Survival in Hostile Environments:
Strategies of Renal Medullary Cells

Cells in the renal medulla exist in a hostile milieu characterized by wide variations in extracellular solute concentrations, low oxygen tensions, and abundant reactive oxygen species. This article reviews the strategies adopted by these cells to allow them to survive and fulfill their functions under these extreme conditions.

The renal medulla is the only place in the mammalian organism where cells are exposed routinely to widely varying extracellular osmolalities (79, 128). In antidiuresis, interstitial osmolalities may be several-fold higher in the papilla than in systemic plasma, whereas in diuresis values close to isoosmolality are found (13). NaCl and urea concentrations of an order of magnitude such as those occurring in the inner medulla in antidiuresis severely compromise the viability of most animal cells. In addition, efficient urinary concentration is inevitably associated with low oxygen tensions within the renal medulla. Medullary hypoxia results from 1) the relatively low blood flow through the medulla, a necessary prerequisite for the maintenance of the high interstitial papillary tonicity, 2) oxygen diffusion from descending to ascending vasa recta (AVR), and 3) the high metabolic demands of medullary cells [particularly the medullary thick ascending limbs (mTAL)] (18, 79, 91). Finally, in addition to osmotic stress and low oxygen tension, medullary cells are exposed to elevated concentrations of reactive oxygen species (ROS) produced by mTAL and vasa recta cells (FIGURE 1) (75, 134).

In the following, we describe some of the strategies employed by renal medullary cells to allow them to survive and fulfill their function in this hostile milieu.

**Tonicity Stress**

When, after prolonged diuresis, the renal concentrating mechanism is activated, extracellular NaCl concentrations, and hence tonicity, may rise steeply. This leads to osmotically obliged water efflux from, and hence shrinkage of, medullary cells, which in turn occasions an increased intracellular solute concentration, i.e., increased intracellular ionic strength (FIGURE 2) (109).

Studies on isolated perfused papillary collecting ducts (PCDs) and mTAL show that such acute hypertonic stress rapidly activates Na⁺-H⁺ and Cl⁻/HCO₃⁻ exchangers, which work in parallel to mediate the net uptake of NaCl. This is followed by water influx and gradual recovery of cell volume (41, 112). However, despite enhanced Na⁺ entry, the intracellular Na⁺ concentration of PCD cells in the kidney in situ rises only moderately in this situation (109), probably because of the simultaneous activation of the Na⁺-K⁺-ATPase. Thus most of the imported Na⁺ is replaced rapidly by K⁺ from the extracellular spaces, thus leading to greatly elevated intracellular K⁺ concentrations and resulting in persistent elevation in cellular ionic strength. Prolonged exposure to an abnormally high intracellular ionic strength, however, may disturb protein structure and function (129). Medullary cells avoid this hazard by exchanging a significant portion of their monovalent inorganic electrolytes for metabolically neutral, low-molecular weight organic substances, termed “organic” or “compatible” osmolytes (FIGURE 2). Although energetically demanding and time consuming, this process leads to normalization of intracellular ionic strength despite persistent elevation of the extracellular NaCl concentration. In the initial phase of adaptation to hypertonic stress, specific heat shock proteins (HSP) may contribute to the attenuation of toxicity-induced protein destabilization (25).

The major organic osmolytes accumulated by medullary cells include the trimethylamines betaine and glycerophosphorylcholine (GPC), the polyols myo-inositol and sorbitol, and, although probably less important, several amino acids (39, 53, 79). Intracellular accumulation of organic osmolytes is accomplished either by uptake from the extracellular space (betaine, myo-inositol, taurine), intracellular production (sorbitol), or reduced degradation (GPC) (79).

