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What Do Aquaporin Knockout Studies Tell Us about Fluid Transport in Epithelia?

Oliver J. Maclaren · James Sneyd · Edmund J. Crampin

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Abstract The investigation of near-isosmotic water transport in epithelia goes back over 100 years; however, debates over mechanism and pathway remain. Aquaporin (AQP) knockouts have been used by various research groups to test the hypothesis of an osmotic mechanism as well as to explore the paracellular versus transcellular pathway debate. Nonproportional reductions in the water permeability of a water-transporting epithelial cell (e.g., a reduction of around 80–90 %) compared to the reduction in overall water transport rate in the knockout animal (e.g., a reduction of 50–60 %) are commonly found. This non-proportionality has led to controversy over whether AQP knockout studies support or contradict the osmotic mechanism. Arguments raised for and against an interpretation supporting the osmotic mechanism typically have partially specified, implicit, or incorrect assumptions. We present a simple mathematical model of the osmotic mechanism with clear assumptions and, for models based on this mechanism, establish a baseline prediction of AQP knockout studies. We allow for deviations from isotonic/isosmotic conditions and utilize dimensional analysis to reduce the number of parameters that must be considered independently. This enables a single prediction curve to be used for multiple epithelial systems. We find that a simple, transcellular-only osmotic mechanism sufficiently predicts the results of knockout studies and find criticisms of this mechanism to be overstated. We note, however, that AQP knockout studies do not give sufficient information to definitively rule out an additional paracellular pathway.

Keywords Aquaporin knockout · Epithelial transport · Osmosis · Aquaporins · Water transport · Osmotic mechanism

O. J. Maclaren (✉) · E. J. Crampin
Auckland Bioengineering Institute, The University of Auckland, 70 Symonds Street, Auckland 1010, New Zealand
e-mail: omac010@aucklanduni.ac.nz

J. Sneyd
Department of Mathematics, The University of Auckland, Auckland, New Zealand

E. J. Crampin
Department of Engineering Science, The University of Auckland, Auckland, New Zealand

Introduction

Water transport in epithelia plays a central role in many physiological processes, including those of the intestines, kidney, stomach, eyes, brain and the salivary glands. When significant concentration gradients exist in the direction of transport, the mechanism is osmotic; when there is transport in the absence of, or against, a gradient between bathing solutions, the mechanism and pathways are less clear (Reuss 2009). Under these latter conditions, the water transport is in the same direction as the net solute transport and approximately isosmotic; the standard explanation is again based on an osmotic mechanism, one which invokes localized coupling compartments and very small osmotic differences—"local osmosis" (Reuss 2009, 2010; Spring 1999). According to this mechanism, water flow follows active solute transport osmotically, due to the establishment of gradients in solute concentration across localized, membrane-separated compartments (such as the lateral intercellular space, the lumen, and the canaliculi). This water flow is taken to occur primarily through transcellular water channels known as aquaporins (AQPs).

The existence of water channels in red blood cells and epithelial systems with high water permeabilities was predicted by biophysical methods at least half a century ago; however, these predictions were only finally fulfilled many years later, in the 1990s, with the discovery of the molecular identity of these channels, the AQP protein family, by the Agre laboratory [see, e.g., the monograph by Finkelstein 1987 for a review of the pre-AQP water pore theory and Agre's Nobel lecture (Agre 2004) for a discussion of the discovery of AQPs]. AQP knockouts (and inhibitions) have been proposed as one method for testing the osmotic hypothesis as well as determining whether water transport is via a primarily paracellular or primarily transcellular pathway. In the context of near-isosmolar transepithelial fluid transport, a series of knockout studies by the Verkman laboratory were interpreted as supporting the view that AQPs play a facilitating role for an osmotic coupling mechanism, with the caveat that the increased permeability provided by the AQPs appears to be only strictly necessary when the fluid transport rate (normalized to surface area) is sufficiently high (Verkman 2011).

