**Cell growth and cell death in renal distal tubules, associated with diuretic treatment**

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**Introduction**

The most effective diuretic drugs inhibit salt transport by renal distal tubular cells by binding to cell-type specific apical transport proteins. For the three successive cortical distal segments (Figure 1) the specific transport proteins have been recently cloned and located: the Na-K-2Cl cotransporter (bumetanide-sensitive cotransporter, BSCl), binding loop diuretics such as furosemide, is limited to the thick ascending limb of Henle’s loop (TAL) [1]; the thiazide-sensitive NaCl cotransporter, TSC, binding thiazide-like diuretics, is present in the distal convoluted tubule (DCT) exclusively [2]; the epithelial sodium channel ENaC, inhibitable by potassium-sparing diuretics, such as amiloride, is characteristic for the cortical segments of the collecting system (CS; comprising the connecting tubule [CNT] and cortical collecting duct [CCD]) [3]. Although at the cellular level the various distally acting diuretics all block Na entry from the tubular fluid into the cell, site and location of the transport inhibition are decisive for the resulting differential patterns of diuresis. Transepithelial Na-reabsorption involves (i) entrance of Na into the cell across the apical cell membrane via specific transport proteins following electrochemical gradients, and (ii) its transport across the basolateral membrane into the interstitium, from where it is taken back into the blood circulation. The transport step out of the cell is mediated by the energy-requiring activity of the Na-K-ATPase, situated in the basolateral cell membrane. The necessary energy is provided by mitochondria. Prolonged altered transepithelial transport rates in a given segment, for instance, following dietary changes in salt intake or diuretic treatment, entail adaptative changes in the extent of the basolateral cell membrane area and of the mitochondrial volume of the cells, which can be assessed by morphological means.

**Effects of enhanced NaCl-entry into distal tubular cells**

One immediate and obligatory consequence of inhibited NaCl-reabsorption in a given segment is the higher solute load, delivered to the downstream segments. This causes higher transport rates in the latter, and eventually adaptative augmentation of transport capacity. For example: inhibition of NaCl-reabsorption in the water-impermeable TAL by furosemide increases by several times the NaCl-load in the downstream DCT. Salt reabsorption in the DCT via the TSC-protein necessarily increases with the load. Six days of furosemide-treatment (with compensation of volume and salt loss) in rats caused massive hypertrophy of the DCT epithelium [4], including hypertrophy of the individual cells as well as important increases in DNA synthesis rate, especially during the initial 24 h of...
treatment [5]. The following two segments, CNT and CCD, were hypertrophied as well, although less than the DCT. It was suggested that the cascade, leading to the observed increased DNA synthesis rate, was triggered by the enhanced entrance rate of Na into the cells. The augmentation of transport capacity in the distal segments downstream the TAL after furosemide treatment might thus contribute (i) to the progressive loss of diuretic efficiency with prolonged treatment, and (ii) to the known potassium loss, occurring under treatment with loop diuretics, since increased Na-reabsorption rates in the CS segments are coupled with enhanced potassium secretion. The adaptive hypertrophy explains, why the functional changes are maintained for some days after clearance of furosemide from the circulation.

Effects of NaCl-entry inhibition into distal tubular cells

If increased Na entry rate into the cell stimulates cellular growth and DNA synthesis rate, does the reduction or inhibition of Na entry rate by diuretics affect cellular growth and structure? Answers to this question have been provided again by morphological studies.

Effects of furosemide-inhibited NaCl-transport on TAL cells

After acute reduction of NaCl-entry into TAL cells by furosemide Bahro et al. [6] reported an increased frequency of autophagocytotic lysosomes in TAL cells, suggesting a break-down of cellular structures, involved in transport. Loffing et al. [5] demonstrated that during the first 24 h of furosemide application the DNA synthesis rate in TAL cells declined to zero, before recovering the basal rate during the following 48 h. The recovery under continuous furosemide treatment was explained by the existence, in addition to the Na-K-2Cl cotransporter, of other pathways for Na-entry in the apical membrane of TAL cells, which might assure a basal Na entry rate across the apical membrane. One candidate is the sodium/proton exchanger, NHE-3 [7].