**Betaine and myo-inositol**

Uptake of both betaine and myo-inositol is mediated by Na⁺-dependent, secondary-active transport, the betaine γ-amino-butyrate transporter (BGT1), and the Na⁺-dependent myo-inositol transporter (SMIT) (67, 95), and thus relies heavily on steep transmembrane Na⁺ gradients. Both transporters are located either in the basolateral and/or apical membrane of renal epithelial cells (37, 124). Although myo-inositol taken up by SMIT stems primarily from nutritional sources, part of the betaine transported by BGT1 may have been produced by proximal tubule cells and, following release, may have reached the site of uptake via either the tubular or vascular route (12, 36, 61, 71, 121). Northern blot analysis of kidneys from antidiuretic animals has demonstrated a much greater abundance of both BGT1 and SMIT mRNA in the outer and inner medulla than in the cortex (20, 119, 125). In both the outer and the inner medulla, BGT1 and SMIT mRNA abundance decreases significantly after induction of diuresis (20, 125). In
Intracellular accumulation of sorbitol in response to osmotic stress is accomplished primarily by enhanced expression of aldose reductase (AR), the enzyme that catalyzes the conversion of glucose to sorbitol (5, 27, 79). Hence, in the cortex and outer medulla of the concentrating kidney, sorbitol is usually not detectable, but, along with the abundance of both AR mRNA and protein, its content increases steeply from the outer-inner medullary boundary to the tip of the papilla, thus paralleling the rise in interstitial solute concentrations (13, 20, 27, 48, 101, 128). Conversely, during states of diuresis, AR expression and sorbitol contents are reduced sharply in the inner medulla (13, 20, 27, 101, 128). Regulation of sorbitol via reduction to fructose by sorbitol dehydrogenase makes only a minor contribution to the adaptation of medullary cells to changes in extracellular tonicity (15, 20, 98).

After birth, when the concentrating ability of the mammalian kidney rises gradually, AR mRNA and AR

**Sorbitol**

**FIGURE 1.** Major stress factors acting on medullar-resident cells

addition to increased transcription, recent data indicate that posttranscriptional modifications (i.e., PKC-mediated phosphorylation) and protein-protein interactions via PDZ motifs are involved in regulating membrane surface abundance of BGT1, thereby possibly providing a rapid mechanism for regulation of transport activity (66, 92).

**FIGURE 2.** Cell volume and intracellular concentrations of inorganic electrolytes and organic osmolytes

Cell volume and intracellular concentrations of inorganic electrolytes and organic osmolytes in the renal medulla in antidiuresis and diuresis, during transition from antidiuresis to diuresis and vice versa. Letter size symbolizes the magnitude of the respective concentrations or the intensity of a specific process (transport, synthesis, degradation).
immunoreactivity in the inner medulla also increase (48), and immature, inner mTALs are transformed into mature ascending thin limbs (ATLs). Interestingly, this differentiation process is restricted to AR-positive thick ascending limb cells, whereas AR-negative cells, not protected from tonicity-induced stress by elevated concentrations of organic osmolytes, are subject to apoptosis (48).

**GPC**

Intracellular accumulation of GPC in response to tonicity stress is accomplished primarily by reduced degradation (7, 51). Increased release from phosphatidylcholine mediated by phospholipase A₁, phospholipase A₂, and/or lysophospholipase is less important. The relevance of low rates of GPC degradation for intracellular GPC content in the intact kidney is highlighted by the much lower activity of GPC-degrading enzymes in papillary than in cortical tissue (120). The activity of GPC:choline phosphodiesterase, which catalyzes the degradation of GPC to choline and glycerol 3-phosphate (51), is reduced by elevated concentrations of both NaCl and urea. In contrast, accumulation of betaine, myo-inositol, and sorbitol is barely influenced by high urea concentrations. The urea-mediated inhibition of GPC:choline phosphodiesterase explains the observation that, in the kidney in vivo, medullary urea and GPC contents rise in parallel in the outer medulla and tip of the papilla (110, 120, 126). Of note, in addition to “compatible,” the trimethylamines GPC and betaine are also termed “counteracting” osmolytes, since these compounds counteract the adverse effects of high urea concentrations (130).

**Transcriptional Regulation of Osmoprotective Genes**

Interestingly, several mechanisms conferring protection against tonicity stress are regulated by a common transcriptional activator, the tonicity-responsive element binding protein (TonEBP/NFAT5), which shows homology to the family of Rel-like transcription factors (122). The genes encoding AR, BGT1, SMIT, taurine transporter (TauT), and HSP70 contain (multiple) consensus sequences through which TonEBP drives the expression of the respective gene in response to osmotic stress (33, 45, 97, 113, 123). Although intracellular ionic strength correlates most closely with TonEBP activity (86), the precise mechanism of tonicity-induced gene expression is not completely understood. Activation of TonEBP requires proteasome activity, nuclear redistribution, dimerization, and possibly phosphorylation, the latter involving several putative kinase pathways (FIGURE 3; reviewed in Refs. 104, 122).