Despite the simplicity and apparent experimental confirmation of the osmotic explanation for fluid transport by epithelia, this theory continues to be brought into question; and a variety of issues with, and alternatives to, the osmotic mechanism have been discussed. For example, Hill (2008) claims that "there is no clear idea of how [fluid transport by epithelia] occurs" (p. 1) and presents five theories that he considers the main candidates, including the osmotic theory. Questions raised about mechanism are closely interlinked with debates over the pathway of water transport: whether transcellular, via AQPs, or paracellular, via the tight junctions. For example, three of the models Hill (2008) presents are based on transcellular water flow, while two are based on a primarily paracellular flux. In particular, as discussed extensively in Hill et al. (2004), a central point of contention is whether the dominant function of AQPs is to provide a pathway for water transport and to what extent AQP knockout studies confirm or contradict this.

The main question that we address here is whether the transcellular local osmosis mechanism gives predictions consistent with AQP knockout studies. As discussed, contrary to the affirmative answer of the Verkman group, Fischbarg (2010), Hill (2008), Hill et al. (2004), and Zeuthen (2010) have all argued that these results are not consistent with what the osmotic mechanism predicts. Hill (2008) considers this claimed inconsistency with AQP knockout data to be the "major problem" (p. 3) of the osmotic theory, and this same objection is repeated by the above-mentioned authors. In particular, focus has centered on the nonproportional reductions in permeability and water transport, as well as apparent complicating reductions in salt transport. For example, a study by Ma et al. (1999) of the effect of knocking out AQP5 on saliva secretion found the reduction in fluid transport to be around 60 %. However, Hill et al. (2004) pointed out that this reduction in water transport needs to be interpreted in the context of a greater reduction in water permeability of 65–90 %, a figure based on other studies of AQP knockouts in cells from salivary glands as well as renal proximal tubules and membrane vesicles obtained from tubules (Krane et al. 2001; Ma et al. 1999; Schnermann et al. 1998) and a drop in salt transport estimated to be around 40 % (Hill et al. 2004). The difficulty here lies in determining exactly what qualitative and quantitative relationships to expect between the various quantities—in particular, between the water transport rate, the solute transport rate, and the water permeability—when an AQP knockout study is carried out in an epithelial system. The arguments of Fischbarg (2010), Hill (2008), Hill et al. (2004), and Zeuthen (2010) appear to rest on assumptions of proportionality or linearity between the various quantities when AQPs are knocked out. We show that this assumption does not hold.

In this article we aim to provide a quantitative baseline for the correct interpretation of the results of AQP knockout studies which makes clear and fair assumptions regarding the osmotic mechanism. Earlier work (Weinstein and Stephenson 1981; Weinstein et al. 1981) developed the general theory describing epithelial fluid and ion transport and conducted systematic explorations model parameter dependence. Other work in addition to the above, such as that of Mathias and Wang (2005), O'Brien (2011), and Segel (1970), has also investigated the basic behavior of simple osmotic models but has focused primarily on the relationship between spatially distributed models—those based on the standing gradient model of Diamond and Bossert (1967)—and compartment-style models—those based on the model of Curran (1960), essentially finding the two to be equivalent for many regimes of interest. Here, we are interested in a comparison to AQP knockout studies. Building on the prior literature, we consider the key features of the equations required to understand the effect of AQP knockout experiments. We allow for nonisosmotic as well as isosmotic transport regimes, which, depending on the transport parameter regimes, is necessary for a proper understanding of the differing effects of AQP knockout studies and the relationship between water permeability, water transport, and ion transport. We make essential use of dimensional analysis to reduce the number of parameters that must be independently considered.

In the next section we develop our basic mathematical model of epithelial transport, based on an osmotic mechanism and a boundary condition appropriate for a directly collected transported solution. We then consider the basic features of our model, first by examining a representative example of an AQP knockout experiment and then by deriving a general functional relationship, implied by our model equations, between the normalized water flux and a parameter defined as a ratio of water and solute transport

parameters. In the following section we use this functional relationship to compare our model with a selection of data from AQP knockout studies. Finally, we discuss the implications of our results in the context of the various interpretations of these data in the current literature.

Basic Model

The differences between epithelial systems, e.g., in the precise geometry and transporters present, complicate the problem of giving a simple yet accurate universal description of epithelial transport; however, it is still necessary to establish a simple baseline of expected behavior. Here, we give the equations for a simple compartment model, shown in Fig. 1, in order to consider what to expect from AQP knockout studies. The basic model considered here is applicable to both forward-facing absorptive epithelia (renal proximal tubule, lung alveolus) and backward-facing secretory epithelia (salivary acini) according to the mappings indicated (see Fig. 1 caption for details). We consider a transcellular-only water flux to establish the baseline behavior of this mechanism. We discuss paracellular water flux later.

