AQP8, was eventually cloned from rat liver and pancreas (Koyama et al., 1997). Given that the ducts are thought to be the predominant site of fluid secretion in the pancreas, we were surprised to find that AQP8 is localized exclusively at the apical membrane of the acinar cells (Hurley et al., 2001). Immunoelectron microscopy confirmed the presence of AQP8 at the apical surface of these cells and also in subapical intracellular vesicles, raising the possibility that there might be regulated trafficking of AQP8 to the apical membrane. Although the apical surface of the acinar cells has a very small area, the fluid output of these cells is also thought to be relatively small, so it is not clear why water-channel expression in the pancreas should be concentrated here, particularly as neither AQP8 nor any of the other AQP isoforms were detected in the ductal epithelium.

In order to determine the functional role of AQP8 in rat pancreatic acinar cells, we measured the osmotic water permeability of the acinar cell plasma membrane (Hurley et al., 2001). Cells were isolated by collagenase digestion of minced rat pancreas (Fig. 1*A*) and video microscopy was used to record the changes in cell volume induced by a hypotonic challenge. In the control experiments illustrated in Fig. 1*B*, the osmolarity of the perfusate was reduced to 50% by withdrawing 145 mM sucrose from an initially isosmotic solution. The cells increased in volume by about 60% in approximately one minute. From the initial rate of swelling and the initial cell radius we estimated the osmotic permeability P_f of the plasma membrane to be approximately 10^{-3} cm s^{-1}. Since water flows across the apical and basolateral membrane domains could not be distinguished, this figure represents a lumped permeability value for the whole surface of the cell. This estimate of P_f is comparable with that of many mammalian cell plasma membranes. It indicates that the acinar cell membrane is more permeable than a phospholipid bilayer but about an order of magnitude less permeable than the erythrocyte membrane. Interestingly, there was no significant difference in the measured P_f values of single cells and coupled pairs of cells, or 'doublets', such as the one shown in Fig. 1*A*. In the doublets, the apical surfaces of the two cells remain juxtaposed and isolated from the bath fluid by a ring

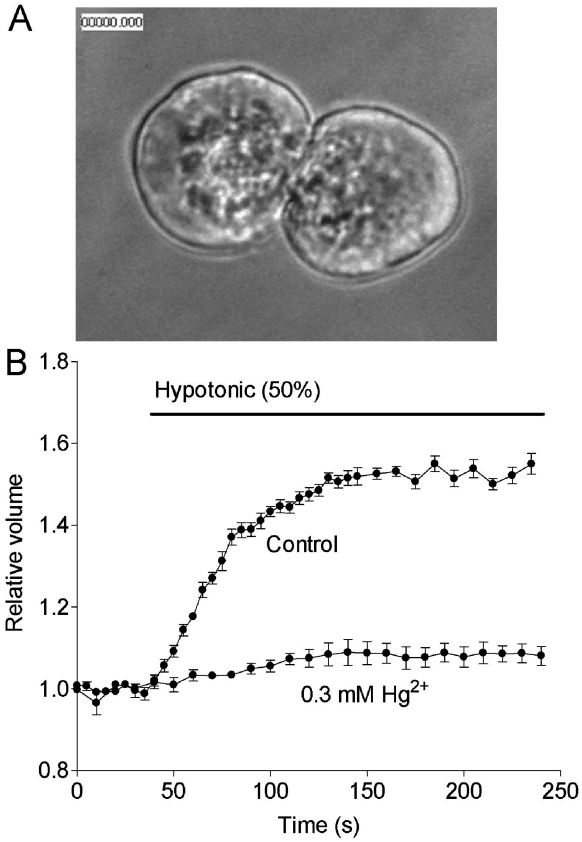

Fig. 1. Measurement of the osmotic water permeability of pancreatic acinar cell membranes. (*A*) Bright field image of acinar cells isolated from rat pancreas by collagenase digestion, and perfused with HEPES-buffered physiological salt solution at 37°C. The image area occupied by single cells, or doublets such as this, was measured in image sequences captured at timed intervals by video microscopy. Relative areas were converted to relative volumes by assuming that the individual cells were spherical. Acinar cell diameters were 15–20 μm. (*B*) Changes in the relative volume of rat pancreatic acinar cells subjected to a hypotonic challenge by withdrawal of 145 mM sucrose from an initially isotonic solution (Control, mean ± SEM, $n = 6$). Data are also shown for cells pre-treated with 0.3 mM $HgCl_2$ for 10 min ($n = 5$).

of contact at the tight junction. If most of the water permeability of the membrane is concentrated at the apical surface, as the AQP8 localization data suggest, then one has to conclude that the tight junction itself has a substantial water permeability.

