Molecular basis for the dialysis disequilibrium syndrome: altered aquaporin and urea transporter expression in the brain

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Abstract

Background. Cerebral disorders caused by brain oedema characterize the dialysis disequilibrium syndrome, a complication of rapid haemodialysis. Brain oedema is presumably caused by the ‘reverse urea effect’, i.e. the significant urea gradient between blood and brain after dialysis, with, as a result, an inflow of water into the brain. To assess the molecular basis of this effect, we examined the expression of urea transporter UT-B1 and aquaporin (AQP) 4 and AQP9 in the brain of uraemic rats.

Methods. Brain, kidneys and one testis were collected from four sham-operated (control) and four uraemic rats, 10 weeks after 5/6 nephrectomy (Nx). Protein abundance was measured by semi-quantitative immunoblotting using affinity-purified rabbit anti-rat antibodies applied on tissue crude homogenates.

Results. The results are expressed as means±SE of band density (arbitrary units). In Nx compared with control rats, the brain expression of UT-B1 was reduced by half (32±3 vs 62±8, P<0.01) whereas that of AQP4 was doubled (251±13 vs 135±5, P<0.001), and that of AQP9 increased by 65% (253±22 vs 154±10, P<0.01). UT-B1 expression was also lowered by Nx in kidney medulla (45±21 vs 141±4, P<0.01) but was unchanged in testis.

Conclusions. The conjunction of a reduced expression of UT-B and an increased expression of AQPs in brain cells may bring a new clue to understanding the DDS mechanism. Because of low UT-B abundance, urea exit from astrocytes is most probably delayed during rapid removal of extracellular urea through fast dialysis. This creates an osmotic driving force that promotes water entry into the cells (favoured by abundant AQPs) and subsequent brain swelling.

Keywords: chronic renal failure; kidney; oedema; rat; subtotal nephrectomy; UT-B1

Introduction

Dialysis disequilibrium syndrome (DDS), a complication of haemodialysis, is characterized by neurological symptoms including headache, disorientation, nausea, seizures and coma. This syndrome is assumed to result from brain swelling occurring as a consequence of a rapid haemodialysis process. Johnson et al. observed that DDS was prevented by the use of dialysate fluid containing urea at a concentration similar to that of the patient’s blood [1]. This observation suggests that brain swelling was due to a significant brain to blood urea gradient after dialysis and led to the concept of the ‘reverse urea effect’ [2]. The involvement of urea in the DDS was supported further by animal experiments which allowed direct measurements of brain water and urea content [2–4]. In binephrectomized rats, haemodialysis reduced urea concentration in the brain far less than in plasma and markedly increased brain water content. This increase was prevented by addition of urea to the dialysate [3,4], thus confirming the mechanism by which the same procedure protected patients from DDS [1].

It is now well established that water and urea movements across plasma membranes are greatly ‘facilitated’ by specific water channels (aquaporins; AQP) and urea transporters (UTs), respectively, which allow fast transmembrane equilibration. In the absence of such proteins, water and urea diffusion through cell membranes is quite slow and equilibration between the cells and their surrounding interstitium is therefore delayed.

Among the AQPs and UTs cloned so far, AQP1, AQP4, AQP9 and UT-B1 are expressed in the brain. AQP1 was detected in epithelial cells of choroidplexus [5], whereas AQP4, AQP9 and UT-B1 have been localized in perivascular astrocyte end feet,
in ependymal cells lining cerebral ventricles and in endothelial cells [6–8]. AQP4, AQP9 and UT-B1 presumably facilitate rapid equilibration of water and urea throughout brain parenchyma. Inasmuch as urea permeability of the blood–brain barrier is increased by a specific transporter (UT-B1), the early finding of Kleeman et al. that urea moves much more slowly into rabbit brain than into other tissues such as muscle is somewhat puzzling [9].