The "coupling compartment" (Fig. 1, label 3) corresponds to the middle compartment of the original model of coupled water transport constructed by Curran (1960), but here the right bounding membrane of this compartment represents a limiting case of no solute reflection and no resistance to water transport; thus, the concentration on the right-hand side of this compartment is the same as that of the coupling compartment. This is natural for a directly collected transported solution where all solute transported into the coupling compartment is convected out with the bulk solution. This setup is also a natural limiting case of the Diamond and Bossert (1967) standing gradient model (discussed, e.g., by Friedman 2008). The coupling compartment corresponds to the lateral intercellular spaces in forward-facing absorptive epithelia and to the lumen/canaliculi in backward-facing secretory epithelia.

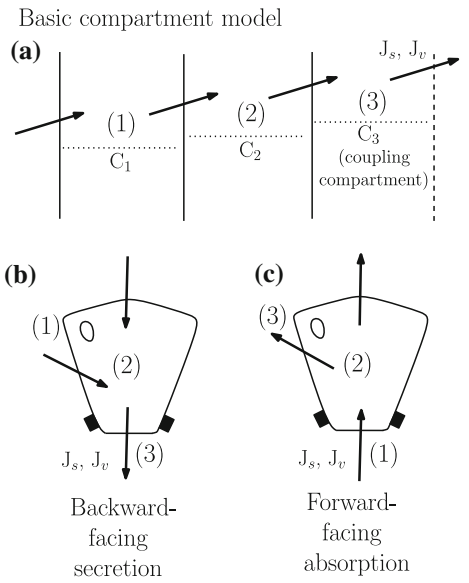

Fig. 1 Basic compartment model (**a**) and mappings of this onto the physiological systems of **b** backward-facing secretion and **c** forward-facing absorption. Label *1* corresponds to the lateral intercellular and extracellular region for the backward-facing secretion system and to the lumen for the forward-facing absorption system. These correspondences are reversed for label *3*; i.e., it corresponds to the lumen in the backward-facing secretion system and to the lateral intercellular/extracellular region for the forward-facing absorption system. In both cases label *2* corresponds to the cell. These mappings allow us to use the same compartment model for both types of system

Osmosis Through a Simple Membrane

We define J_{vi} and J_{si} to be the total volume (water) flux and total ion flux, respectively, out of compartment i and C_i to represent the total osmolyte concentration in region i. For bathing media this is simply the total ion concentration in region i, while for a cell compartment cell-impermeant species are included. The general form of an osmotically driven flux through a simple membrane out of a compartment i into a neighboring compartment j is, assuming a linear dependence between driving force and flow,

$$J_{vi} = P_i\left(C_j - C_i\right) \tag{1}$$

where P_i is the water permeability of membrane i through which the outward water flux, J_{vi}, flows. This form is quite general and requires no assumptions on mechanisms of solute flux. For example, if there is molecular sieving due to the membrane, the permeability term P_i is multiplied by a "reflection coefficient," in the terminology of Kedem and Katchalsky (1958). We have, however, neglected hydrostatic pressure effects. It is convenient for the analysis to write

$$\Delta C := (C_3 - C_1) \tag{2}$$

i.e., the total concentration difference across the cell. At steady state the osmotically driven volume fluxes are equal, which means

$$J_{v1} = P_1(C_2 - C_1) = J_{v2} = P_2(C_3 - C_2) = J_v \tag{3}$$

From these relations, C_2 can be eliminated from the volume flux equations, which gives

$$J_v = P_T(C_3 - C_1) = P_T\Delta C \tag{4}$$

where $P_T := \frac{P_1P_2}{P_1+P_2}$ is a lumped permeability parameter.

Collection Boundary Condition

As discussed, we assume that the transported solution is directly collected. In steady state, neglecting, e.g., oscillatory effects (discussed in the context of saliva secretion

by Maclaren et al. 2012), this means the concentration of the transported solution C_R is given by

$$C_R = C_3 = J_s/J_v \tag{5}$$

This can be combined with Eq. 2, giving

$$J_s = J_v(C_1 + \Delta C) \tag{6}$$

This equation can also be interpreted as determining the nonequilibrium steady-state concentration, ΔC, established because of the active salt transport, J_s, into the coupling compartment and the convective removal of salt out of the end of the compartment. To the extent that this boundary condition is applicable, it is also independent of the assumption of an osmotic mechanism. Thus, we will use this condition to relate the quantities J_s, J_v and ΔC for both the theoretical model and knockout data, generally to estimate J_s given J_v and ΔC, regardless of whether ΔC, say, is measured or predicted based on the osmotic mechanism. This is consistent with the method used by Hill et al. (2004) to estimate J_s in criticizing the osmotic mechanism.