To evaluate the contribution of AQP water channels to the osmotic permeability of the acinar cell membrane, we examined the effect of a 10 min pre-treatment with 0.3 mM Hg^{2+}, which is known to effectively block most of the AQP isoforms, including AQP8 (Ishibashi et al., 1997). Exposure to Hg^{2+} dramatically reduced the rate of swelling of both single cells and doublets in response to the hypotonic challenge (Fig. 1*B*). We estimated that the value of P_f was reduced by an order of magnitude to approximately 10^{-4} cm s^{-1}, a value that would be more typical of a simple phospholipid bilayer. If the drop in P_f were entirely attributable to block of the apical AQP8 channels, this would seem to be a rather large change, given that AQP8 is confined to such a small area. It raises the possibility that there might also be water-channel expression at the basolateral membrane, although there is no independent evidence of this, and immunohistochemistry has so far failed to detect any of the other known AQP isoforms.

Perhaps surprisingly, AQP8 knockout mice follow normal growth patterns and show no obvious defect in the processing of dietary fat (Yang et al., 2005). This finding raises questions about the significance of AQP8 in acinar cell function. A defect in pancreatic enzyme secretion resulting from the absence of AQP8 would certainly have affected the growth and development of the animals. No measurements of pancreatic secretion were reported in this study, so it remains possible that CCK-evoked fluid secretion is impaired in these animals, but this clearly has little physiological impact under normal conditions.

Water Channel Expression in Pancreatic Ducts

In considering fluid secretion in the ductal system of the pancreas, it is dangerous to regard the ductal epithelium as a homogeneous tissue. There are distinct differences in structure and function along the length of the ductal system, and it has become clear that some regions are more important than others in the process of fluid secretion. This heterogeneity has now become particularly apparent in the expression pattern of aquaporin water channels.

Aquaporin 1

Although earlier immunohistochemical studies had failed to detect any AQP expression in rat pancreatic ducts (Hurley et al., 2001), Ko et al. reported AQP1 immunoreactivity in the interlobular ducts of the rat pancreas, both in tissue sections and in isolated duct segments (Ko et al., 2002). Their results were supported by positive RT-PCR data which confirmed the presence of mRNA for AQP1 in isolated interlobular ducts. In addition, immunoelectron microscopy revealed patchy immunolabelling of AQP1 in some of the larger intralobular ducts, but none at all in the acinar cells, centroacinar cells and small intralobular ducts (Furuya et al., 2002).

Although we had previously reported strong AQP1 labeling in the vasculature of the rat pancreas, in contrast to Ko et al., we had not seen any labeling in the ductal system (Hurley et al., 2001). The reason for the discrepancy was finally resolved when we raised the AQP1 antibody concentration used for the labeling reaction by a significant margin (Burghardt et al., 2003). It then became possible to detect labeling at both the apical and basolateral surfaces of the rat interlobular duct cells (Fig. 2*B*). The need to use such high antibody concentrations, however, indicates that the expression of AQP1 in the duct cells is probably at a much lower level than in the capillaries. It was also evident that, apart from the strong labeling of the capillaries and venules, there was no AQP1 labeling of acinar or ductal structures within the secretory lobules (Fig. 2*A*).

In the human pancreas, the situation is quite different (Burghardt et al., 2003). There is abundant immunolabelling of AQP1 in all of the smaller ducts but rather less in the interlobular ducts. Within the secretory lobules, AQP1 is readily detected at both the apical and basolateral membranes of the intercalated ducts and intralobular ducts (Fig. 2*C*). It is also abundant in the centroacinar cells, which are interspersed amongst the acinar cells and located not only at the centre of the acini but also extending to their margins. Outside the secretory lobules, AQP1 is present in some of the smaller interlobular ducts but in these it seems to be confined to the apical membrane of the epithelial cells (Fig. 2*D*). Downstream, the expression of AQP1 quickly diminishes with distance, and most of the medium-sized and larger interlobular ducts show no labeling.

The distribution of AQP1 along the ductal system matches quite closely with that of the key transport proteins involved in electrolyte secretion. Thus CFTR, the anion channel that plays a major role in secretin-evoked secretion, is largely confined to the apical membrane of the intercalated and intralobular ducts in the human pancreas (Crawford et al., 1991; Marino et al., 1991). Its co-localization with AQP1 (Burghardt et al., 2003) in these proximal elements of the ductal system suggests that the bulk of the secretin-evoked secretion may be generated there. Conversely, the sparseness of AQP expression in the larger, distal interlobular ducts suggests that they have a more passive role in the human pancreas,

