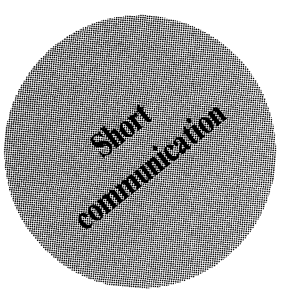
ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 545-548

Rat cerebral mitochondrial glutaminase activity is unaffected by moderate hyperammonemia in two models

Jan Albrecht, Wojciech Hilgier and **Lidia Faff**

Department of Neurotoxicology, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa St., 00-784 Warsaw, Poland



Abstract. The phosphate-dependent (PAG) and phosphate-independent (PIndG) glutaminase activities were measured in cerebral perikaryal mitochondria derived from rats subjected to ammonium acetate- induced "simple" hyperammonemia (SHA) or thioacetamide-induced hepatic encephalopathy (HE). These two moderately hyperammonemic conditions were previously found to be accompanied by pronounced changes in virtually all the enzyme activities coupling the tricarboxylic acid cycle to the synthesis and metabolism of the excitatory neurotransmitter glutamate. Both PAG and PIndG remained unaffected by SHA or HE, indicating that they do not contribute to the cerebral glutamine/glutamate imbalance associated with both conditions.

Key words: hyperammonemia, hepatic encephalopathy, mitochondria, phosphate-activated glutaminase

The major route of ammonia metabolism in the CNS is the glutamate (GLU)-glutamine (GLN) cycle. It consists of GLN synthesis from GLU that is mediated by an astrocytic, cytoplasmic enzyme, glutamine synthetase, and subsequent GLN conversion to neurotransmitter or metabolic pools of GLU, which includes direct GLN hydrolysis by a mitochondrial enzyme, phosphate-activated glutaminase (PAG), and complex transamination and dehydrogenation loops that couple GLU metabolism to energy metabolism, and are also catalyzed by enzymes located in mitochondria (for reviews and references see Cooper and Plum 1987, Aoki et al. 1991, Albrecht and Faff 1994). Expectedly, overloading of this route by excess ammonia entering the brain in hyperammonemic conditions causes derangements of glutamatergic or GABAergic neurotransmission and energy metabolism (literature listed in the references above). In this laboratory, we have investigated the effects of ammonium acetate-induced simple hyperammonemia (SHA), and thioacetatamide (TAA)-induced hepatic encephalopathy (HE) following toxic liver damage, on the activities of mitochondrial enzymes involved in GLU synthesis and metabolism. Either treatment resulted in moderate hyperammonemia of comparable degree (Albrecht and Hilgier 1984, Hilgier and Olson 1994), and in marked changes in virtually all the mitochondrial enzymes involved in GLU metabolism. The enzymes affected included: the enzymes of the malate-aspartate shuttle (Faff--Michalak and Albrecht 1991), 2-oxoglutarate dehydrogenase (Faff-Michalak and Albrecht 1993a), glutamate dehydrogenase acting towards both GLU synthesis and degradation either direction (Faff--Michalak and Albrecht 1993b), and the CO_2-fixing enzyme, pyruvate carboxylase (Faff-Michalak and Albrecht 1991). The changes involved both synaptic and perikaryal mitochondria and were of the character indicating depression of both GLU synthesis and energy metabolism (reviewed in Albrecht and Faff 1994). The only mitochondrial enzyme pertinent to GLU not yet considered in our investigations was PAG. PAG is located in the inner mitochondrial membrane of neuronal perikarya, nerve endings, and glial cells, but by immunocytochemical evidence predominates in perikaryal mitochondria (Aoki et al. 1991, and references therein). In the present study, therefore, we measured the activity of PAG in SHA and HE rats. In addition, we measured the phosphate-independent form of glutaminase (later referred to as PIndG), which is active in cerebral mitochondria (Dennis et al. 1977), albeit its physiologic significance is uncertain (Kovacevic and McGivan 1983, Aoki et al. 1991).