Model Features

Representative Example of Nonproportional Changes

Here, we consider a particular example to simply and directly address the question of whether we should expect the osmotic mechanism to produce proportional changes in permeability and water transport when an AQP knockout study is conducted. We also consider what to expect of salt transport changes. We use Eqs. 4 and 6, considering their consistency with knockout data. In the next subsection we consider more general features of these equations.

Consider a water-transporting epithelium such as a salivary acinus initially transporting a solution deviating 5–10 % from isosmotic to a reference solution of osmolarity 300×10^{-6} osm/cm^3, i.e., a transporting a solution of osmolarity 315–330 $\times 10^{-6}$ osm/cm^3. This gives $\Delta C = 15$ to 30×10^{-6} osm/cm^3. With a volume flux of $J_v = 1 \times 10^{-4}$ cm/s, the osmotic assumption (Eq. 4) gives a lumped transcellular permeability of $P_T = 3.3$ to 6.7 cm^4/(s·osm).

Now, considering a reasonable upper limit on the reduction in permeability of 90 % (i.e., reduced to 10 % of its wild-type value) and a reduction in volume flow of 60 % (to 40 % of its wild-type value), we should expect, if the osmotic mechanism Eq. 4 continues to hold, to obtain a knockout osmotic gradient of

$$\Delta C^k = \frac{0.4J_T^w}{0.1P_T^w} = 4\Delta C^w = 60 \text{ to } 120\,\text{osm/cm}^3 \tag{7}$$

where we have used the superscript w for wild-type quantities and k for knockout quantities. This gives a transported solution concentration of

$$C_R = 360 \text{ to } 420\,\text{osm/cm}^3 \tag{8}$$

i.e., a change in transported solution osmolarity ranging about 14–27 %. In the study by Ma et al. (1999) of AQP-5 knockouts in whole-animal (mouse) saliva secretion the authors found a post knockout saliva osmolarity of about 420 osm/cm^3 compared to a wild-type saliva osmolarity of about 300 osm/cm^3.

Considering the point of Hill et al. (2004) regarding an unaccounted for reduction in salt transport, we note that according to the boundary condition (Eq. 6) and assuming that the osmotic mechanism (Eq. 4) is valid, we expect a change in salt transport determined by

$$\frac{J_s^k}{J_s^w} = \frac{r_v J_v^w \left(1 + \frac{r_v}{r_p}\frac{\Delta C^w}{C_1}\right) C_1}{J_v^w \left(1 + \frac{\Delta C^w}{C_1}\right) C_1} = \frac{r_v \left(1 + \frac{r_v}{r_p}\frac{\Delta C^w}{C_1}\right)}{\left(1 + \frac{\Delta C^w}{C_1}\right)} \tag{9}$$

for a fraction r_v of water transport and r_p of water permeability remaining in the knockout system. Note that for sufficiently small $\frac{\Delta C^w}{C_1}$ this can be approximated by

$$\frac{J_s^k}{J_s^w} = r_v\left(1 + \left(\frac{r_v}{r_p} - 1\right)\frac{\Delta C^w}{C_1}\right). \tag{10}$$

Based on the numbers above, Eq. 9 gives a decrease in salt transport of about 49–54 %. Again, this is in the direction expected and comparable to the 41 % estimated by Hill et al. (2004) from measured values. Note that our analysis does not specify why or by what mechanism there is a reduction in salt transport but simply that self-consistency of the osmotic mechanism requires that this should be observed. In an analysis of a simulation model of saliva secretion (Maclaren et al. 2012) we noted that the increased concentration gradients tend to reduce the driving force for ion secretion in the direction of flow and that this can account for a large portion of the reduction required for self-consistency of the osmotic mechanism. In the next section we consider a comparison of the osmotic mechanism to more knockout data. First, we derive a simpler representation of our equations to aid in this.

