Importance of Organic Osmolytes for Osmoregulation by Renal Medullary Cells

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The cells in the renal medulla protect themselves from the extracellular hypertonicity in that region of the kidney by accumulating large amounts of sorbitol, inositol, glycerophosphorylcholine, and betaine. The system is uniquely active in this part of the body, but it represents a throwback to primitive mechanisms by which cells in virtually all organisms, including bacteria, yeasts, plants, and lower animals counteract water stress. In this brief review, we summarize how these “compatible organic osmolytes” help the renal medullary cells to survive, the mechanisms by which the organic osmolytes are accumulated, and how the accumulation is controlled to adjust for changing extracellular NaCl and urea concentrations. The compatible organic osmolytes are all intermediates in important biochemical pathways, and although the medical consequences are not yet fully worked out, it is already apparent that inappropriate accumulation of these solutes has major pathophysiological consequences. (Hypertension 1990;16:595–602)

Cells in the medulla of the kidney contain high concentrations of glycerophosphorylcholine (GPC),¹ ² inositol,² ³ sorbitol,⁴ and betaine.⁵ All four compounds are intermediates in important biochemical pathways, but that is not the reason there is so much of them in these particular cells. Rather, these “organic osmolytes” protect the cells from the hyperosmotic environment of the renal medulla.

The balance between extracellular and intracellular solutes in the renal medulla of rats during antidiuresis is illustrated in Figure 1. The extracellular fluid contains high concentrations of NaCl and urea coincident with operation of the urinary concentrating mechanism. The osmolality and urea concentration are just as high in the cells as outside. However, the electrolyte concentration is much lower in the cells,⁶ ⁷ similar to the level in cells elsewhere in the body. The osmotic difference is made up by organic osmolytes, which evidently help regulate cell volume by opposing external hypertonicity. The question remains, however, why the cells accumulate these particular solutes, rather than inorganic salts, which are more generally used to regulate the volume of mammalian cells. The answer⁸ comes from theories derived by comparative physiologists and biochemists.
with the state of diuresis. We expect the organic osmolytes to vary in the same manner if they are to serve as compatible solutes. Figure 2 shows the distribution of osmolytes in kidneys of antidiuretic rabbits. The results in other studies of rabbits and additional species are similar.\(^5,11,18\) NaCl increases toward the tip of the renal papilla. The distribution of sorbitol, betaine, and GPC closely follows this gradient. Inositol, on the other hand, is higher in the outer than in the inner medulla, suggesting an additional role for inositol, besides acting as a compatible osmolyte. Figure 3 shows how osmolytes differ between diuresis and antidiuresis in rabbits. Sodium is higher during antidiuresis, as are the compatible organic osmolytes. This result is also similar in the other species studied. Considering both location in the kidney and the state of diuresis, the sum of the renal organic osmolytes correlates strongly with extracellular sodium, consistent with their role as compatible solutes.\(^10,17\) Interestingly, free amino acids are abundant in renal medullary cells, but their level does not change with antidiuresis.\(^11\)

**Methylamines Protect Against Denaturing Effects of High Urea Concentrations**

The concentration of urea in renal medullary cells varies considerably and generally is high. Urea is a potent destabilizer of protein structure and an inhibitor of function. Methylamines are effective stabilizers of protein structure and generally activators of functional properties (\(K_m, V_{max}\)). When combined with urea at a concentration ratio of about 1:2 methylamine:urea, their effects can often offset those of...
urea.\textsuperscript{9,19} Thus, an additional role as "counteracting" osmolytes has been proposed for methylamines. Renal medullary urea varies both with location in the medulla and with the state of diuresis (Figures 2 and 3). We expect betaine and GPC to vary in the same manner if they are to serve as counteracting osmolytes. In fact, in chronic states renal medullary GPC concentration correlates very well with urea, and much better than betaine does.\textsuperscript{10} Based on this and other studies,\textsuperscript{17} GPC apparently is the main counteracting organic osmolyte in kidneys.