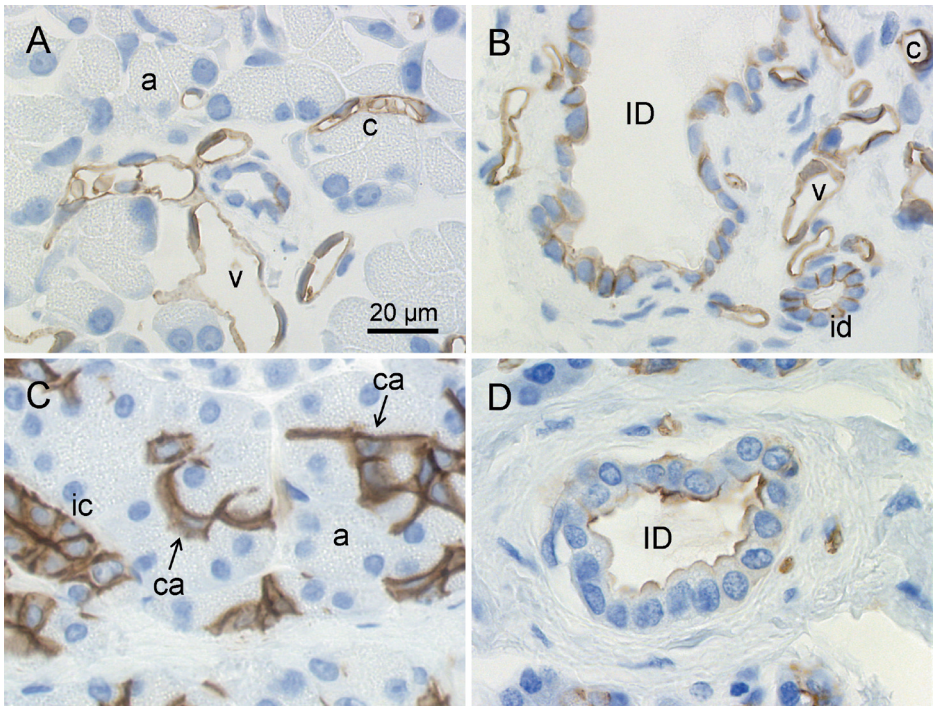

Fig. 2. Localization of AQP1 expression in paraffin sections of rat (*A, B*) and human (*C, D*) pancreas by immunoperoxidase labeling. (*A*) Strong labeling of the microvasculature (*c*, capillaries; *v*, venules) but not of the acinar cells (*a*) in rat pancreas. (*B*) Apical and basolateral labeling of rat interlobular duct (*ID*) and a smaller intralobular duct (*id*). (*C*) Strong labeling of centroacinar cells (*ca*) and intercalated ducts (*ic*) in human pancreas. (*D*) Apical membrane labeling of a small human interlobular duct (*ID*). Full methodology is described in Burghardt et al. (2003). All images are at the same magnification.

perhaps acting chiefly as conduits for the secreted fluid.

A similar correlation may apply in the rat pancreas because it is well established that the interlobular ducts, where AQP1 is primarily located, express CFTR and are capable of secreting fluid in response to stimulation with secretin (Argent et al., 1986; Gray, Greenwell & Argent, 1988). The lower level of AQP1 expression in the rat ducts may be linked to the fact that the fluid output from the rat pancreas is relatively low compared with other species (Case, 2006). Alternatively, it may reflect the much larger apical surface area of the interlobular duct cells in the rat pancreas compared with the intercalated duct cells in the human pancreas, which secrete into a very narrow luminal space and consequently have a very small apical surface area.

It is interesting to note that rat cholangiocytes, the epithelial cells that line the bile ductules within the liver, also express AQP1 at both apical and basolateral membranes (Nielsen et al., 1993). These cells closely resemble pancreatic duct cells in being responsive to secretin and in secreting a HCO_3^--rich fluid. It has also been suggested that AQP1 trafficking to the cholangiocyte plasma membrane may be promoted by secretin (Marinelli et al., 1997, 1999). It would be interesting to see whether the same is true in the pancreatic duct.

Given the importance of the liver and pancreas in fat digestion, the observation that dietary fat processing is defective in AQP1 knockout mice (Ma et al., 2001) would appear to support the idea that AQP1 is required for normal pancreatic and biliary secretion. However, the same study also reported that neither the secretory flow rate nor the pH of the bile and pancreatic juice was significantly altered in the knockouts compared with wild-type animals.