General Model Features

Equations 4 and 6 relate the quantities J_v, J_s, ΔC, C_1 and P_T. Here, we combine these equations and perform a dimensional analysis to understand the general features of the relationship between these quantities. Firstly, combining Eqs. 4 and 6 to eliminate ΔC gives

$$J_v\left(\frac{J_v}{P_T} + C_1\right) - J_s = 0 \tag{11}$$

This determines an implicit function of the form $F(J_v, J_s, P_T, C_1) = 0$ between $n = 4$ physical quantities. There are

$r = 2$ independent dimensions among these quantities, a velocity (flux) and a concentration (length and time only ever appear together in a ratio of one to the other). Hence, by the Buckingham Pi theorem of dimensional analysis (Buckingham 1914; Logan 1997), we can reduce this to a relationship between $k = n - r = 2$ dimensionless quantities. We can obtain this relationship by choosing two quantities by which to nondimensionalize the others; here, we choose the salt flux, J_s, and the reference concentration, C_1, defining

$$J_v^* := J_v / \left(\frac{J_s}{C_1} \right) \tag{12}$$

which defines the nondimensional variable J_v^* as the ratio of the actual volume flux, J_v, to its isosmotic limiting value (for a given salt flux) $\frac{J_s}{C_1}$, and

$$L := \frac{P_T C_1}{J_s / C_1} \tag{13}$$

which represents the relative importance of water transport to solute transport (the steady-state salt flux given by J_s). L gives a systematic way of deciding whether the water permeability is "large"; as measured relative to solute transport, permeability is relatively large when $L \gg 1$ (also discussed by Mathias and Wang 2005). Hence, we will refer to L as the "transport ratio." This gives a single universal equation relating the two nondimensional quantities J_v^* and L representing the basic behavior of osmotically driven fluid transport in any simple epithelium, expressed as

$$\frac{1}{L} \left(J_v^* \right)^2 + J_v^* - 1 = 0 \tag{14}$$

We use this to further explore the dependence of water transport on water permeability and solute transport in the next section.

Comparison of the Universal Curve to Available AQP Knockout Data

The effect of an AQP knockout is to reduce the lumped permeability parameter P_T. A reduction in P_T gives a proportional change in the nondimensional transport parameter L when all else is held fixed. The reductions in P_T, and hence L, due to knockouts will be large, around an order of magnitude. In addition, as discussed above, changes in salt transport, J_s, are also observed in knockouts (either directly or indirectly via the boundary condition relating ΔC and J_s). J_s and L are inversely related, and changes in J_s are generally expected to be up to around 50 % or so, less than the changes in water permeability but still significant. The main quantity we expect to differ most significantly between the different epithelial systems (in wild type) is the overall transport, which is captured by J_s (note that in the isosmotic regime water transport is proportional to salt transport).

The relationship (Eq. 14) between water transport and L is shown in Fig. 2. As discussed above, this relationship provides a universal prediction of the osmotic fluid transport given the ratio of solute flux and water permeability parameters, expressed as a single curve through appropriate nondimensionalization. Hence, we would expect any epithelial system to which our simple compartment model is an adequate approximation, whether wild-type or knockout system, to give results that lie on the same single curve. We have indicated representative experimental data (discussed below) in the figure.

Specifically, we considered three epithelial systems: the kidney proximal tubule (absorptive, expressing AQP1), the salivary gland acini (secretory, expressing AQP5) and the lung alveolus (type I cells, absorptive, expressing AQP5). The key quantities of interest are the (changes in) permeability, fluid transport and net ion transport. The numerical estimates that we used for these systems are given in Tables 1 and 2 in the appendix, and we discuss the assumptions on wild-type data in the text there. Both the proximal tubule (Schnermann et al. 1998) and salivary acini (Ma et al. 1999) have significant but nonproportional reductions in fluid transport compared with the reductions in permeability when AQP1 and AQP5, respectively, are knocked out, while fluid transport in the lung alveolus is unaffected when AQP5 is knocked out (Ma et al. 2000). A follow-up study on the proximal tubule (Vallon et al. 2000) found an increase in the concentration gradient in the knockout system, similar to the findings of increased osmolarity of collected saliva in the salivary glands (Ma et al. 1999).