Renal Cells in Tissue Culture Accumulate Organic Osmolytes When Exposed to High NaCl and Urea

It is much easier to determine the mechanisms involved in accumulation of organic osmolytes and how they are controlled in tissue culture than in vivo. Two renal epithelial cell lines have mainly been used, PAP-HT25 (from rabbit inner medulla)\textsuperscript{20} and MDCK (from dog kidney).\textsuperscript{21} When the medium NaCl and urea levels are high, PAP-HT25 cells accumulate all four renal organic osmolytes and MDCK accumulate all but sorbitol (Figure 4).

The mechanisms of osmoregulatory accumulation of the various renal organic osmolytes, as defined in tissue culture studies, are summarized in Figure 5. Sorbitol is synthesized from glucose in a reaction catalyzed by aldose reductase.\textsuperscript{24} GPC is synthesized from choline.\textsuperscript{25} Inositol\textsuperscript{26} and betaine\textsuperscript{22} are accumulated by transport. The tissue culture studies have provided considerable information about how accumulation is regulated in response to changes in NaCl and urea. Some of the processes discovered in these tissue culture experiments have also been found in vivo, but confirmation of the others awaits further evidence.

Sorbitol Is Synthesized From Glucose in a Reaction Catalyzed by Aldose Reductase, the Amount of Which Is Osmotically Regulated

When PAP-HT25 cells, grown at a normal osmolality, are switched to high NaCl medium, aldose reductase activity rises. The induction of aldose reductase activity and subsequent sorbitol accumulation are relatively slow.\textsuperscript{27} There is a lag of 6 hours before a rise in enzyme activity is detected and sorbitol continues to accumulate for 3–4 days. The signal for induction of aldose reductase activity appears to be an increase in intracellular ionic strength (mainly potassium salts).\textsuperscript{27} When extracellular NaCl increases, the cells shrink and their internal ionic strength rises, remaining elevated for at least 24 hours. The increase in ionic strength apparently triggers a series of events that result in accumulation of sorbitol. As the sorbitol accumulates, the ionic strength is reduced toward normal. Increasing the medium osmolality with raffinose, which also raises intracellular ionic strength, has the same effect as adding NaCl, but increasing urea or glycerol, which do not raise the intracellular ionic strength, does not elevate aldose reductase or sorbitol.

The earliest event that has been characterized is a rise in aldose reductase messenger RNA (mRNA) abundance.\textsuperscript{28} The level of mRNA begins to rise after 3 hours, peaks at 18–24 hours, then falls to a steady level some six times higher than in isotonic medium, as sorbitol is accumulated by the cells. The increase in aldose reductase mRNA involves more rapid transcription of the aldose reductase gene (A. Garcia-Perez, F.L. Smardo, J.D. Ferraris, B.M. Martin, and M.B. Burg, unpublished observations). This im-
plies that ionic strength may specifically regulate transcription of a particular gene. Osmotic regulation of transcription in bacterial cells is an active area of investigation, and the existence and mechanism of such gene regulation in mammalian cells is an intriguing subject for future research.

The rate of synthesis of aldose reductase protein rises, then falls, proportional to the changes in aldose reductase mRNA. As more protein is synthesized, the amount in the cells increases, accounting for the rise in aldose reductase activity. Degradation of aldose reductase protein, on the other hand, is slow (half-life of approximately 1 week) and is unaffected by osmolality. Therefore, the increase in enzyme protein is entirely due to more rapid production.

As already mentioned, the rate of synthesis of aldose reductase protein peaks 18–24 hours after medium NaCl is increased, then falls. The fall coincides with accumulation of sorbitol. If the rise in sorbitol is prevented by inhibiting aldose reductase, the rate of synthesis continues to increase for more than 24 hours. The most likely link between sorbitol and aldose reductase protein synthesis rate is the ionic strength, which apparently regulates transcription of the aldose reductase gene.