The involvement of AQP4 in brain oedema has been stressed by the findings that AQP4 mRNA is upregulated in rats with focal cerebral ischaemia [10] and that mice lacking AQP4 are partially protected from brain oedema in water intoxication and ischaemic models of brain injury [11]. We previously have reported decreased UT-B1 mRNA abundance in the brain of uraemic rats [12]. In contrast, UT-B1 protein expression increases in brain of aged rats [13].

Because DDS involves abnormal equilibration of water and urea between blood and brain tissue, we evaluated the expression of AQP4, AQP9 and UT-B1 proteins in the brain of rats with chronic renal failure. Immunoblotting was used for semi-quantitative analysis of these proteins in the brain of uraemic rats studied 10 weeks after 5/6 renal ablation. Our study reveals opposite changes in the abundance of the UT and the water channels (fall in UT-B1 and rise in AQPs). Such disturbances may explain urea retention and water accumulation in the brain when haemodialysis of uraemic patients results in a rapid fall in blood urea level.

**Methods**

**Animals and induction of chronic renal failure**

Four male 7-week-old Sprague–Dawley rats (Charles River, France) weighing ~220 g were subjected to subtotal nephrectomy (Nx) achieved by surgical ablation of 5/6 of the renal mass in two steps. Under pentobarbital anaesthesia (Sanofi Santé Animale, France), the right kidney was decapsulated to preserve the adrenal gland, and its two poles (about two-thirds of kidney mass) were excised. Bleeding was minimized by application of collagen powder (Pangen, Fournier, France) on the cut surfaces and by mild pressure for 2–3 min. One week later, the left kidney was decapsulated and removed after ligature of the renal pedicle. Four control rats underwent laparotomy and kidney decapsulation, but both kidneys were left intact. Rats had free access to tap water and standard rat chow during the whole experiment (M25, Extralabo, France).

Ten weeks after subtotal nephrectomy, rats were anaesthetized with pentobarbital, and a blood sample was drawn from the jugular vein. Kidneys were removed and weighed. The kidney medulla, encompassing the inner stripe of outer medulla and the inner medulla, was excised from coronal kidney slices. The whole right cerebral hemisphere of each rat was collected as well as one testis. Chronic renal failure was confirmed by measuring blood urea concentration, using a commercial kit (Urea-Kit, BioMérieux, Marcy-L’Etoile, France).

**Western blot analysis**

Affinity-purified rabbit polyclonal antibodies were used as primary antibodies to reveal AQP4, AQP9, and UT-B1 proteins selectively. Glial fibrillary acidic protein (GFAP) was also studied as a marker of astrocytes. For AQP4 and AQP9, commercial polyclonal antibodies raised against the 19 amino acid C-terminal peptide sequences of rat AQP4 or rat AQP9 were used (Alpha Diagnostic International, San Antonio, TX). For UT-B1, the antibody used here was raised against a 20 amino acid peptide corresponding to the C-terminal sequence of rat UT-B1, as characterized elsewhere [8]. GFAP was revealed with a protein A-purified rabbit polyclonal antibody (Promega, Madison, WI).

Tissues were finely minced with a razor blade and then thoroughly homogenized in ice-cold lysis buffer (250 mMm/1 sucrose, 10 mMm/l triethanolamine, pH 7.6) containing protease inhibitors (Complete Mini EDTA-free protease inhibitor cocktail tablets, Roche Diagnostics, Meylan, France). Protein concentration was determined by the Bradford method (Biorad, Hercules, CA). Samples were then solubilized in Laemmli buffer, and heated at 65 °C for 10 min before loading. Proteins (15 μg) of each tissue for each rat were separated by SDS–PAGE (10%) and transferred to PVDF membranes. Blots were blocked for 45 min at room temperature with phosphate-buffered saline containing 5% non-fat dry milk, followed by incubation with one of the specific primary antibodies (0.25 μg/ml) for 2 h at room temperature. The membranes were then thoroughly washed and incubated for 60 min with the secondary antibody, a goat peroxidase-conjugated anti-rabbit IgG polyclonal antibody (0.2 μg/ml) (Promega, Madison, WI).