The knockout effect for each system is shown in Fig. 2. As can be seen, the theoretical prediction based on a simple osmotic mechanism captures the trend of the knockout effects well, including the size of the knockout effects for systems in which there is a reduction (salivary and renal) as well as the difference between systems which do and those which do not respond to knockouts. The shape of the curve explains the behavior of systems for which there is no knockout effect despite, e.g., a drop in permeability of an order of magnitude (lung); these would be expected to lie around $L \gg 10^2$, i.e., well into the region for which the relationship between fluid flow and L plateaus.

Discussion

The Simple Osmotic Mechanism is Consistent with AQP Knockout Experiments

Our goal here was to demonstrate in a straightforward manner that the baseline behavior of a simple osmotic

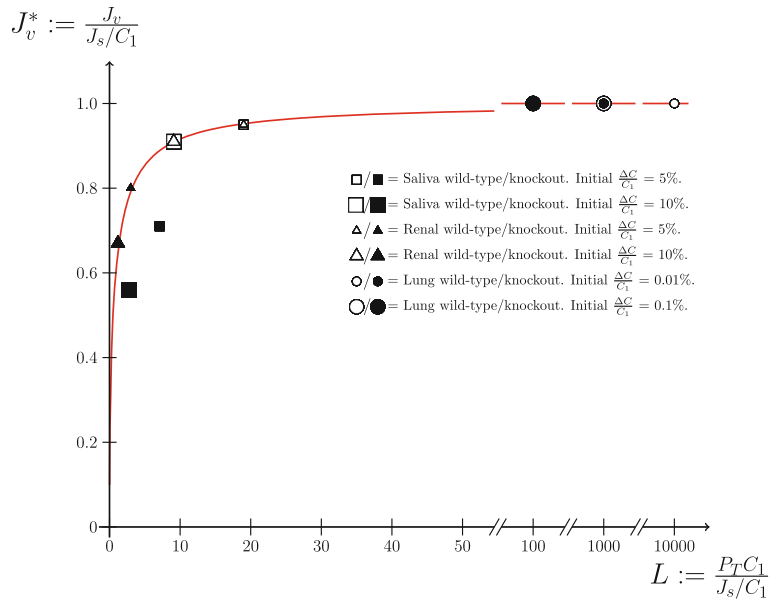

Fig. 2 Relationship between normalized water volume flux and the transport ratio (water permeability relative to salt transport rate). There is a region of relatively steep change, where AQP knockout effects are significant, and a plateau region, in which AQP knockout effects are not significant. Representative data (given in Tables 1 and 2 in the appendix) are shown for wild-type and knockout experiments for a salivary gland acinus and a renal proximal tubule, both of which have significant but nonproportional knockout effects, and a lung alveolus, which has no significant effect of knockout on volume flux. Both the salivary glands and renal proximal tubule (since they have similar overall transport rates and water permeabilities) have data pairs (wild-type and knockout) shown for assumptions of both 5 and 10 % deviation from isosmotic conditions. The two lung data point pairs were generated by assuming, for each case, a water permeability value equal to the maximum and minimum water permeabilities of the salivary gland and renal proximal tubule systems, along with a much smaller overall transport rate. These assumptions give the much lower initial deviations from isosmotic conditions (0.01 and 0.1 %, respectively). Further discussion and justification of these assumptions is given in the appendix

mechanism is capable of accounting for the results of AQP knockout studies, contrary to the criticisms raised by other researchers (Fischbarg 2010; Hill 2008; Hill et al. 2004; Zeuthen 2010). We have shown that the simple osmotic mechanism gives quantitative predictions consistent with a range of knockout data from a variety of epithelial tissues. It is a mistake to assume or expect proportional relationships between water transport, water permeability or salt transport in a knockout experiment. A natural steady state arises in the coupling compartment (this feature of the osmotic mechanism is also discussed by Mathias and Wang 2005), which involves an interaction between flow and concentration. This gives rise to nonlinearities in the relationships between the various quantities. The key feature of this steady state is that the osmotic gradient across the cell increases as water permeability decreases. This is consistent with experimental results finding an increase in the osmotic gradient after AQP1 knockouts in mouse proximal tubules (Vallon et al. 2000); the authors conclude that "we believe that, in view of the markedly enhanced transtubular osmotic gradient alternative, nonosmotic mechanisms of fluid absorption need not be invoked in the proximal tubule of AQP1-knockout mice" (Vallon et al. 2000, p. F1032).