Several kinases are known to be activated on hyper-tonic stress (PKA, Fyn, p38), but their role in phosphorylation and in TonEBP transactivation has not been established unambiguously, because TonEBP does not appear to be a direct substrate for tonicity-inducible protein kinases (56). On the other hand, Lee et al. (57) have suggested that dimerization is required for phosphorylation of TonEBP and DNA binding. In addition, signal transduction via integrin-mediated cell-matrix...
interactions may contribute to toxicity-induced TonEBP activation (72).

Increased HSP70 mRNA stability in response to hypertonicity has been shown recently (3). This may contribute to increased abundance of HSP70 after prolonged exposure to osmotic stress.

Osmolyte Release

When, after a period of water shortage, large amounts of fluid are ingested, medullary NaCl and urea concentrations may fall precipitously, resulting in water influx and substantial swelling of medullary cells (13). In this situation, medullary cells release organic osmolytes (and, in consequence, water) rapidly, thus reducing cell volume toward normal (14, 101). The molecular identity of the osmolyte efflux pathway(s) is at present not clear, but the pathway displays the characteristics of a channel protein and allows the passage not only of organic osmolytes but also anions, specifically Cl\(^-\) (93, 111, 115). In addition to enhanced release of osmolytes, toxicity-sensitive genes, such as those encoding BGT1, AR, SMIT, and HSP70, are gradually downregulated (9, 20, 27, 78, 125).

**Na\(^+-\)K\(^+-\)ATPase**

The maintenance of low intracellular Na concentrations, and hence of an adequate driving force for Na-coupled transporters, is promoted by hypertonicity-induced increase in Na\(^+-\)K\(^+-\)ATPase expression and activity (16, 87). Specifically, the enhanced expression of the \(\gamma\)-subunit, a phenomenon dependent on Cl entry and activation of JNK2 and phosphatidylinositol 3-kinase (PI3K), appears to be vitally important for cultured medullary cells exposed to hypertonic stress (21, 22).

Interestingly, hypertonicity-induced activation of the Na\(^+-\)K\(^+-\)ATPase is associated with increased oxidative stress in mTAL cells (76).

**Urea Stress**

In contrast to NaCl, the other major medullary solute, urea, penetrates most cellular membranes readily and is thus not considered a major osmotic stressor. Renal papillary interstitial urea concentrations may reach 600 mM in the human kidney and may exceed 2,000 mM in desert rodents. Urea, in concentrations reached in the papilla of many mammals including humans during antidiuresis, may lead to structural and functional alterations of intracellular macromolecules, i.e., nucleic acids and enzymes (28, 80, 130). In cultured renal medullary cells, urea concentrations exceeding 400–600 mM (depending on the cell type) compromise cell viability or even induce apoptosis (83, 84, 127). Experimental evidence for a protective function against high urea concentrations has been obtained for two classes of molecules. These are the trimethylamines (betaine and GPC), which may be assumed to function as counteracting osmolytes or chemical chaperones, and specific HSPs (e.g., HSP70), also known to be molecular chaperones. Trimethylamine osmolytes require a molar ratio of trimethylamines to urea of 1:2 to exert optimal protection (19, 60, 108). During recent years, it has become increasingly clear that HSP70, which is constitutively expressed at high levels in the renal medulla, contributes significantly to the adaptation of medullary cells to high urea concentrations (10, 11, 82). This effect is probably related to the chaperone activity of HSP70, since molecular chaperones bind to the hydrophobic residues that may be exposed by the denaturing potential of urea, thereby preventing intermolecular aggregation and loss of function (11, 34, 84). Indeed, HSP70 counteracts urea-induced inhibition of lactate dehydrogenase, an enzyme that is metabolically important for medullary cells (80). Additionally, HSP70 blocks the apoptotic signalling cascade at several levels (96), which may contribute further to HSP70-mediated resistance against high urea concentrations. Other HSPs, the expression of which increases along the corticomedullary axis, include the small HSPs HSP25 and \(\alpha\)-crystallin, and osmotic stress protein (OSP) 94 (11), suggesting a role similar to that of HSP70.

