Abstract Cells of the renal medulla, which are exposed under normal physiological conditions to widely fluctuating extracellular solute concentrations, respond to hypertonic stress by accumulating the organic osmolytes glycerophosphorylcholine (GPC), betaine, myo-inositol, sorbitol and free amino acids. Increased intracellular contents of these osmolytes are achieved by a combination of increased uptake (myo-inositol and betaine) and synthesis (sorbitol, possibly GPC), decreased degradation (GPC) and reduced osmolyte release. In the medulla of the concentrating kidney, accumulation of organic osmolytes, which do not perturb cell function even at high concentrations, allows the maintenance of "normal" intracellular concentrations of inorganic electrolytes. Adaptation to decreasing extracellular solute concentrations, e.g. diuresis, is achieved primarily by activation of pathways allowing the efflux of organic osmolytes, and secondarily by inactivation of production (sorbitol) and uptake (betaine, myo-inositol) and stimulation of degradation (GPC). Apart from modulation of the osmolyte content, osmolality-dependent reorganization of the cytoskeleton and expression of specific stress proteins (heat shock proteins) may be further, as yet poorly characterized, components of the regulatory systems involved in the adaptation of medullary cells to osmotic stress.

Key words Antidiuresis · Diuresis · Heat shock proteins · Ionic strength · Organic osmolytes · Osmotic stress · Renal medulla

Introduction

Constancy of intracellular solute concentrations and cell volume is a prerequisite for optimal cell function. Since, in general, water crosses cell membranes readily, changes in extracellular tonicity (i.e. in the concentrations of solutes to which membranes are poorly permeable) are usually followed by corresponding changes in intracellular solute concentration due to transmembrane water flow [79]. Most animals, however, are able to maintain their extracellular tonicity within narrow limits, despite widely varying intake of solutes and water. In mammals, the kidney is a key component of the defence system against major changes in intra-/extracellular solute concentrations. This is due to the kidney’s ability to produce urine of highly variable osmolality depending on the hydration status. This remarkable feature of the kidney results from the operation of the countercurrent system in the renal medulla. During water deprivation, when a concentrated urine is excreted, NaCl and urea are accumulated in the medullary interstitium and provide the driving force for water reabsorption across a collecting duct epithelium rendered water-permeable by antidiuretic hormone [60]. Conversely, during excessive water intake extracellular solute concentrations in the renal medulla decline, and a dilute urine is excreted. Hypoosmolality of the urine results from absorption of electrolytes along tubule segments displaying either constitutively poor water permeability (the thick ascending limb of the loop of Henle and the distal convoluted tubule), or a decreased water permeability due to greatly reduced plasma concentrations of antidiuretic hormone (the collecting duct system) [60]. Thus in the mammalian renal medulla interstitial solute concentrations widely fluctuate.

These highly variable extracellular NaCl and urea concentrations confront renal medullary cells with three major problems. First, in the concentrating kidney, severe osmotic stress is exerted on these cells by the high extracellular concentrations of Na⁺, which, in contrast to urea, does not readily penetrate cell membranes, and its major accompanying anion Cl⁻. In renomedullary cells, osmotic equilibrium with these elevated extracellular NaCl concentrations is achieved primarily by intracellular accumulation of small organic solutes ("organic osmolytes") [8, 42]. In contrast to inorganic electrolytes (Na⁺, Cl⁻, K⁺), organic osmolytes do not severely impair
cells is the extraordinarily high urea concentration (excretion and release of organic osmolytes and on the effects of solute concentrations. Emphasis will be on the accumulation of organic osmolytes. In the following we will consider mechanisms that allow the effective accumulation and reduction of extracellular osmolality necessitate the transition from diuresis, i.e. low medullary solute concentrations, to antidiuresis, i.e. high medullary solute concentrations. This leads to an unspecific elevation of the concentrations of organic osmolytes, trimethylamines are also called “counteracting” osmolytes.

The second challenge encountered by renomedullary cells is the extraordinarily high urea concentration (exceeding 2000 mM) in the papilla of some mammals in antidiuresis. Although urea readily penetrates most cell membranes, and consequently does not exert a major osmotic stress, such high urea concentrations per se affect cell viability seriously by disturbing the structure and function of proteins and nucleic acids [25, 122]. These deleterious effects are believed to be counterbalanced specifically by trimethylamines and, possibly, by several stress-inducible proteins, the so-called heat shock proteins.

The third issue is that these cells must cope with extreme changes in external solute concentrations during the transition from diuresis, i.e. low medullary solute concentrations, to antidiuresis, i.e. high medullary solute concentrations, and vice versa. These physiological fluctuations in extracellular osmolality necessitate mechanisms that allow the effective accumulation and reduction of organic osmolytes. In the following we will consider the cellular events associated with the adaptation of medullary cells to increases or decreases in extracellular solute concentrations. Emphasis will be on the accumulation and release of organic osmolytes and on the effect of the fluctuating extracellular solute concentrations on heat shock proteins.

**Adaptation to increasing extracellular solute concentrations**

When the renal concentrating mechanism is activated after long-term diuresis, the cells of the renal medulla are exposed to an increasing extracellular osmolality resulting in osmotically induced water loss, and hence cell shrinkage. This leads to an unspecific elevation of the concentrations of all intracellular solutes, including Na⁺, K⁺ and Cl⁻, which make a major contribution to total intracellular osmolality under isotonic conditions. A prominent feature of this initial response to hypertonic stress is thus the increase in intracellular ionic strength (Figs. 1, 2). Restoration of cell volume towards normal, the so-called regulatory volume increase, is initiated by the rapid activation of ion transport systems that import Na⁺ and Cl⁻; either via Na⁺/2Cl⁻/K⁺ symport [40] or Na⁺/H⁺ antiport in parallel with Cl⁻/HCO₃⁻ antiport [20, 55, 126]. The net influx of Na⁺ and Cl⁻ obliges osmotic water entry and is the first stage of cell volume recovery. The enhanced entry of Na⁺ associated with this initial adaptation process leads to increased intracellular Na⁺ concentrations [10, 123] and, hence, activation of the Na⁺/K⁺-ATPase [123]. Most of the Na⁺ ions entering the cell in the course of this regulatory volume increase are thus replaced by K⁺ [10, 123]. This explains the observation that after an abrupt rise in extracellular tonicity, the cells in the renal medulla exhibit only a moderate increase in the intracellular Na⁺ concentration but a substantial rise in the intracellular K⁺ concentration [123]. Activation of the Na⁺/K⁺-ATPase following hypertonic stress results, however, not only from an elevated intracellular Na⁺ concentration, but also from enhanced transcription of the Na⁺/K⁺-ATPase α1- and β1-subunit [103] genes. The increase in the abundance of α1- and β1-subunit mRNA observed in cultured inner medullary collecting duct cells is absent when these cells are exposed to Na⁺-free, mannitol-containing hypertonic medium, indicating that the hypertonicity-induced stimulation of subunit mRNA expression depends on the presence of Na⁺ in the extracellular medium, and, possibly, in the intracellular compartment [103]. Although accelerated influx of inorganic electrolytes in response to shrinkage may lead, in principle, to complete volume restoration of medullary cells, this would not attenuate the elevated intracellular ionic...