We have primarily considered the consistency of the model equations with knockout data by substituting in known or estimated values; Maclaren et al. (2012) carried out simulations of a mathematical model of saliva secretion and found that the increase in lumenal concentrations caused by a decreased ratio of fluid to solute transport parameters naturally led to a decrease in the solute current due to decreased favorable driving force and that these balancing factors led to the establishment of a new (quasi-) steady state which was consistent with knockout data. Other factors decreasing salt transport rates should be investigated to further improve the mechanistic understanding of knockout studies.

More detailed simulation models of various epithelial systems exist—e.g., see the discussions of tubular absorption by Weinstein (1994, 2003) as well as simulations and analysis of saliva secretion produced by our group (Gin et al. 2007; Maclaren et al. 2012; Palk et al. 2010). These models provide more mechanistic understanding and will hopefully provide the ability in the future to predict the results of knockout studies in more detail, e.g., accounting for ductal modifications of the primary secretion in saliva secretion models. Deviations from steady-state behavior

may also be a factor, as considered by Maclaren et al. (2012) in the context of saliva secretion.

In summary, far from unexplained or unusual as put forward by, e.g., Hill (2008) and Hill et al. (2004), non-proportional changes in water permeability, water transport rates, concentration gradients and salt transport rates are natural consequences or aspects of the osmotic mechanism. We also note that the key determinant of the effect of an AQP knockout is the ratio of solute to solvent transport quantities, which can also be interpreted as the ratio of permeability to overall transport rate quantities, consistent with the discussion by Verkman (2011).

A Paracellular Water Flux is not Required to Explain Knockout Data but Cannot be Ruled Out by These Data

We did not include a paracellular water flux in our model yet were able to match the trend of knockout data; the straightforward conclusion is that a paracellular water pathway is not required to explain knockout data. That isolated cells maintain around 10 % of their permeability after AQP knockouts is sufficient to account for the remaining water flux. Thus, assuming an osmotic mechanism, attributing the remainder of the fluid flow after a knockout experiment to the paracellular pathway is an invalid inference. On the other hand, a paracellular component of water flow cannot be ruled out by these studies either. We found when attempting to include a paracellular flux (not shown; see Maclaren et al. 2012) that there is no obvious restriction on a significant fraction of the total volume flux being paracellular (with mechanism left unspecified) and the remainder then attributed (as above) to transcellular osmotic flow.

Despite these points, many studies can be found in the literature which interpret knockout studies as both providing evidence in support of a predominantly transcellular water pathway (e.g., those of Krane et al. 2001; Ma et al. 1999; Schnermann et al. 1998) and, contradicting this, as providing evidence in support of a predominantly paracellular pathway (e.g., those of Fischbarg 2010; Hill et al. 2004; Zeuthen 2010). AQP knockout studies, while a significant contribution to the study of epithelial water transport, actually give us little definite information about the pathway for water transport, directly or indirectly. The review articles by Spring (1998, 1999) discuss the strengths and weaknesses of various attempts to directly determine the water flux fractions through each pathway, concluding (Spring 1998, p. 116) that the fraction of the transepithelial fluid that flows directly across the tight junction remains to be accurately determined in any leaky epithelium, a conclusion again supported by our analysis. More data, along with further experimental innovation, are still required to fully settle this issue, though we find the criticisms of the primarily transcellular osmotic mechanism to be overstated.

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Appendix: Wild-Type and AQP Knockout Data

The wild-type renal proximal tubule water flux we used is similar to that given by Whittembury and Reuss (1992) and that used by Mathias and Wang (2005). We took the water flux to be five times bigger than that in the proximal tubule in the case of the salivary glands and 100 times smaller than that in the proximal tubule in the case of the lung alveolus, similar to the rough estimates given by Ma et al. (1999, 2000) and Schnermann et al. (1998) [though Ma et al.(1999) gave an estimate of the maximum rate of saliva secretion of up to 20 times the rate of absorption in the proximal tubule, we used a more conservative estimate]. We assumed that the salivary glands and proximal tubule systems transport under near-isosmolar conditions, 5–10 % from isosmotic. The assumptions for water flux and osmolarity of transport are our main wild-type parameter assumptions, and the wild-type renal proximal tubule transport quantities derived from these assumptions—water permeability and salt transport—are also similar to those given by Whittembury and Reuss (1992) and those used by Mathias and Wang (2005). Because the lung alveolus has a high water permeability but low water transport and