The lack of effect of AQP1 deletion on pancreatic secretion prompted us to explore the localization of AQP1 in the normal mouse pancreas by immunohistochemistry. Our main conclusion was that AQP1 expression levels were even lower in the mouse pancreas than in the rat. Not surprisingly, there was no AQP1 labeling of the acinar cells or ductal structures within the secretory lobules (Fig. 3*A*) although the strong labeling of the microvasculature provided a positive internal control. But even in the interlobular ducts, AQP1 labeling was sparse and confined to the apical membranes of just a limited number of cells (*arrows*, Fig. 3*B*). Given this result (and others described below) it is perhaps not surprising that pancreatic secretion in the mouse is not significantly affected by AQP1 deletion. On the other hand, we predict that a lack of AQP1 would have a more pronounced effect on pancreatic secretion in humans than in the mouse.

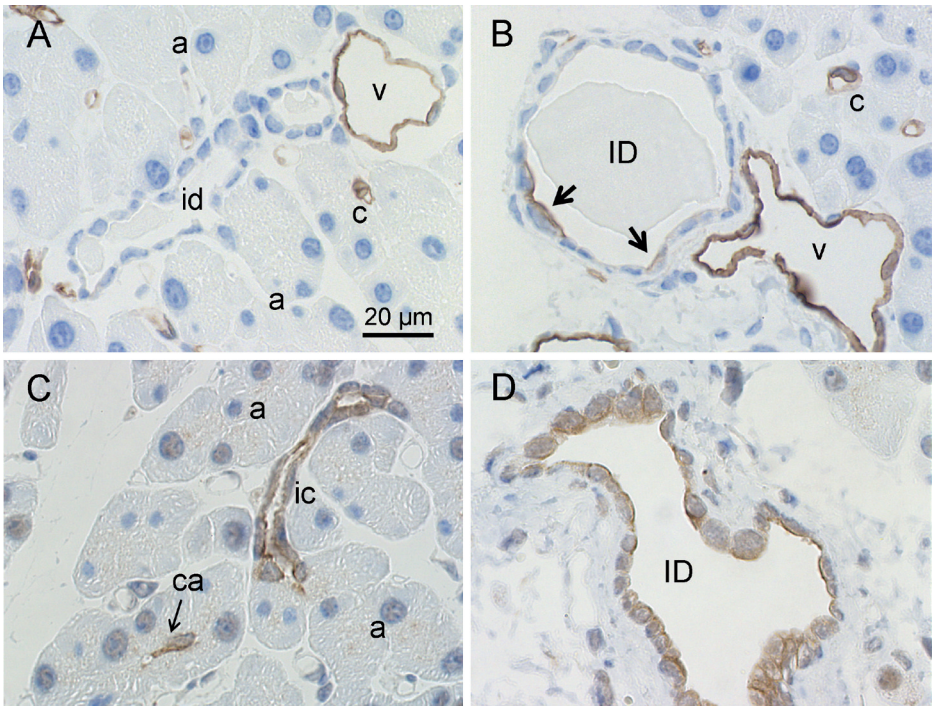

Fig. 3. Localization of AQP1 (*A, B*) and AQP5 (*C, D*) in paraffin sections of mouse pancreas by immunoperoxidase labeling. (*A*) Strong AQP1 labeling of the microvasculature (*c*, capillaries; *v*, venules) but not of the acinar cells (*a*) or intralobular ducts (*id*). (*B*) Sparse AQP1 labeling of apical membranes of interlobular duct (*ID*) cells. (*C*) AQP5 labeling of centroacinar cells (*ca*) and intercalated duct (*ic*) cells. (*D*) AQP5 labeling of the apical membranes of interlobular duct (*ID*) cells. Full methodology is described in Burghardt et al. (2003). All images are at the same magnification.

Aquaporin 5

Although both we and Ko et al. detected mRNA for AQP5 in rat pancreas by RT-PCR, neither laboratory was able to demonstrate protein expression by immunohistochemistry (Hurley et al., 2001; Ko et al., 2002). In the human pancreas, however, we did find AQP5 labeling but it was confined almost exclusively to the apical membrane of the intercalated duct cells (Burghardt et al., 2003). It co-localized almost exactly with CFTR, and therefore, by implication, with the AQP1 that is also present at the apical membrane of these cells. Unlike AQP1, however, AQP5 was absent from the basolateral membrane of the intercalated duct cells and centroacinar cells, and did not appear to co-localize with CFTR at the apical membrane of the acinar cells. Furthermore, AQP5 expression did not extend as far down the ductal system as AQP1.

The presence of both AQP1 and AQP5 at the apical membrane of the intercalated duct cells in the human pancreas is perhaps surprising. This degree of redundancy, not seen in other exocrine glands, may indicate that the tiny apical surface area of these cells would otherwise pose a significant barrier to water transport. The presence of AQP5 would certainly help to explain the lack of any obvious defect in pancreatic function in Colton antigen-null individuals who lack functional AQP1 (Preston et al., 1994).

