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Research Report

Brain purine metabolism and xanthine dehydrogenase/oxidase conversion in hyperammonemia are under control of NMDA receptors and nitric oxide

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ABSTRACT

In hyperammonemia, a decrease in brain ATP can be a result of adenine nucleotide catabolism. Xanthine dehydrogenase (XD) and xanthine oxidase (XO) are the end steps in the purine catabolic pathway and directly involved in depletion of the adenylate pool in the cell. Besides, XD can easily be converted to XO to produce reactive oxygen species in the cell. In this study, the effects of acute ammonia intoxication in vivo on brain adenine nucleotide pool and xanthine and hypoxanthine, the end degradation products of adenine nucleotides, during the conversion of XD to XO were studied. Injection of rats with ammonium acetate was shown to lead to the dramatic decrease in the ATP level, adenine nucleotide pool size and adenylate energy charge and to the great increase in hypoxanthine and xanthine 11 min after the lethal dose indicating rapid degradation of adenylates. Conversion of XD to XO in hyperammonemic rat brain was evidenced by elevated XO/XD activity ratio. Injection of MK-801, a NMDA receptor blocker, prevented ammonia-induced catabolism of adenine nucleotides and conversion of XD to XO suggesting that in vivo these processes are mediated by activation of NMDA receptors. The in vitro dose-dependent effects of sodium nitroprusside, a NO donor, on XD and XO activities are indicative of the direct modification of the enzymes by nitric oxide. This is the first report evidencing the increase in brain xanthine and hypoxanthine levels and adenine nucleotide breakdown in acute ammonia intoxication and NMDA receptor-mediated prevention of these alterations.

1. Introduction

Ammonia (in both electrically neutral NH_3 and charged NH_4^+ forms) is a common degradation product of proteins, amino acids, and other nitrogen bases. However, extra ammonia is a neurotoxin fast-acting via an increase in the ROS production (Kosenko et al., 1997). Increased brain ammonia concentrations are a hallmark feature of several neurological disorders including congenital urea cycle disorders, Reye's syndrome and hepatic encephalopathy associated with liver failure. Administration of large doses of ammonia can be considered as a model for study at an early stage of acute hepatic

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Abbreviations: NO, nitric oxide; ROS, reactive oxygen species; SNP, sodium nitroprusside; XD, xanthine dehydrogenase; XO, xanthine oxidase; XDH, xanthine dehydrogenase/oxidase complex

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encephalopathy. Metabolic basis of hyperammonemia and hepatic encephalopathy has been studied by us for as long as 20 years (for example, Kosenko et al., 1994, 1996, Kosenko et al., 1997, 1998, Kosenko et al., 2003, 2004), using animal models and was well described by Zwingmann (2007).

Ammonia injection to rats induces depletion of brain ATP which is associated with increased activity of ATP phosphohydrolase (Na^+/K^+-exchanging) (EC 3.6.3.9, Na^+/K^+-ATPase) and consumption of ATP (Kosenko et al., 1994). Besides, depletion of brain ATP in hyperammonemia can be a result of adenine nucleotide catabolism and accompanied by depletion of total adenylate pool and accumulation of dephosphorylated oxypurines. This hypothesis was not tested to date.

Xanthine:NAD^+ oxidoreductase (EC 1.17.1.4, xanthine dehydrogenase, XD) and xanthine oxidase (EC 1.17.3.2, XO), two enzyme forms of the xanthine dehydrogenase/oxidase (XDH) enzyme complex, are the end steps in the purine catabolic pathway and directly involved in depletion of the adenylate pool (AN pool) in the cell. XD and XO have also been implicated as a source of reactive oxygen species (ROS) inducing neuronal cell injury (Haorah et al., 2008). XD predominates in healthy tissue, but under pathological conditions XD may be readily converted to XO through the reversible thiol oxidation of sulfhydryl residues on XD or by the irreversible proteolytic cleavage of a fragment of XD (Corte and Stirpe, 1972; Nishino et al., 2008). A decrease in the ATP level was proposed to be necessary for transformation of XD to XO (Roy and McCord, 1983). It is unknown whether conversion of XD to XO is associated with the AN pool and levels of hypoxanthine and xanthine, substrates of the two enzyme and the end degradation products of adenylates.