The mechanism(s) by which medullary cells sense elevated interstitial urea concentrations and transduce these signals into adequate protective responses are far less understood than those for hypertonicity. There is even evidence that urea diminishes toxicity-induced responses, since urea has been shown to inhibit expression and action of TonEBP (114). Accordingly, intracellular accumulation of urea-countering betaine is increased by virtue of toxicity-stimulated expression of BGT1, whereas urea itself lacks or even blunts this effect (52, 114). The same phenomenon is observed with respect to HSP70, which protects Madin-Darby canine kidney (MDCK) cells against high urea concentrations (82). Induction of HSP70, however, involves toxicity-induced activation of TonEBP (123), whereas urea alone is insufficient (85). Exposure of MDCK cells to high urea concentrations in the absence of hypertonic NaCl kills the majority of cells, whereas pre-exposure with hypertonic medium prevents this effect (84, 99). Thus elevated extracellular toxicity is apparently a prerequisite for adaptation to high urea concentrations; however, urea-mediated protection from toxicity-induced apoptosis has been reported also (see below).

Cohen and coworkers (24, 136) demonstrated that the effects elicited by urea involve characteristics of extracellular-regulated kinase (ERK) and epidermal growth factor (EGF) receptor pathways. Urea induces immediate early genes (Egr-1) at the transcriptional level, whereas inhibition of EGF receptor kinase abolishes this effect (136). Furthermore, preexposure to 200
mM urea protects iMCD3 cells from the proapoptotic effects of hypertonic stress (additional 200 mosmol/kgH2O) (135), whereas EGF receptor kinase inhibition attenuates this effect significantly (136). The mechanisms underlying these observations and the physiological relevance of diminished TonEBP abundance and action by urea are, however, poorly understood.

Collectively, urea stress and tonicity stress activate distinct signal transduction pathways, although the interrelationship between NaCl and urea and the coexistence of these two solutes in the renal medulla apparently is important for cell function and survival.

Oxidative Stress

Recent evidence suggests that renal medullary cells produce abundant superoxide, derived primarily from NADH oxidase and mitochondrial respiratory chain enzymes (40, 138). In isolated descending vasa recta (DVR) and mTAL, angiotensin II (ANG II) increases superoxide formation (75, 134). In DVR, this effect apparently involves PKC activation, whereas in mTAL cells ANG II-mediated stimulation of the Na+-H+ exchanger appears to be involved critically in superoxide production by NADPH oxidase (76).

In agreement with the latter notion, elevated extracellular solute concentrations increase superoxide formation in isolated mTAL by virtue of stimulation of the Na⁺⁻⁻⁻⁻H⁺ exchanger (76). ROS may not only cause tubulo-interstitial injury in pathophysiological settings but may also modulate tubulo-vascular nitric oxide (NO) cross talk under normal conditions. Superoxide constricts DVR and enhances sodium reabsorption in the mTAL (32, 89), most likely via reduced bioavailability of NO, which combines readily with superoxide to form peroxynitrite (32). Thus a sustained increase in medullary ROS production may well result in salt retention and systemic hypertension.

Hypoxic Stress

The microcirculation of the renal medulla has to satisfy partially conflicting requirements. On the one hand, the cortico-papillary osmotic gradient achieved by countercurrent multiplication of tubular solute transport, by low blood flow rates, and by countercurrent exchange of solutes and water between DVR and AVR has to be maintained, whereas on the other hand the nutrient supply to the medullary tissue must not be compromised. Counterflow arrangement of DVR and AVR not only provides the structural basis for countercurrent exchange, thereby preserving high medullary solute concentrations, but also allows diffusion of metabolic substrates from the DVR to the AVR (91). As a consequence, oxygen tension in the medulla is much lower than that in the renal cortex (58, 91). To deal with the threat of medullary hypoxia, the kidney has evolved a number of strategies aiming at maintaining the concentrating capacity without endangering the medulla-resident cells.

