Experimental Evidence That the L-Aspartate Microproteinuria Actually Results from a Tubular 'Washout'

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Dear Sir,

Ødum et al. [1] have recently reported that the infusion of the acidic amino acid L-aspartate to volunteers produces a transient increase of the urinary excretion of β_2 -microglobulin and of the major urinary trypsin inhibitor. The albumin excretion was unchanged, indicating that these effects are presumably due to an impaired tubular reabsorption. The mechanism involved, however, seems to differ from that of the tubular proteinuria induced by cationic amino acids, in particular L-argi-nine. The inhibitory effect of L-aspartate was delayed, occurring after the infusion period, whereas that of argi-nine was immediate and also much more pronounced. These results are at variance with those published by Mogensen and Soiling [2] in 1977. These authors showed that cationic amino acids such as L-lysine or L-arginine instantaneously inhibit the tubular reabsorption of proteins, whereas neutral or acidic amino acids (e.g. L-aspartate) are ineffective. These observations, supported by subsequent studies [3, 4], led to the current view that the initial step in the tubular reabsorption of proteins is the binding of positive charges of the protein to the negative surface charges of tubular cells [5].

The explanation for this discrepancy seems to be the lack of adequate control data in the experiments of Ødum et al. [1]. The proteinuria of volunteers infused with L-aspartate should have been compared not with the preinfusion levels but with the pattern of protein excretion of subjects infused with an equimolar dose of NaCl. The authors exclude the possibility of a 'washout' of proteins caused by an increased water flow in the tubule, because a 'late' proteinuria was not detected after infusion of sodium bicarbonate or L-arginine. Sodium bicarbonate,

however, was infused at a molar dose (83 mmol) and in a total volume which were, respectively, 2 and 3 times lower than in the L-aspartate experiment (56 mmol L-aspartate + 128 mmol NaCl). In the case of L-arginine, the massive increase in protein excretion induced by this amino acid precluded the detection of any 'late' proteinuria. Ødum et al. [1] also refute the hypothesis of a tubular 'washout' by referring to the studies by Macbeth and Pope [6] and Holstein-Rathlou et al. [7]. These studies, however, are of little relevance since the latter has not measured protein excretion and the former deals with the preservation of tubular function during abdominal surgery.

In order to assess whether the L-aspartate-induced proteinuria was caused by a tubular 'washout' or by a more specific mechanism, we have studied the urinary excretion of β_2 -microglobulin in

rats injected intravenously with equimolar doses (5.7 mmol) of NaCl, L-aspartic acid or L-arginine. The pH of the amino acid solutions was adjusted to 7.4 with NaOH or HCl. The dose of L-aspartate was about 7 times higher than that perfused to volunteers by Ødum et al. [1]. A control group given physiological saline was also included. The salts were given as a single injection in a volume of 5 ml/kg. Immediately after the injection, urine was collected for a period of 2 h. A second collection was also performed between 2 and 6 h to disclose a possible delayed effect on tubular reabsorption. Rat β_2 -microglobulin was determined with a sensitive immunoassay described previously [8]. Data were analyzed by one-way analysis of variance followed by Dunnett's [5] multiple comparison test.

Compared to rats given an equimolar dose of NaCl, those injected with L-aspartate showed no significant change in the β_2 -microglobulin excretion whereas under

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Table 1. Urinary flow and β_2 -microglobulin excretion during 2 h following a single intravenous injection of physiological saline or equimolar doses of hypertonic saline, L-aspartate or L-arginine to rats (mean $\pm$ SD)

Urinary flow β_2 -Microglobulinuria ml/2 h μ g/2 h

Physiological saline (NaCl, 45 mg/kg)

1 $\pm$ 0.4 0.95 $\pm$ 0.3

Hypertonic saline (NaCl, 0.33 g/kg)

4 $\pm$ 0.82^a 10.1 $\pm$ 6.6^a

L-Aspartic acid,

sodium salt (L-aspartic acid, 0.76 g/kg)

5.2 $\pm$ 0.18^a 4.6 $\pm$ 4.7^a

L-Arginine chloride (L-arginine, 1 g/kg)

2.35 $\pm$ 1^b 96 $\pm$ 37^b

^a Significantly different from the physiological saline group. ^b Significantly different from groups treated with physiological or hypertonic saline.

the same conditions, L-arginine produced about a 10-fold increase of β_2 -microglobulinuria (table 1). Both hypertonic saline and L-aspartate infusions depressed the tubular reabsorption of β_2 -microglobulin. But, this was evidently due to an unspecific osmotic effect causing an increase of urinary flow and a 'washout' of proteins from the proximal tubule. Four hours after the treatment, the β_2 -microglobulinuria of rats given hypertonic saline or L-aspartate had returned to baseline levels while that of rats given L-arginine was still 3 times higher than the pretreatment value. These results demonstrate that contrarily to basic amino acids, L-aspartate is not able to specifically interfere with the tubular reabsorption of proteins.

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