Effects of thiazide-inhibited NaCl-entry in DCT cells

Recent data on rats, treated with thiazide-like diuretics, illustrate the dramatic effects of complete blocking of NaCl entry into DCT cells [8]. During the first 24 h thiazide treatment provoked a transient decrease to zero of the DNA synthesis rate in DCT cells, similar to that observed in TAL cells under furosemide. After 24–36 h the polar distribution of the TSC and the Na-K-ATPase in DCT cells was broken down and mitochondria were digested by autophagocytosis. The Ca2+ ATPase, another basolateral transport enzyme, and the cytoplasmic calcium binding protein calbindin D28K , both normally present in the DCT and CNT and involved in Ca2+ reabsorption by these segments, were no more detectable in DCT cells. The cellular dedifferentiation excluded any regulated transepithelial transport activity by DCT cells. After 72–96 h DCT cells in all DCT profiles underwent massive apoptotic death, which was preceded by transient upregulation in DCT cells of the protooncogen c-myc and the tumour suppressor gene p53 [9]. Focal interstitial inflammatory reactions were associated with the tubular lesions. Interestingly, under continuous treatment for several weeks the frequency of apoptoses in DCT cells and of interstitial inflammatory reactions regressed. However, the regenerated DCT epithelium displayed a reduced transport capacity. Also in mice several days of thiazide-treatment resulted in structural hypertrophy of the DCT epithelium. Yet, in contrast to rats, apoptoses in DCT cells were not detectable (unpublished observation).

Pathways for cellular lesions

It is only possible to speculate on the pathways leading to the cellular lesions after impaired apical NaCl-entry. We propose the following hypothesis: complete blocking of Na-entry leads to alteration of the intracellular electrolyte composition, e.g. increased intracellular Ca2+, and impaired volume regulation [8]. These changes in intracellular electrolyte composition might trigger a cascade, resulting in altered gene regulation, and finally in dedifferentiation and/or apoptosis of the cells. The lack of severe cellular lesions after NaCl-entry inhibition in TAL, in mouse DCT, and the recovery of the DCT epithelium in rats under continuous thiazide-treatment, might be explained by the presence of alternative Na-entry pathways in the respective apical cell membrane. These might be constitutive, such as NHE-3 in the TAL, or upregulated during application of the diuretic. In line with our hypothesis, the absence of lesions in the DCT cells of thiazide-treated mice might reflect the presence of other electrolyte transport proteins, e.g. ENaC in their apical membrane, in addition to the TSC [10]. Also an observation from rats may support our suggestion: the very last TSC-positive cells in rat DCT, that are intermingled with CNT cells, regularly escape lesions under thiazide treatment. Some data indicate that these particular cells express both TSC and ENaC [11]. Whether the regenerated DCT cells during prolonged thiazide-treatment have induced alternative transport proteins is still an open question.

Conclusion

The data derived from experimental studies in animals clearly reveal that modulation of solute entry into distal tubular cells profoundly affects their metabolism. Increased solute entry rates, for instance secondary to inhibited salt reabsorption in an upstream segment, trigger cellular growth and hyperplasia, and chronically enhance the transport capacity of the segment. Such changes probably contribute to the breaking of the
diuretic effect. Lowering of salt entry rates into the target cells slows down the replicating rate of the cells and chronically results in a reduction of transport capacity of the given epithelium. Complete blocking of salt entry into the cells seems to entail cell death by apoptosis.

With respect to the human nephron precise data on the distribution of transport proteins along the distal tubules are lacking. It is unknown whether thiazide treatment has a similar effect on human DCT cells as in rat. However, there are a few clinical case reports, demonstrating focal interstitial inflammation, associated with distal tubules, following treatment with a combination of thiazides and potassium-sparing diuretics [12,13]. These were interpreted as immunological events, but according to our experimental findings, a more direct effect of the diuretics might constitute an alternative explanation. The potential risk of severe tubular or tubulointerstitial lesions during therapeutic use of combinations of different classes of diuretics should be considered.

References

Oxidative stress as the triggering event for vascular remodelling

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Vascular remodelling is an important physiological process that takes place during embryogenesis as well as during adult life. In the adult, vascular remodelling is a necessary adaptive process in response to either modified haemodynamic conditions or to angioplasty. Chronic changes in blood flow and pressure induce adjustments that tend to maintain shear stress and wall stress constant [1–3].

Vessel wall properties during remodelling

New non-invasive methodology that allows measurement of geometrical and mechanical properties of arteries, led to the characterization of vascular remodelling in animal models as well as in human hypertension. High resolution echotracking devices can be used to assess the biomechanical consequences of vascular remodelling. Using these devices an autoregulation of the mechanical properties of conduit vessels in the forearm of hypertensive patients as well as in the larger conduit vessels of hypertensive rat strains has been observed [2,3]. Only Young’s elastic modulus appeared to be modified during hypertension [3], implying that the normalization of the circumferential wall stress is achieved by increased wall tissue mass. The thickened wall initially retains the same intrinsic properties as those of normotensive controls. However, over time the wall tissues become stiffer, as reflected by the increased incremental elastic modulus-stress relationship [3].