Compared with cortical cells, medullary cells display a much higher glycolytic capacity, rendering them less dependent on oxygen (4, 54, 100). In addi-
tion, medullary cells have much higher quantities of HSPs, such as HSP70 or HSP25, at their disposal, enhancing their resistance against hypoxia-induced cell injury (see below and above).

In addition, there are autocrine and paracrine signaling pathways interconnecting the various structures of the renal medulla, thus avoiding undue hypoxia. For example, vasopressin stimulates NaCl transport and, in consequence, the energy requirement of the mTAL (38, 42), but also reduces blood flow to and solute washout from the medulla by pericyte-mediated constriction of DVR (35, 116). Undue hypoxia in this situation is avoided by the vasopressin-mediated increase in medullary toxicity that, in turn, stimulates cyclooxygenase (COX)-1/2 expression with a rise in prostaglandin E2 (PGE2) production (133) and a decrease in the production of vasoconstrictory endothelin-1 (133). PGE2 inhibits NaCl absorption in the mTAL and collecting duct (44, 46, 49, 117), attenuates vasopressin-mediated constriction of DVR, and enhances expression of cytoprotective HSPs (77). Moreover, vasopressin also causes neuronal NO synthase (nNOS) expression to rise in a V2 receptor-dependent fashion (65). In water deprivation, expression of endothelial NO synthase (eNOS) is also enhanced in the outer medulla (65). In this situation, increased NO production may help to mitigate vasopressin-induced medullary hypoperfusion and lessen the energy requirement of the mTAL by inhibiting NaCl absorption along this nephron segment (26, 88).

Similarly, ANG II, at concentrations that significantly reduce cortical blood flow, either barely affects medullary circulation or even causes medullary blood flow to rise (Ref. 30, and further references in Ref. 31), although this mediator potently constricts isolated DVR (91, 134). This relative resistance of the medullary circulation to ANG II has been ascribed to ANG II-induced enhancement of NO production primarily in the mTAL (30) and to an ANG II-induced and COX-2-mediated rise of prostaglandin (mainly PGE2) synthesis (94). Both NO and PGE2 not only counteract the vasoconstrictor effect of ANG II but also inhibit tubular NaCl absorption in the outer and inner medulla (see above). Of specific interest with respect to the effects of ANG II in the outer medulla is the finding that ANG II stimulates NO synthesis in mTAL cells and, by inhibiting apical Na+-K+-2Cl– cotransporter (NKCC)-cotransporter activity in these cells, may reduce oxygen consumption in an autocrine feedback loop (59, 90). Adenosine provides another example of tubulo-vascular cross talk, a term initially coined by Cowley and co-workers for NO (26, 30); released by tubular cells in situations of restricted energy supply or/and tonicity stress (6), adenosine dilates preconstricted DVR, thus increasing medullary blood flow (1, 106, 107, 139) and reducing NaCl absorption in the mTAL (8). Hence, the mTAL may help to adjust, actively and appropriately, energy requirement and oxygen supply in a situation of general vasoconstriction, thus avoiding medullary hypoperfusion and hypoxia (FIGURE 4).

Transcriptional Response to Hypoxia

Given the physiologically hypoxic environment in the renal medulla, hypoxia-inducible factor (HIF)-1 may play a significant role in preventing hypoxic cell injury in this kidney region. HIF-1 is a basic helix-loop-helix transcriptional activator that stimulates the expression of various genes in response to reduced oxygen availability (118). This process requires stabilization, nuclear translocation, and heterodimerization of the hypoxia-inducible HIF-1α/HIF-1β subunit with its constitutively expressed binding partner HIF-1β (118). A physiological role for HIF-1 is supported by the observation that HIF-1α abundance increases along the cortico-

![FIGURE 5. Mechanistic network of stress factors and protective/adaptive responses](http://physiologyonline.physiology.org) Simplified mechanistic network of stress factors (A) and protective/adaptive responses (B) in the renal medulla. Black lines/arrows indicate stimulation; red lines/arrows indicate inhibition.
medullary axis, whereas HIF-1α expression declines in outer and inner medulla after furosemide administration (140). Conversely, water deprivation increases the number of HIF-1α-positive cells in the renal medulla (64). In vitro, hypoxia, but not hyperosmolality, increases HIF-1α expression in renal medullary interstitial cells and inner medullary collecting duct cells (140).