Expression of aldose reductase mRNA and the enzyme activity vary in vivo in response to changes in renal medullary extracellular NaCl concentration, similar to the responses observed in tissue culture. Because Brattleboro rats lack vasopressin, they excrete large quantities of dilute urine and have low renal medullary Na+. After they have been antidiuretic for 5 days because of treatment with vasopressin, renal medullary Na+, aldose reductase mRNA, and aldose reductase activity are all greatly increased. The response of the enzyme system is relatively slow, however. When normal rats become diuretic because they are given furosemide, renal medullary Na+ and sorbitol decrease markedly within 2 hours, but aldose reductase mRNA and activity are not affected at that time, similar to the results in tissue culture.

Glycerophosphorylcholine Is Synthesized From Choline

GPC is higher in MDCK cells grown in medium made hyperosmotic with NaCl or NaCl plus urea than in medium of normal osmolality. When MDCK cells, grown at normal osmolality, are switched to medium made hyperosmotic with NaCl plus urea or urea alone, GPC rises over a week or more. This increase in GPC was studied in detail to determine the mechanism involved.

The signal that triggers GPC accumulation must differ, at least in part, from that for sorbitol. Like sorbitol, GPC increases when NaCl is added to the medium. Unlike sorbitol, GPC increases in MDCK and PAP-HT25 cells also when urea is added to the medium. Elevated urea concentration does not increase the intracellular ionic strength. Thus, although high intracellular ionic strength may induce sorbitol accumulation (see above), it is not necessary to induce GPC accumulation.

GPC is a methylamine. Although there is no direct evidence that it counteracts the destabilizing effects of urea, this is believed to be the case, judging from its chemical structure. Assuming that methylamines in the renal medulla counteract the destabilizing effect of urea, it is understandable that GPC accumulation should be induced by high urea, even in the absence of high NaCl.

Tissue culture medium ordinarily contains choline, and it was present in the experiments listed above. When cells grown in medium of normal osmolality are switched to hyperosmotic medium that does not contain choline, GPC rises slightly by the second day, but it then falls back to the baseline level. In contrast, if choline is present in the hyperosmotic medium the level of GPC continues to rise. Thus, GPC apparently is synthesized from choline taken up from the medium, and even when there is no exogenous choline, some metabolic intermediate (probably phosphatidylcholine) already formed in the cells serves as the source for the transient increase in GPC. Choline is taken up into MDCK cells by sodium-independent transport, but this choline transport is not affected by medium osmolality. Therefore, substrate transport is not a control point for osmoregulation of GPC accumulation.

The step that is osmotically regulated in the metabolism of GPC is unknown. The generally accepted pathway of synthesis is from phosphatidylcholine catalyzed by phospholipase A and lysophospholipase. GPC can be degraded by a specific diesterase. Most attention has been directed to the possibility that activity of this diesterase is controlled. The original evidence was that the amount of GPC diesterase concentration is lower in renal medulla than cortex and its activity is directly inhibited by NaCl plus urea. Thus, a rise in medullary NaCl and urea would inhibit the enzyme and increase GPC, as is observed. There is little evidence for the theory, however. When MDCK cells are adapted to high NaCl or high NaCl plus urea, GPC diesterase activity decreases approximately 25%, but this decrease is small compared with the associated change in GPC content. Also, GPC diesterase activity does not differ significantly between antidiuretic and diuretic rats. The question of osmotic regulation of GPC diesterase remains to be resolved, and there are numerous other possible control points in the GPC metabolic pathways that need to be investigated.