We have also found evidence for AQP5 expression in the rat and mouse pancreas. Compared with salivary gland controls, where AQP5 is abundantly expressed at the apical and canalicular membranes of the acinar cells (Gresz et al., 2004), expression levels in the rodent pancreas seem to be significantly lower. By raising antibody concentrations, however, specific labeling can be detected at the apical membranes of the intercalated duct cells in the mouse pancreas and there is also some labeling associated with the centroacinar cells (Fig. 3*C*). Apical membrane labeling for AQP5 continues along the intralobular and interlobular ducts (Fig. 3*D*) and appears to be more uniformly distributed than for AQP1 (Fig. 3*B*). The labeling pattern for AQP5 in the rat pancreas (*not shown*) is very similar to that in the mouse. Although it is impossible to draw any conclusions about relative expression levels, the presence of AQP5 in these ducts might account for the apparently normal secretory rates evoked by secretin in the AQP1 knockout mice (Ma et al., 2001).

Taken together, these results suggest that, in the ductal system of the pancreas, both AQP1 and AQP5 are expressed at the apical membrane in those regions of the ductal tree where secretin-evoked fluid and electrolyte secretion is most pronounced: the intercalated ducts in humans and the interlobular ducts in rodents. AQP1 is also present at the basolateral

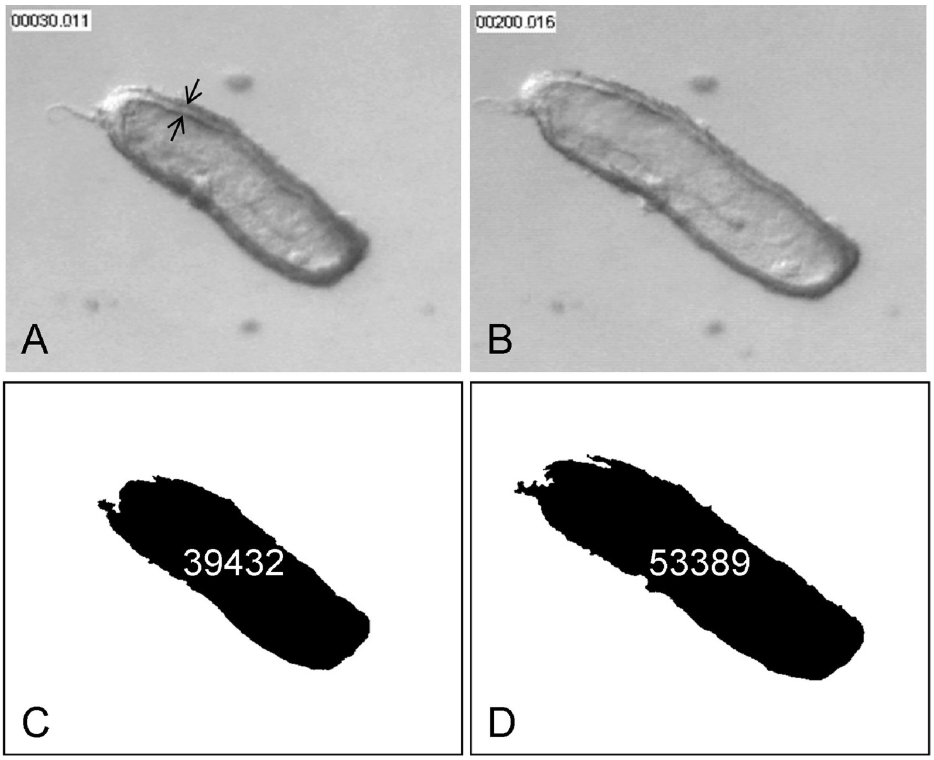

Fig. 4. Measurement of transepithelial water flow in isolated interlobular ducts isolated from rat pancreas by collagenase digestion and microdissection. The duct ends seal following overnight culture, thus fluid movement into the enclosed lumen results in duct swelling. *Upper panels* show bright field images captured 20 s before (*A*) and 150 s after (*B*) exposure to a hypotonic challenge by withdrawal of 145 mM sucrose from an initially isotonic perfusate. The thickness of the epithelium is indicated by the arrows. *Lower panels* (*C*, *D*) show the corresponding images after binary transformation together with pixel counts for the duct image area. Full methodology is described in Fernández-Salazar et al. (2004).

membrane in most of these ductal segments and, in semi-quantitative terms, AQP expression levels appear to be higher in the human intercalated ducts than in the rodent interlobular ducts.