Table 1 Representative wild-type epithelial data: see text for sources

System	J_v (cm/s)	C_1 (osm/cm^3)	$\frac{\Delta C}{C_1}$ (no units) (%)	P_T (cm^4/[s·osm])	J_s(osm/[cm^2·s])	L (no units)	J_v^* (no units)
Salivary gland acinus	$1 \times 10^{-4\dagger}$	$300 \times 10^{-6\dagger}$	5 (10)†	6.7 (3.3)	3.2×10^{-8} (3.3×10^{-8})	19 (9.1)	0.95 (0.91)
Renal proximal tubule	$2 \times 10^{-5\dagger}$	$300 \times 10^{-6\dagger}$	5 (10)†	1.3 (0.7)	6.3×10^{-9} (6.6×10^{-9})	19 (9.1)	0.95 (0.91)
Lung alveolus	$2 \times 10^{-7\dagger}$	$300 \times 10^{-6\dagger}$	0.1 (0.01)	0.7 (6.7)†	6.0×10^{-11} (6.0×10^{-11})	1,000 (10,000)	1.0 (1.0)

† Assumed wild-type parameter values; the remaining parameters are derived from these. Note that $\frac{\Delta C}{C_1}$ is assumed and P_T is derived for the salivary and renal systems, while this is reversed for the lung alveolus. This is discussed further in the main text and the appendix

Table 2 Representative knockout effect data: see text for sources

System	$\frac{\Delta_k(P_T)}{P_T^w}$† (no units) (%)	$\frac{\Delta_k(J_v)}{J_v^w}$† (no units) (%)	$\frac{\Delta_k(\Delta C)}{\Delta C^w}$† (no units) (%)	$\frac{\Delta_k(J_s)}{J_s^w}$ (no units) (%)	L (no units)	J_v^* (no units)
Salivary gland acinus	–80	–60	+700	–47 (–35)	7.1 (2.8)	0.71 (0.56)
Renal proximal tubule	–90	–46	+400	–36 (–26)	3.0 (1.2)	0.80 (0.67)
Lung alveolus	–90	0	0	0	100 (1,000)	1.0 (1.0)

† Knockout effects based on experimental data; the remaining effects and knockout parameters are derived from these. This is discussed further in the main text and the appendix

negligible measurable changes in transport rates and osmolarity in knockouts (Ma et al. 2000), we instead assumed a value of water permeability between the lower and upper values of salivary gland and renal proximal tubule water permeabilities and calculated the osmolarity of transport from this; the deviation from isosmotic transport was significantly lower than that assumed in the salivary glands and renal proximal tubule, as would be expected (Table 1).

Given the assumption on the degree of wild-type osmolarity, the solute transport rates were calculated assuming the validity of the collection boundary condition (Eq. 6). This condition was also used to estimate the measured solute transport rate given the measured osmolarity in knockouts as it is assumed to be valid independently of the osmotic mechanism (the osmotic mechanism predicts a possibly different osmolarity from that measured).

The knockout effects were based primarily on those given by Schnermann et al. (1998) and Ma et al. (1999, 2000) for the renal proximal tubule, salivary gland acini, and lung alveolus. There were no measured changes in transport in the lung alveolus. The estimate of the reduction in permeability for the proximal tubule was taken to be 90 %, as an upper-limit case, higher than the 78 % reduction found by Schnermann et al. (1998) but in line with the 89 % reduction in permeability found in proximal tubule vesicles by Ma et al. (1998) in another AQP1 knockout study. The reduction in permeability for the salivary glands was not measured by Ma et al. (1999), but the reduction was found to be 65 and 77 % for cells isolated from the parotid and sublingual glands, respectively, by Krane et al. (2001); we took the reduction to be 80 % as a representative upper value (Table 2).

Importantly, because the studies of Ma et al. (1998) and Krane et al. (2001) measured the reductions in isolated cells and vesicles, we have some confidence that these reductions are directly representative of the changes in the permeability of the transcellular-only pathway. We could not find measurements of the changes in permeability of single cells or vesicles for AQP5-deficient alveolar cells but made the assumption of a similar effect to that in salivary cells and proximal tubule vesicles.

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