Inositol Is Taken Up From the Medium by Sodium-Dependent Transport, Which Is Osmotically Regulated

MDCK and PAP-HT25 cells accumulate increased amounts of inositol in medium made hypertonic by addition of NaCl. Inositol can be synthesized by mammalian cells, but many types of cells accumulate it by sodium-dependent transport. Inositol usually is included in tissue culture media in plasmalike
concentrations (=0.1 mM). When MDCK cells are switched to hypertonic medium, their inositol levels rise over a week or more to approximately three times the initial value. Increasing the osmolality with NaCl, raffinose, sorbitol, or dextrose has essentially the same effect. However, increasing the osmolality with urea or glycerol does not induce inositol accumulation, which is similar to the result for sorbitol. Increase of cell inositol in hypertonic medium requires that inositol be present in the medium. The cells accumulate inositol by active sodium-dependent transport. When the medium is made hypertonic by adding NaCl, the sodium gradient into the cells rises for a while, which increases the sodium-dependent transport. This is only a small part of the effect, however. Most of the increased transport is still found when medium sodium is decreased back to the normal level during transport measurement (hyperosmolality maintained with mannitol). Also, when the osmolality of the tissue culture medium is increased with raffinose, the transport rate increases to even higher levels than when osmolality is increased with NaCl, although the sodium gradient is not elevated. The rise in transport rate occurs over several days. $V_{\text{max}}$ rises without change in $K_m$. Hypertonicity probably increases the number of functioning inositol transporters. The evidence for this is indirect. Increased $V_{\text{max}}$ is consistent with the existence of more transporters but does not prove it. Additional support for the theory comes from recent experiments using toad oocytes as an expression system. When mRNA from MDCK cells grown in hypertonic medium is injected into the oocytes, sodium-dependent $[\text{H}]$inositol uptake by the oocytes increases, but this does not occur with mRNA from oocytes grown under isotonic conditions. Most likely, hypertonicity increases inositol transporter mRNA, resulting in more inositol transporters being synthesized and activated.

There is some preliminary evidence for sodium-dependent inositol transport in rat renal outer and inner medullas; however, there are no reports indicating whether this process is osmotically regulated in vivo and fully accounts for medullary inositol accumulation that is observed.

**Betaine Is Taken Up From the Medium by Sodium-Dependent Transport, Which Is Osmotically Regulated**

Osmoregulatory uptake of betaine by MDCK cells is similar in most respects to that of inositol. In brief, although there is betaine synthesis in some tissues, this does not account for its osmoregulatory accumulation by renal cells in tissue culture. In these cells the accumulation depends on the presence of betaine in the medium and occurs by sodium-dependent transport. The plasma betaine concentration probably is similar to that of inositol (=0.1 mM), but there have been few measurements. Hypertonicity increases betaine transport $V_{\text{max}}$ (but not $K_m$). Only a small part of the increment in transport is due to increased sodium gradient. High NaCl or raffinose induces betaine uptake, but urea and glycerol do not. The rise in betaine transport occurs over 12–24 hours.

Although urea has little effect on inositol in MDCK cells, urea inhibits the accumulation of betaine. This finding is striking in view of the theory of counteracting osmolytes, which was discussed earlier. Remember that methylamines (including betaine) can reverse harmful effects of urea. Yet urea does not induce accumulation of betaine, as it does that of GPC. Rather, urea suppresses betaine uptake. Evidently, renal cells prefer to use GPC as a counteracting osmolyte for reasons that we do not understand.

At present it is not clear that accumulation of betaine by renal medullary cells in vivo is regulated by transport from the extracellular fluid, as it is in tissue culture. There is evidence that renal medullary cells can not only synthesize betaine from choline but also that they can actively transport betaine. Neither process has been shown to be osmotically regulated in vivo.

**Accumulation of Organic Osmolytes Contributes to the Survival and Growth of Renal Cells Exposed to Hyperosmolality, but Excessive Accumulation Can Be Harmful**

The compatible and counteracting osmolytes hypotheses imply that cells exposed to high NaCl or urea will be harmed if they do not accumulate organic osmolytes. Study of cloning efficiency, which measures survival and growth of cells in culture, provides direct evidence for this and for a harmful effect of excessive accumulation of organic osmolytes.