Water Permeability of the Ductal Epithelium

Isolated ductal epithelial cells are very small and, so far, no measurements of the osmotic permeability of the plasma membrane have appeared in the literature. However, a unique feature of the pancreatic duct means that it is relatively easy to obtain estimates of the transepithelial osmotic permeability: when short segments of interlobular duct are isolated by collagenase digestion and microdissection, and are then stored overnight in tissue culture, the ends of the ducts seal to create a closed luminal space. This was first demonstrated using interlobular ducts from the rat pancreas (Argent et al., 1986) and the same procedure has subsequently proved successful with ducts from guinea-pig (Ishiguro et al., 1996) and mouse (Fernández-Salazar et al., 2004). As far as we can tell, the sealing of the ends of the duct results in complete restoration of epithelial integrity, and the ducts will swell to two or three times their initial volume, without leaking, when fluid is secreted or osmotically driven into the luminal space (Fig. 4). By calculating the rate of swelling from bright-field images captured by video microscopy it is possible to estimate volume flow per unit area of epithelium. Typical values for secretin- or forskolin-evoked secretion are 200–300 pl min^{-1} mm^{-2} in rat and mouse ducts and 600–800 pl min^{-1} mm^{-2} in the guinea-pig ducts (Fernández-Salazar et al., 2004).

Basal secretory rates are low in unstimulated rat ducts, so it is possible to examine the changes in duct volume that occur in response to step changes in the osmolarity of the bath solution. The experiments illustrated in Fig. 5 show that isolated rat ducts behave like near-perfect osmometers when exposed to solutions with a range of osmolarities. Bathed initially in an isotonic, HEPES-buffered salt solution containing sucrose, the ducts swelled reversibly when the sucrose concentration was reduced (Fig. 5*A*) and they shrank reversibly when the sucrose concentration was raised (Fig. 5*B*). Over the range of volume changes examined, there was little evidence of the ducts approaching their elastic limit, or of the luminal hydrostatic pressure rising to a level where it might reduce the inwardly directed osmotic flow. Assuming a reflection coefficient of 1 for sucrose, the volume

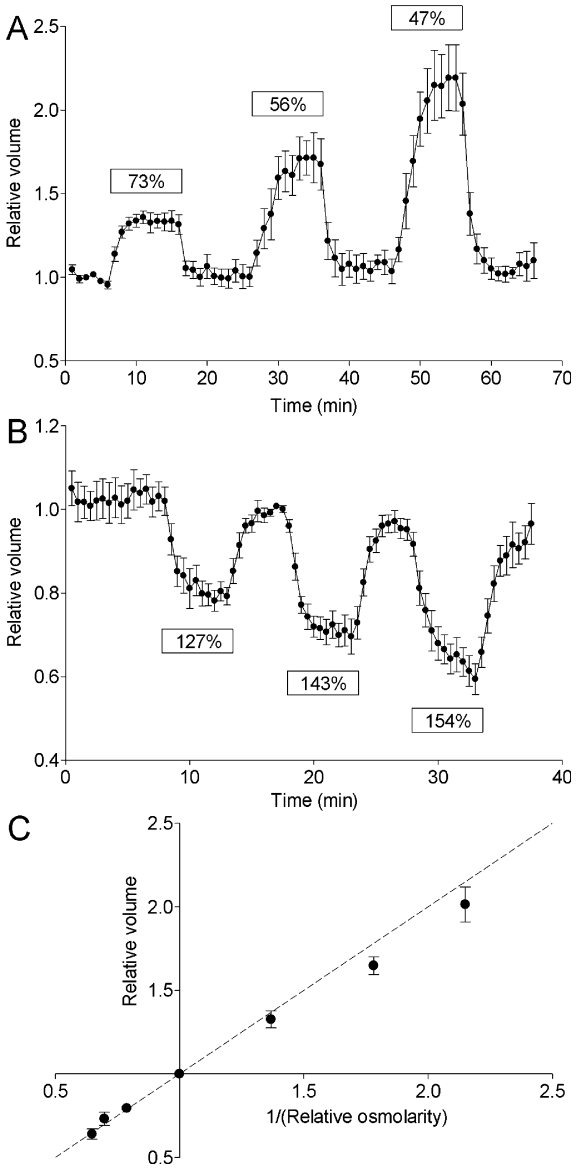

Fig. 5. Changes in the relative volume of rat interlobular ducts following step changes in the osmolarity of the perfusate (expressed as a percentage of the isotonic solution osmolarity). Bright field images were captured at 5 s intervals and subjected to image analysis as shown in Fig. 4. (***A***) Swelling of ducts exposed to increasingly hypotonic solutions achieved by removal of sucrose from an initially isotonic solution (mean ± SEM, $n = 8$). (***B***) Shrinkage of ducts exposed to increasingly hypertonic solutions achieved by addition of sucrose ($n = 12$). (***C***) Equilibrium volumes, attained with the three hypertonic and three hypotonic solutions, plotted against the reciprocal of the osmolarity normalized to that of the isotonic solution. The dashed line shows the predicted behavior of a perfect osmometer.

changes of the ducts appeared to lie close to ideal van t' Hoff behavior (Fig. 5*C*).