Bands were visualized on Hyperfilm-ECL (Amersham) by chemiluminescence (ECL+, NEN, Boston, MA). Apparent molecular weights were determined using pre-stained protein markers, broad range (New England Biolabs, Beverly, MA). Equal protein loading was ascertained by Coomassie blue staining of the PVDF membranes at the end of the experiment. After scanning, the density of the bands on the films was quantified using NIH image software. The abundance of the corresponding water channels and UT was expressed in arbitrary units of density. The abundance of each protein in each tissue includes both unglycosylated and glycosylated forms.

**Statistics**

Data are expressed as means ± SE. Differences were analysed by Student’s *t*-test. They were considered significant for *P* < 0.05.

**Results**

About 85% of total kidney mass was removed during the 5/6 Nx procedure. Ten weeks later, the remnant kidney was markedly hypertrophied, reaching about half of the total kidney mass of control rats (1643 ± 208 vs 3170 ± 196 mg). Plasma urea concentration was increased >4-fold, from 4.9 ± 1.3 to 22.8 ± 2.6 mmol/l (*P* < 0.001).

In the brain, AQP4 is expressed as a monomeric and a dimeric form of mol. wt 32 and 62 kDa,
respectively. AQP9 is expressed as a faint unglycosylated form of 31 kDa and two major glycosylated forms at ~48 kDa [14]. UT-B1 is expressed as an unglycosylated form of 29 kDa, and glycosylated forms of 33 kDa (Figure 1). Experimentally induced chronic renal failure altered the expression of UT and AQPs in the brain. In uraemic rats, the 62 kDa band of AQP4 was much more intense than in control rats, whereas the faint 32 kDa band was reduced. Altogether, these changes resulted in a doubling of total AQP4 protein (251 ± 13 vs 135 ± 5 arbitrary units, *P < 0.001) (Figure 1). Glycosylated forms of AQP9 were significantly enhanced in the brain of uraemic rats, resulting in a 1.6-fold increase in the abundance of total AQP9 protein (253 ± 22 vs 154 ± 10, *P < 0.01) (Figure 1). Both forms of UT-B1 were reduced by half, from 62 ± 8 to 32 ± 3, *P < 0.01 (Figure 1). GFAP, the marker of glial cells, is revealed as a doublet, the protein of lower molecular weight being a degraded form [15]. In uraemic rats, the 1.4-fold increase in GFAP abundance was not statistically significant (from 30 ± 2 to 42 ± 5, *P < 0.10) (Figure 1).

In the kidney, UT-B1 is revealed as an unglycosylated form of 29 kDa, as in the brain, and as glycosylated forms identified as a broad band at ~47.5 kDa. The abundance of UT-B1 was markedly reduced in the kidney medulla of three out of four Nx rats, resulting in a mean abundance of 45 ± 21 and 141 ± 4 for Nx and control rats, respectively (Figure 2). The inter-individual differences in UT-B1 abundance were not correlated with the level of uraemia. In the testis, UT-B1 is expressed solely as a non-glycosylated protein of 48 kDa that is presumably a new longer form of UT-B, not yet cloned [8]. It is noteworthy that testis UT-B was unchanged by chronic renal failure (Figure 2).

**Discussion**

The present study reveals that major changes occur in the expression of AQPs and UT in the brain of rats with chronic renal failure. Ten weeks after severe reduction of kidney mass, the brain exhibits a large fall in the expression of UT-B1 and a marked increase in the expression of AQP4 and AQP9.