Acute intoxication with large doses of ammonia has been shown to be mediated by activation of NMDA receptors in rat brain in vivo (Hermenegildo et al., 2000). Excessive activation of NMDA receptors is involved in the neuronal damage found in brain ischaemia and some neurodegenerative diseases (Beal, 1992; Lafon-Cazal et al., 1993). The mechanisms by which overactivation of NMDA receptors leads to neuronal degeneration and death involve activation of L-arginine, NADPH:oxygen oxidoreductase (nitric-oxide-forming) (EC 1.14.13.39, NO synthase) (Kitano et al., 2004), the formation of nitric oxide (NO) and other ROS (Gunasekar et al., 1995; Mattson et al., 1995). It is unknown whether NMDA receptors and NO contribute to adenylate metabolism and conversion of XD to XO.

The aim of the present work was to study the effects of acute ammonia intoxication in vivo on brain AN pool and xanthine and hypoxanthine levels during the conversion of XD to XO. Another aim was to explore the effects of MK-801, a NMDA receptor blocker, and sodium nitroprusside (SNP), a NO donor, on above metabolites and enzymes.

2. Results

2.1. *Brain purine levels*

We determined the effects of ammonia and/or MK-801 administration to animals on brain adenine nucleotides as well as on xanthine and hypoxanthine, products of adenine nucleotide degradation.

Injection of rats with ammonium acetate led to the pronounced reduction (by 65%) of brain ATP, rises of ADP (by 30%) and AMP (by 56%) (Fig. 1), and to the decrease in the AN pool by 38% and the adenylate energy charge (EC) by 20% (Fig. 2). Ammonia-induced changes in all parameters of adenine nucleotide metabolism were prevented

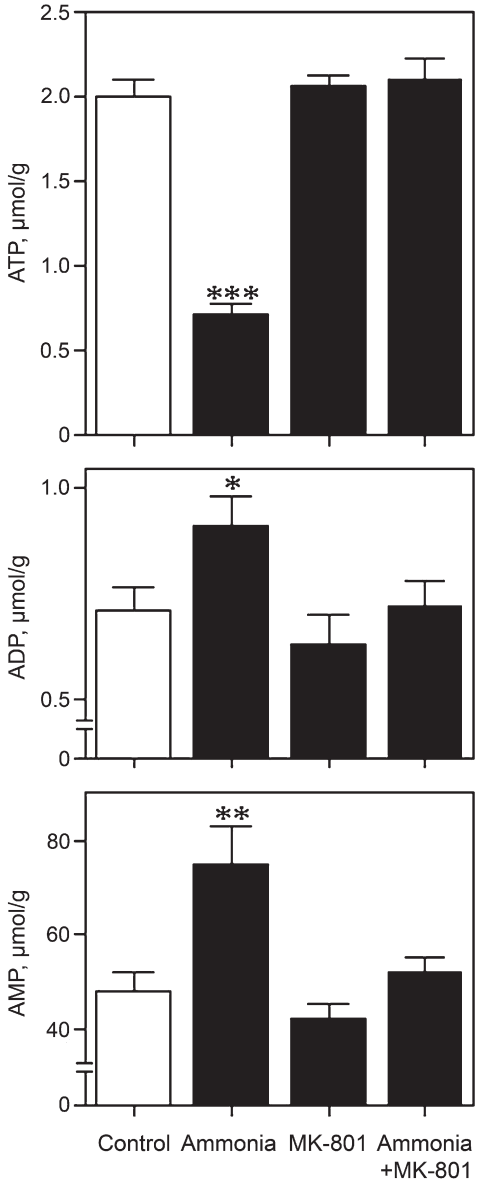

Fig. 1 – Brain ATP, ADP and AMP in control rats and rats injected with ammonia and/or MK-801. Rats were injected with either saline (Control), 12 mmol/kg of ammonium acetate (Ammonia), 1.5 mg/kg of MK-801, or 12 mmol/kg of ammonium acetate followed by 1.5 mg/kg of MK-801 (Ammonia + MK-801). Then the acid extract was prepared from forebrain and ATP, ADP and AMP were measured and expressed as nmol/g or mmol/g wet weight. Results are mean ± S.E.M. of 6 to 8 rats ($^*p<0.05$, $^{**}p=0.01$, $^{***}p=0.0001$, as compared to control, Student's t-test).