To estimate the transepithelial osmotic permeability in this preparation it is essential to use a tissue chamber optimized for rapid solution changes. In the experiments described here we measured the initial rate of swelling of the duct in response to a sudden drop in bath osmolarity achieved by withdrawing sucrose from an initially isosmotic solution. As shown in Fig. 6*A*, the ducts swelled rapidly and the volume increase was largely complete in less than one minute. Varying the flow rate through the tissue chamber did not significantly alter the initial rate of swelling, so we can be reasonably confident that the limiting factor here was the osmotic permeability of the duct epithelium rather than the time constant of the change in bath osmolarity. Unstirred layer effects were minimal because there was very little connective tissue covering the basal surface of the epithelium and the luminal space was itself only about 100 μm in diameter.

From the initial rate of swelling in response to a 145 mosM gradient of osmolarity, we estimate the osmotic permeability P_f of the ductal epithelium to be 1.7×10^{-2} cm s^{-1}. This is almost identical to the value obtained by Ko et al. (2002) and is comparable with values from other fluid-transporting epithelia (although it is an order of magnitude lower than the P_f of the renal proximal tubule). On the basis of this figure, and assuming a simple osmotic model for fluid secretion, we calculate that an osmotic gradient of just 1 mosM would be sufficient to drive the volume flow evoked by secretin stimulation. Experimentally, a small hypertonicity of this magnitude would be difficult to detect, so this result is entirely consistent with the apparent isotonicity of pancreatic juice. Compared with other epithelia that achieve near-isosmotic fluid transport, the volume flow across the epithelium of an interlobular duct isolated from rat pancreas is relatively low: 1–2 μl cm^{-2} h^{-1} compared with more than 50 μl cm^{-2} h^{-1} in mammalian gallbladder (Steward, 1982). It is therefore not surprising that a relatively small osmotic gradient is sufficient to generate a near-isosmotic secretion. But it remains to be seen whether higher secretory rates are observed, for example, in the intercalated ducts of the human pancreas, and whether the transepithelial P_f values there are also correspondingly greater.

Although the transepithelial osmotic permeability of the rat interlobular duct is clearly sufficient to support near-isosmotic secretion, none of the findings discussed so far allow us to distinguish between transcellular and paracellular osmotic flow. The transepithelial P_f value could be due either to a high transcellular permeability, enhanced by the presence of aquaporins at the apical and basolateral membranes, or to a high paracellular permeability via the tight junctions. On the basis of electrical resistance measurements, the ductal epithelium is a classical leaky epithelium (Novak & Greger, 1988) and it is widely accepted that pores through the tight junctions provide the main pathway for the secreted cations (Steward et al., 2005). We cannot therefore exclude

