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Long-term ingestion of ammonium inhibits lysosomal proteolysis in rat liver

Vicente Felipo, María-Dolores Miñana, Ruth Wallace and Santiago Grisolía

Instituto de Investigaciones Citológicas de la Caja de Ahorros de Valencia, Centro Asociado del CSIC, Amadeo de Saboya, 4, 46010 Valencia, Spain

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A standard diet was supplemented with ammonium acetate (20%, w/w). The effect on liver protein degradation of oral administration of the ammonium diet to rats for 6 weeks has been studied. It is shown that lysosomal proteolysis is markedly decreased (by 62%) while non-lysosomal proteolysis is inhibited by 11%. This is the first report showing that ammonium ingestion inhibits liver proteolysis.

Ammonia; Lysosomal proteolysis

1. INTRODUCTION

The concentration of ammonia in most animal tissues is maintained at low levels. A 5–10-fold increase of the normal ammonia concentration in blood induces toxic effects in most species, with functional disturbances of the central nervous system. In ureotelic animals the main ammonia detoxication mechanism is the production of urea in the liver. Severe hepatic diseases usually lead to hyperammonemia which in turn is thought to play an important role in encephalopathy.

To study hyperammonemia the main models used are: the porta-cava shunt [1,2] and urease treatment [3]. We have recently developed a new model consisting of oral administration to rats of a diet containing ammonium acetate (20%, w/w). Although it has been often shown that ammonia inhibits lysosomal proteolysis in cultured hepatocytes [4,5] and partially in cultured human glial cells [6], this is the first report showing that oral administration of ammonium to the animal inhibits liver proteolysis.

Correspondence address: S. Grisolía, Instituto de Investigaciones Citológicas de la Caja de Ahorros de Valencia, Centro Asociado del CSIC, Amadeo de Saboya, 4, 46010 Valencia, Spain

2. MATERIALS AND METHODS

2.1. *Treatment of animals*

Male Wistar rats weighing 250–275 g were used. Ammonium acetate was mixed in the food (20%, w/w). A mineral and vitamin supplement was added to the drinking water. This mixture contained (in mg/l of drinking water): 140 NaCl, 226 KCl, 120 calcium lactate·$5H_2O$, 36 $MgSO_4 \cdot 7H_2O$, 300 $NaHCO_2$, 150 citric acid·$1H_2O$, 4520 glucose, 8.5 sodium saccharine, 0.2 thiamine, 0.1 riboflavine, 1 nicotinamide, 0.1 pyridoxine, 1 dexpanthenol, 0.01 biotin, 5 ascorbic acid, 0.3 DL-α-tocopherol acetate, 500 IU retinol, 100 IU ergocalciferol.

Control and experimental rats were killed after 42 days on the diet.

2.2. *Effect of ammonium ingestion on proteolysis in liver cytosol*

Rats were fed control or ammonium containing diet for 42 days. Livers were homogenized in 2.5 vols of 0.15 M NaCl containing 5 mM 2-mercaptoethanol. The homogenates were centrifuged for 20 min at 12000 × g at 4°C and the supernatants were used. 10 mg of protein were incubated at 37°C in a final volume of 2.5 ml containing 60 mM NaCl, 30 mM sodium phosphate, pH 7.4. At indicated times, 500 μl portions were taken and trichloroacetic acid-soluble amino acids were determined with a ninhydrin assay [7]. Inhibitors of lysosomal proteolysis were added to a set of tubes, final concentrations were 100 μM chloroquine and 300 μM leupeptin.

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3. RESULTS AND DISCUSSION

The effect of long-term (6 weeks) ammonium ingestion on liver protein degradation has been studied. As shown in fig.1, ammonium ingestion inhibits liver protein degradation. To assess the effect of ammonium ingestion on lysosomal and non-lysosomal proteolysis, the assays were carried out in the absence or the presence of inhibitors of lysosomal proteolysis (chloroquine plus leupeptin). The results presented in table 1 indicate that ammonium ingestion inhibits only slightly non-lysosomal proteolysis (11% inhibition), however, lysosomal proteolysis is markedly inhibited by 62%.

It has been shown that addition of 4 mM ammonium chloride to hepatocyte suspensions inhibits protein degradation 50% [5]. The levels of ammonia in livers of our control and experimental rats were 0.67 and 0.90 μmol/g liver, respectively; therefore a slight increase in the ammonia content of the liver results in a large (62%) inhibition of lysosomal proteolysis. As indicated in [5], ammonia, a weak base, accumulates in the acidic interior of the lysosomes, elevating the intralysosomal pH and inactivating the lysosomal proteases.

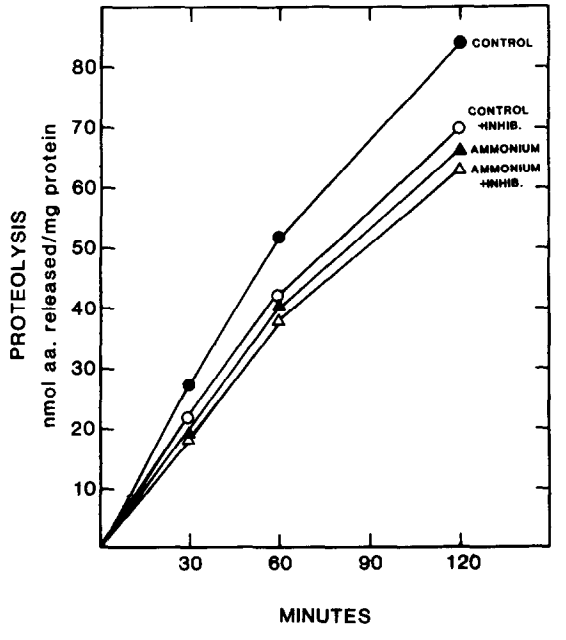

Fig.1. Effect of ammonium ingestion on proteolysis in rat liver. Assays were carried out as described in section 2 without and with addition of inhibitors of lysosomal proteolysis (100 μM chloroquine and 300 μM leupeptin). Values are the mean of six assays.

Table 1

Effect of ammonium ingestion on lysosomal and non-lysosomal proteolysis in rat liver

Diet	Proteolysis (in nmol aa. released/mg protein)		
	Without inhibitors	With inhibitors	Lysosomal
Control	83 ± 7	70 ± 7	13
Ammonium	67 ± 6	62 ± 6	5

Proteolysis was assayed as described in section 2. The values (mean of six assays) after 2 h of incubation at 37°C are given. The lysosomal proteolysis was calculated as the difference in proteolysis without and with addition of inhibitors of lysosomal proteolysis (100 μM chloroquine and 300 μM leupeptin)

Relative to the reported inhibition of protein degradation by ammonia in isolated hepatocytes mentioned above [4,5], it should be noted that isolated hepatocytes prepared by the method of collagenase perfusion are in a highly protein-catabolic state, the intracellular content of lysosomes and autophagosomes increases rapidly during incubation [5] and the rate of protein synthesis is only one-tenth the rate of protein degradation [8]. Therefore isolated hepatocytes do not behave as they do in vivo. The present paper shows for the first time that administration of ammonium in vivo markedly inhibits lysosomal proteolysis.

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