If the level of expression of AQPs and UT-B1 represents the limiting factor for rapid osmotic and urea equilibration, the changes seen in uraemic rats should affect the kinetics of water and urea movements between blood and brain. The reduction in UT-B1 abundance should slow down urea egress from the brain, while the increase in AQPs should facilitate osmotically driven water influx in the brain. These changes probably do not matter much in a chronic state because the composition of extracellular fluids does not change very rapidly, and simple diffusion allows equilibration with surrounding tissues, even if more slowly. However, if similar changes occur in the brain of patients with end-stage renal failure, it may be assumed that, during rapid removal of urea from blood and extracellular fluids by haemodialysis,
The testis was not affected by advanced renal failure. Nephrectomy than in control rats. In contrast, UT-B1 abundance in brain and kidney of rats with subtotal nephrectomy was significantly lower in brain and kidney of rats with subtotal nephrectomy than in control rats. *P < 0.05 vs the control group. The abundance of UT-B1 was significantly lower in brain and kidney of rats with subtotal nephrectomy than in control rats. In contrast, UT-B1 abundance in the testis was not affected by advanced renal failure.

Increased expression of brain AQPs (AQP4 and AQP9) has been reported in various aetiological conditions of brain oedema such as brain tumours, head trauma, stroke and liver failure [10,16,17]. The present study reveals that renal failure is an additional aetiology leading to increased AQP expression in the brain. This increase surpasses that of GFAP, the marker of astroglial cells in which AQP4 and AQP9 are expressed, thereby probably reflecting an overexpression of AQPs in perivascular astrocytic end feet. In contrast to their brain counterparts, kidney AQPs (AQP1, 2 and 3) are reduced in rats with chronic renal failure induced by similar surgical reduction of the renal mass [18]. Therefore, the changes in the expression of cell membrane water channels in uraemic rats appear to be organ specific.

AQPs and UT-B1 are predominantly expressed as glycosylated forms, but the functional consequence of their glycosylation is not clear. For AQP4 and AQP9, no study has examined this question yet, to our knowledge. As concerns UT-B1, its membrane targeting and urea transport functions do not seem to be altered by mutation of the N-glycosylation consensus site [19].

The signal and the mechanism(s) involved in the overexpression of AQPs in the brain of uraemic rats are still unknown. This overexpression might be ascribed to the elevated vasopressin plasma level associated with severe chronic renal failure [20]. Indeed, it has been reported that vasopressin, through V1a receptors, induced an increased water flux through the astrocytic synctium [21]. Such an increased water flux may rely on upregulation of AQP abundance as observed here. Elevated AQP levels may pertain to P38-mitogen activated protein kinase that upregulates AQP4 and AQP9 in rat astrocytes cultured under mannitol-induced hyperosmotic conditions [22]. The possibility that an elevated serum sodium concentration might play a role in the changes observed in the abundance of AQPs and UT in uraemic rats is unlikely because the serum concentration of sodium was shown to be unaltered in rats with uraemia achieved with identical surgical Nx and studied at a similar stage of chronic renal failure [23].

In conclusion, the findings reported herein reveal that chronic renal failure results in dramatic changes in the expression of water channels and UT in the brain. A marked decline in UT-B1 is associated with a doubling of AQP4 and AQP9 abundance in the brain of uraemic rats. These results provide new clues for the understanding DDS and, more generally, call for more studies addressing the detailed alterations in

**Fig. 2.** Semi-quantitative immunoblotting analysis of protein abundance of UT-B1 in brain, kidney medulla and testis of control (Co) and 5/6 nephrectomized (Nx) rats (the blot for brain UT-B1 is the same as that in Figure 1, but is shown again here for comparison with the other two organs). (A) Each lane, corresponding to a single rat, was loaded with 15 µg of protein. MW = molecular weight markers. (B) Quantification of band densities (means ± SEM of four rats). *P < 0.05 vs the control group. The abundance of UT-B1 was significantly lower in brain and kidney of rats with subtotal nephrectomy than in control rats. In contrast, UT-B1 abundance in the testis was not affected by advanced renal failure.
UTs and water channels in brain and other tissues during chronic renal failure.

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