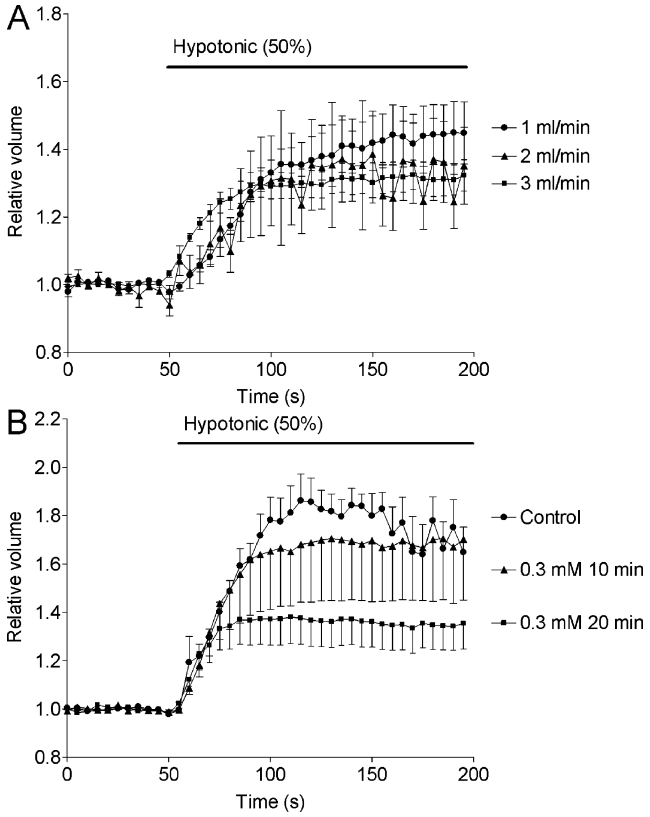

Fig. 6. Measurement of the transepithelial osmotic permeability of rat interlobular duct epithelium. Changes in duct volume induced by a 50% reduction in perfusate osmolarity were measured by the method illustrated in Fig. 4. (***A***) Varying the flow rate of perfusate (1–3 ml min^{-1}) through the tissue chamber had little effect on the initial rate of swelling of the ducts following the hypotonic challenge. The volume of the slot-shaped chamber was 220 µl. Data are means ± SEM (n = 4–5). (***B***) Swelling of ducts exposed to hypotonic challenge alone (*Control*) or following pre-treatment with 0.3 mM $HgCl_2$ for 10 min or 20 min (n = 4 in each case).

the possibility that these leaky tight junctions also provide a pathway for paracellular water flow.

Rat interlobular ducts express AQP1 water channels at both apical and basolateral surfaces, so the contribution of the basolateral channels can be evaluated by blocking them with Hg^{2+}. In our hands, pre-treatment of the isolated ducts with 0.3 mM Hg^{2+} for 10 or 20 min, which is normally sufficient to block AQP1 (Roberts et al., 1994), had no significant effect on the initial rate of duct swelling (Fig. 6*B*). It did, however, limit the final equilibrium volume of the duct, particularly after the longer period of Hg^{2+} treatment. If P_f had been significantly reduced by Hg^{2+}, we would have expected a slower initial swelling leading to a similar final volume. The fact that the final volume was reduced, rather than the initial rate, suggests that Hg^{2+} was reducing the distensibility of the duct wall rather than the osmotic permeability of the epithelium. We therefore conclude that basolateral AQP1 contributes relatively little to the transepithelial osmotic permeability of the duct.

In contrast, microinjection of 0.3 mM Hg^{2+} into the duct lumen reduces the transepithelial P_f of rat interlobular ducts by around 80% (Ko et al., 2002). The simplest interpretation of this result is that, without AQP1 and AQP5, the osmotic permeability of the apical membrane drops significantly and becomes rate limiting for transepithelial water flow in the interlobular duct. Although this result appears quite conclusive, it remains possible that luminal Hg^{2+} also interferes with the water permeability of the paracellular pathway. This question should be readily resolved by osmotic permeability measurements comparing isolated ducts from wild-type and AQP knockout mice.

Conclusions

Of the various mammalian epithelia that have the capacity to secrete or absorb a near-isotonic fluid, the pancreatic duct has been one of the slowest to yield to experimental investigation. This stems partly from prolonged uncertainty about the exact site of fluid secretion in the gland, and partly from the inaccessibility of the secretory epithelium for experimental studies. In salivary, lacrimal and sweat

glands, most of the secreted fluid is thought to arise as a plasma-like primary secretion in the secretory endpieces or acini — structures which comprise much of the mass of the gland. In the pancreas, however, most of the fluid secretion evoked by secretin derives from ductal epithelial cells that comprise only a small fraction of the gland mass. Furthermore, the secretion itself, although approximately isotonic, has a strikingly different anion composition from plasma, being particularly rich in HCO_3^- ions. As an added complication, the ductal system is longitudinally divided into several functionally and histologically distinct sections, only some of which are actively involved in fluid secretion. To make matters worse, the duct regions that play the predominant role in fluid secretion vary between species.

In all of the species examined so far, it seems that a relatively large volume of fluid is produced by a relatively small number of epithelial cells, so these could reasonably be expected to be a major site of water-channel expression. The first aquaporin to be firmly identified in the pancreas, AQP8, was surprisingly found to be located in the acinar cells, which secrete rather little fluid. However, in the tiny intercalated ducts of the human pancreas, which are probably the main site of fluid secretion, there is now evidence for abundant expression of AQP1 and AQP5. In rodents, where the larger interlobular ducts are thought to be the main site of fluid secretion, AQP expression levels appear to be relatively low, and knockout mouse models have not yet shown any abnormality of pancreatic secretion. This is not the first instance where there appears to be a disparity between AQP expression and water transport capacity (Verkman, 2002) and the possible reasons for this have been reviewed in detail elsewhere (Hill et al., 2004). Nonetheless, isolated duct preparations have revealed a marked inhibition of transepithelial osmotic permeability by luminal Hg^{2+}, and these clearly provide an attractive model system for further exploring the pathways and driving forces for transepithelial water flow. A careful dissection of the transepithelial osmotic permeability into its trans- and paracellular components should be readily achievable in such a system and the alterations in these parameters that occur in AQP knockout animals will be highly informative.

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