**Evidence for the Compatible Osmolytes Hypothesis**

When PAP-HT25 cells are switched to medium made hypertonic by adding NaCl under conditions in which mainly sorbitol is accumulated (i.e., no betaine or urea in the medium), prevention of the sorbitol accumulation by inhibition of aldose reductase greatly reduces cloning efficiency. This result was surprising at first because in numerous tests of aldose reductase inhibitors used for preventing complications of diabetes, no evidence of renal toxicity emerged. The explanation is that when betaine is available, more of it is accumulated by the cells during aldose reductase inhibition, compensating for the lower level of sorbitol. This accumulation of betaine reverses the decrease in cloning efficiency caused by aldose reductase inhibitors (T. Moriyama, A. Garcia-Perez, and M.B. Burg, unpublished observation). The effect probably also occurs in vivo. Antidiuretic rats given aldose reductase inhibitors accumulate more betaine in their renal medullas, compensating for the decrease in sorbitol (P. Yancey, personal communication).
**FIGURE 6.** Schematic drawing showing osmoregulation of sorbitol in renal medullary cells. In the state of chronic antidiuresis, the cells are adapted, meaning that they contain high levels of aldose reductase (AR). During a transient diuresis, sorbitol concentration falls rapidly by efflux (steps 1 to 3). Then, when antidiuresis is reestablished, sorbitol is accumulated again by synthesis and catalyzed by the aldose reductase, which has persisted during the diuresis (step 4). If the diuresis is prolonged, however, as in diabetes insipidus, aldose reductase falls, and the cells become unadapted (step 5). Then, for sorbitol to reaccumulate, antidiuresis must induce synthesis of new aldose reductase (steps 6-8), a process requiring more than 1 day. See text for additional details. mRNA, messenger RNA.

High Extracellular Glucose Damages Renal Cells by Causing Excessive Accumulation of Sorbitol

Glucose is the substrate for synthesis of sorbitol. When blood glucose is high, as in diabetes, cells in the eyes and peripheral nerves that contain aldose reductase are harmed by excess accumulation of sorbitol (see Reference 45 for review). This is one of the major complications of diabetes. Despite the beneficial effects of sorbitol in renal cells, discussed above, excessive accumulation of sorbitol also harms them. When glucose concentration is elevated in the range found in uncontrolled diabetes, PAP-HT25 cell sorbitol increases, and cloning efficiency falls. The fall in cloning efficiency is even greater when medium NaCl is elevated than when it is normal. Under these conditions, an aldose reductase inhibitor at concentrations that reduce cell sorbitol to a level appropriate for the external NaCl concentration increases cloning efficiency. However, with high medium NaCl, if the inhibition of aldose reductase is too great and sorbitol falls too much, cloning efficiency is greatly reduced again. Evidently, osmoregulation requires appropriate accumulation of compatible organic osmolytes, and either too little or too much can be harmful.

Evidence for the Counteracting Osmolytes Hypothesis

When MDCK cells are switched to medium containing a high concentration of urea, it accumulates in the cells, and their cloning efficiency is greatly reduced. Betaine, which when added to the medium increases the osmolality even further, accumulates in the cells and protects them from the harmful effect of urea, as shown by an increase in cloning efficiency from the low level with urea alone. Urea stimulates the accumulation of GPC, but reduces betaine (see above). Thus, methylamines protect renal cells from harmful effects of urea, but GPC is the methylamine that is used by kidney cells as the principal counteracting osmolyte in vivo.

