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ORIGINAL ARTICLE

Opposite effects on regulation of urea synthesis by early and late uraemia in rats

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Summary
Background & aims: Acute and chronic kidney failure lead to catabolism with loss of lean body mass. Up-regulation of hepatic urea synthesis may play a role for the loss of body nitrogen and for the level of uraemia. The aims were to investigate the effects of early and late experimental renal failure on the regulation of hepatic urea synthesis and the expression of urea cycle enzyme genes in the liver.
Methods: We examined the in vivo capacity of urea nitrogen synthesis, mRNA levels of urea cycle enzyme genes, and N-balances 6 days and 21 days after 5/6th partial nephrectomy in rats, and compared these data with pair- and free-fed control animals.
Results: Compared with pair-fed animals, early uraemia halved the in vivo urea synthesis capacity and decreased urea gene expressions ($P<0.05$). In contrast, late uraemia up-regulated in vivo urea synthesis and expression of all urea genes ($P<0.05$), save that of the flux-generating enzyme carbamoyl phosphate synthetase. The N-balance in rats with early uraemia was markedly negative ($P<0.05$) and near zero in late uraemia.
Conclusions: Early uraemia down-regulated urea synthesis, so hepatic ureagenesis was not in itself involved in the negative N-balance. In contrast, late uraemia up-regulated urea synthesis, which probably contributed towards the reduced N-balance of this condition. These time-dependent, opposite effects on the uraemia-induced regulation of urea synthesis in vivo were not related to food restriction and probably mostly reflected regulation on gene level.

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Introduction

Loss of lean body mass during acute and chronic uraemia contributes to the morbidity associated with kidney failure. Protein muscle degradation accelerates, among others, due to metabolic acidosis, insulin resistance and elevated glucocorticoids.[1–4]

However, little is known about the role of changes in the hepatic urea synthesis in the development of negative nitrogen (N)-balance during uraemia. Urea synthesis is the only way the body can irreversibly eliminate amino-N and this way plays a crucial role in whole body N homoeostasis. Stressful conditions such as diabetes, surgical trauma, pain, and infection all up-regulate urea synthesis,[5–8] i.e. increase the capacity of conversion of amino-N into urea-N. Amino-N once incorporated into urea is lost for protein synthesis. The ability of urea synthesis may, therefore, actively influence N-metabolism of extra-hepatic tissue via effects on the size or composition of the blood free pool of amino-N. Thus, the catabolism during stressful events or severe illness may be due partly to such hepatic response to stress.

An earlier study from our group suggested that experimental renal failure up-regulated urea synthesis in rats.[9] However, we did not at that time take into account the effects of the associated decreased food intake, even though we have later shown that this does, in itself, indeed up-regulate urea synthesis.[10] Furthermore, this study provided no information on changes in the gene expression for the urea cycle enzymes that seem to induce the up-regulation of urea synthesis in several stress situations.[11,12]

The aims of the present study were to investigate the effects of early and late experimental renal failure on the capacity of urea-N synthesis (CUNS), and the expression of urea cycle enzyme genes in the liver beyond the influence of food restriction.

Material and methods

Animals

Male Wistar rats (body weight 200–210 g; Moellegaard Breeding Center, Ejby, Denmark) were housed at $22 \pm 2\ ^{\circ}C$, $55 \pm 10\%$ relative humidity, air change 8–10 times per hour, on a 12:12 h light–dark cycle (6:30 AM–6:30 PM light) and with 3 animals per cage. The animals had free access to standard food (Altromin diet no. 1324; Chr. Petersen, Slagelse, Denmark) and tap water.

Uraemia

A 5/6th partial nephrectomy (PNX) was performed as previously described.[13] Following anaesthesia (cf. below), 2/3 of the right kidney was removed retroperitoneally by resecting both poles. During the same operation, the entire left kidney was removed. Both adrenal glands with their vascular connections were preserved. All controls were sham operated, i.e. the animals underwent the same surgical procedure as the PNX animals, except both kidneys were only manipulated.

Design

For each of the six study groups, we determined CUNS, liver mRNAs, blood urea-N concentration (BUN), basal blood α-amino-N (AAN) concentration, whole body N-balance and the change of body weight during experiment (BW). CUNS, BUN, AAN and N-balance were determined in one population and mRNAs were measured in other but otherwise identically treated animals.

(1) Control group of the PNX rats examined on day 6 ($n = 16$) (CUNS, $n = 5$; liver mRNA, $n = 9$; BUN and AAN, $n = 6$; N-balance, $n = 7$; BW, $n = 6$).
(2) Pair-fed control group of the PNX rats examined on day 6 ($n = 15$) (CUNS, $n = 5$; liver mRNA $n = 9$; BUN, AAN and N-balance, $n = 6$; BW, $n = 8$).
(3) PNX rats examined on day 6 ($n = 16$) (CUNS, $n = 5$; liver mRNA, $n = 8$; BUN and AAN, $n = 8$; N-balance, $n = 6$; BW, $n = 10$).
(4) Control group of the PNX rats examined on day 21 ($n = 16$) (CUNS, $n = 5$; liver mRNA, $n = 9$; BUN and AAN, $n = 6$; N-balance, $n = 7$; BW, $n = 6$).
(5) Pair-fed control group of the PNX rats examined on day 21 ($n = 17$) (CUNS, $n = 5$; liver mRNA, $n = 8$; BUN and AAN, $n = 9$; N-balance $n = 6$; BW, $n = 8$).
(6) PNX rats examined on day 21 ($n = 18$) (CUNS, $n = 7$; liver mRNA, $n = 8$; BUN and AAN, $n = 10$; N-balance, $n = 6$; BW, $n = 11$).

In each study group, liver tissue mRNA levels of the five urea enzymes, carbamoyl phosphate synthetase (CPS), ornithine transcarbamylase (OTC), arginino succinate synthetase (ASS), arginino succinate lyase (ASL) and arginase (ARG) were determined.

Anaesthesia and surgical procedures

Following anaesthesia with 0.75 ml/kg Hypnorm s.c. (fentanyl/fluanisone; Jansen Pharma, Birkeroed, Denmark) and 4 mg/kg Midazolam s.c. (dormicum; La Roche, Basel, Switzerland), a catheter (Neoflon 0.6 mm;Viggo-Spectramed, Helsingborg, Sweden) was inserted into the femoral vein for continuous alanine infusion. All animals were retroperitoneally nephrectomised (PNX animals one-sided) immediately before investigation to simplify urea synthesis measurements to only accumulation in body water.[14] This procedure does not acutely influence the CUNS.[14] Blood samples were taken from a retrobulbar venous plexus using heparinised micropipettes (Vitrex; Horsens Laboratory Equipment, Horsens, Denmark).

Capacity of urea nitrogen synthesis

Analysis of non-substrate regulation of urea synthesis in vivo requires standardisation of the process rate in relation to substrate drive. In rats, CUNS can be measured during alanine loading.[14]

The CUNS method has been validated against established quantitative liver function tests,[15,16] and it has been successfully used in studies of N homoeostasis in experimental disease