Interestingly, several HIF-1 target genes putatively favor the adaptation of medullary cells to their hostile environment. These include inducible NO synthase (iNOS) (70), heme-oxygenase-1 (HO-1) (55), and glycolytic enzymes (103), including lactate dehydrogenase-A (102). The homeostatic effects of the latter two are obvious, since they promote glycolytic ATP production under conditions with reduced oxygen availability, as present in the renal medulla during antidiuresis.

Locally produced NO is important for the integrity of the renal medulla because it both dilates medullary vessels and reduces tubular solute reabsorption, thereby improving the medullary oxygen balance. iNOS is expressed abundantly in the S3 segment of proximal tubules, mTAL, and collecting ducts (2, 74). Mattson and co-workers (69) have demonstrated that infusion of a selective iNOS inhibitor in rats reduces total medullary NO synthase activity by 49% with a concomitant reduction in urine flow and sodium excretion. This, and the observation that iNOS protein levels increase along the cortico-medullary axis (68), suggest that HIF-1α-driven iNOS expression in the renal medulla plays a role in preserving medullary integrity and function.

As noted above, renal medullary cells are exposed physiologically to oxidative stress (138). Observations indicate that hypoxia-induced production of ROS contributes to activation of HIF-1 via stabilization of HIF-1α (23, 43). Interestingly, HO-1 (HSP32) is a HIF-1 target gene, and, consistently, its abundance increases along the cortico-medullary axis (132, 137). A physiological role for HO-1 in the renal medulla is substantiated by the observation that the HO-1 product carbon monoxide induces vasodilation via activation of soluble guanylate cyclase (137), whereas HO-1 inhibition in rats results in hypertension (47). In addition, the other HO-1 product, biliverdin, is a potent antioxidant (63) that may limit cellular injury in the renal medulla in the presence of oxidative stress.

Collectively, these data indicate that HIF-1, by virtue of several prohomeostatic target genes, contributes significantly to the integrity and function of the renal medulla, particularly during antidiuresis.

Implications for Pathophysiology

The importance of the adaptive/protective mechanisms described above for the integrity and function of the renal medulla is underscored by several experimental models. Disruption of the TonEBP/NFAT5 gene in mice is associated with reduced expression of several osmoprotective genes in the renal medulla (AR, BGT, SMIT), progressive and profound medullary atrophy, and growth retardation (62). Accordingly, pharmacological inhibition of myo-inositol uptake induces acute renal failure with severe injury to the mTAL (50). Furthermore, targeted disruption of the toxicity-inducible hsp70.1 gene causes loss of tolerance to osmotic stress both in vitro and in vivo (105).

During antidiuresis, COX-2 accounts for the majority of prostanoids formed in the renal medulla (131). Recent evidence suggests that COX-2 products not only modulate medullary blood flow and solute reabsorption but also favor the adaptation of medullary cells to high interstitial solute concentrations in vivo and in vitro (73, 81). In humans, COX inhibition is associated with salt retention, hypertension, edema, and even papillary necrosis, particularly in situations with stimulation of the renin-angiotensin system (17, 29).

As noted above, NO is involved critically in the adjustment of medullary perfusion and solute reabsorption, linking nutrient and oxygen supply to metabolic demands. Accordingly, pharmacological inhibition of NO production causes systemic hypertension, as is the case in genetic models with reduced NO production (Dahl salt-sensitive rats) (26). Recent in vivo evidence further underscores the relevance of HIF-1 and its target genes for the renal medulla, since retrograde gene transfer of a dominant negative HIF-1α isoform via the ureter results in severe medullary injury (64).

Collectively, several factors involved in osmoadaptation, regulation of medullary blood flow, and cell metabolism are essential for the integrity and proper function of the renal medulla, and their malfunction entails severe systemic consequences (FIGURE 5).

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