In the Antidiuretic State Renal Medullary Cells Are Adapted to High NaCl and Urea and Change Their Levels of Transporters and Enzymes for Organic Osmolytes Slowly; However, They Respond to Acute Decrease in NaCl by Rapid Efflux of the Organic Osmolytes

Unadapted cells accumulate organic osmolytes slowly, after exposure to hyperosmotic medium, limited by slow induction of transporters and enzymes. When cells in culture that have been grown at (and adapted to) an osmolality of approximately 300 mosmol/kg are switched to hyperosmotic medium, the accumulation of organic osmolytes requires days, and at least part of the delay is due to the slow rate at which enzymes and transporters are induced (see above). The accumulation is also slow in vivo. Brattleboro rats have low levels of organic osmolytes in their inner medullas because of congenital diabetes insipidus. After treatment with antidiuretic hormone, their urine rapidly becomes more concentrated. The renal medullary organic osmolytes also increase, but much more slowly. The osmolytes rise gradually during the first 3 days, and most of them are even higher after 12 days. Decrease of transporters and enzymes also lags after a decrease in hypertonicity. When PAP-HT25 cells that have been adapted to hypertonicity are returned to isotonic medium, aldose reductase activity falls very slowly (half-time of 6 days). Although the level of aldose reductase mRNA falls rapidly, the enzyme protein is long lived. In contrast, the level of sorbitol decreases within minutes by efflux from the cells. Similarly, in MDCK cells betaine transport decreases over 1–2 days and inositol transport over 6–12 hours when the osmolality is reduced, but both inositol and betaine efflux from the cells within minutes, as does GPC. Similarly, organic osmolytes efflux rapidly from cells in freshly prepared suspensions of renal medullary tubules in vitro when NaCl concentration is decreased. The organic osmolytes also fall rapidly in vivo during furosemide...
diuresis, which acutely lowers renal medullary extracellular NaCl concentration. The mechanism of sorbitol efflux is a large, transient increase in permeability. While sorbitol is exiting the cells at a fast rate, there is also a large increase in its influx, measured with [14C]sorbitol. The rapid efflux of organic osmolytes is volume regulatory. It limits cell swelling when osmolality decreases abruptly. The response is analogous to the rapid efflux of KCl that occurs from many different cell types during the volume regulatory decrease that follows a fall in osmolality. Within 15 minutes after the osmolality is decreased, cell sorbitol is greatly reduced, and the sorbitol permeability falls, but not back to its original low level in the hyperosmotic medium. A slower efflux of sorbitol continues, which balances its continued production (remember that aldose reductase takes many days to fall), so that excess sorbitol does not reaccumulate in the cells.

Mammals that do not consume large amounts of fluid and are chronically antidiuretic adapt their renal medullary cells in the sense that the cells maintain high levels of the enzymes and transporters responsible for organic osmolyte accumulation (shown for aldose reductase in the model proposed in Figure 6). Then, when renal medullary extracellular NaCl concentration falls during a diuresis, the cells swell immediately (1 in Figure 6), but within seconds there is an increase in the permeability to organic osmolytes, including sorbitol (2 in Figure 6). The efflux of solute, followed by water, restores cell volume within minutes (3 in Figure 6). Under physiological conditions, diuresis is likely to be transient. With the subsequent return to antidiuresis, sorbitol can be accumulated again rapidly (4 in Figure 6) because aldose reductase remains high. The same is true of other organic osmolytes whose transporters and enzymes have remained adapted.

If diuresis persists chronically, as in diabetes insipidus, the renal medullary cells become unadapted (5 in Figure 6). They lose the enzymes and transporters required to accumulate organic osmolytes. Then, should the animals become antidiuretic again, there is a lag during which the enzymes and transporters adapt again (6 through 8 in Figure 6). This transition from the unadapted to adapted state has been informative to study in tissue culture and animal models, but it is not the normal physiological regulation, which depends heavily on changes in permeability and is in fact much faster than the time required to go from the unadapted to adapted state and back again.

In conclusion, the large amounts of sorbitol, betaine, GPC, and inositol in renal medullary cells act as compatible and counteracting osmolytes, protecting the cells from the high NaCl and urea in the medulla. Their osmoregulation is important for proper function of these cells.

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KEY WORDS • sorbitol • inositol • glycerophosphorylcholine • betaine • osmotic regulation
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Hypertension. 1990;16:595-602
doi: 10.1161/01.HYP.16.6.595

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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