Male Wistar rats weighing 150-220 g were used throughout. Simple hyperammonemia (SHA) was produced by three intraperitoneal (i.p.) injections of 600 mg/kg body weight of ammonium acetate in physiological saline at 24 h intervals, and the animals were killed 30 min after the last injection (Hilgier et al. 1990, Hilgier and Olson 1994). HE was induced in identical rats by three i.p. injections of 250 mg/kg body weight of a hepatotoxin, thioacetamide, and the animals were sacrificed 24 h after the last injection of the drug (Albrecht and Hilgier 1994). Perikaryal (nonsynaptic) mitochondria were isolated with the Ficoll-sucrose gradient centrifugation method of Lai and Clark (1976), slightly modified (Faff-Michalak and Albrecht 1991). Glutaminase activities were assayed by measuring oxidation of NADH spectrophotometrically at 340 nm with continuous recording, in an incubation mixture designed by Dennis et al. (1977), containing (final concentrations): 0.4 mM EDTA (potassium salt), 0.4 mM NADH, 15 mM 2-oxoglutarate, 19 mM glutamine, 0.015% (v/v) Triton X-100, 9 IU of glutamate dehydrogenase in 50% (v/v) glycerol and 0.3 mg of mitochondrial protein, in a final volume of 3 ml. The PAG assays were carried out in 19 mM potassium phosphate buffer, pH 7.4, and PIndG activity was determined in 15 mM HEPES buffer, pH 8.8.

In agreement with the report of Dennis et al. (1977), the nonsynaptic mitochondrial PAG activity at saturating substrate concentration was about 1.4 times higher than the PIndG activity (Table I). Neither of the two enzyme activities was affected by SHA or HE (Table I).

TABLE I

The phosphate-activated (PAG) and phosphate-independent (PIndG) glutaminase activity in cerebral perikaryal mitochondria from control rats and rats with simple hyperammonemia (SHA) or hepatic encephalopathy (HE)

Group	PAG[a]	PIndG[a]
control	33±6 (6)	24±5 (6)
SHA	30±7 (6)	25±6 (3)
HE	33±6 (7)	31±8 (6)

[a] nmoles/min/mg protein. Results are mean ± SD for the number of independent experiments in parentheses.

In vitro studies with brain slices or homogenates (Benjamin 1981), synaptosomes (Bradford and Ward 1976), or astrocytes in culture (Rao and Murthy 1992a) revealed an exceptionally high vulnerability of PAG to ammonia at low milimolar concentrations known to produce acute toxic effects *in vivo*. In this context it may appear surprising that PAG and PIndG are the only mitochondrial GLU-metabolizing enzymes that are resistant to moderate hyperammonemia. Both in SHA and HE rats, brain ammonia concentration was found to increase to 0.5 mM (Albrecht and Hilgier 1984, Hilgier and Olson 1994), which is only slightly below the concentrations observed to produce *in vitro* inhibition in the above cited studies. It would thus appear that PAG adapts to continuous exposure to ammonia. Consistent with this concept, the enzyme activity was found unchanged in the brains of rats with long-term hyperammonemia following portacaval shunt (Cooper et al. 1985), but was markedly inhibited in synaptosomes (Subbalakshmi and Murthy 1985) or astrocytes (Rao and Murthy 1992b), derived from acutely hyperammonemic rats which were in the convulsive stage. The mechanism of adaptation in the models used in the present study is unknown. It is noteworthy in this context that, toxic liver damage in a model similar to that used in the present study resulted in the increase of the cerebral PAG mRNA level (Thomas et al. 1988).

SHA and HE in the present models are associated with GLN/GLU imbalance in favor of GLN in different brain regions, being most pronounced in the cerebral cortex (Hilgier and Olson 1994). GLN content was increased in either model: 2.5-fold in HE and 1.5-fold in SHA, whereas GLU was decreased by 17% in SHA, and was not different from control in HE. The lack of changes in PAG activity would indicate that other enzymes than PAG must be involved in producing the imbalance. At least in the HE model, excessive accumulation of GLN may, in addition to the disturbances of other mitochondrial enzymes (see the introductory paragraph), be related to the increased cerebral glutamine synthetase (GS) activity (Hilgier et al. 1983). It must be noted however, that neither GS nor PAG activities *in vitro* genuinely reflect the rate of GLN synthesis or degradation *in vivo*. Studies of ^{15}N ammonia fluxes in hyperammonemic rats revealed that the rate of hydrolysis of ^{15}N GLN to ^{15}N GLU is less than 1% that predicted from PAG activity measurements (Kanamori and Ross 1995), whereas ^{15}N GLN synthesis from ^{15}N ammonia occurs at a rate corresponding to <5% of the reported optimum GS activity *in vitro* (Kanamori and Ross 1993). A similar inconsistency between the metabolic fluxes of ammonia and the activities of ammonia metabolizing enzymes in a portacaval shunt model in rat was earlier reported by Cooper et al. (1985). The present result supports the general notion that, while enzyme activity changes appear to inform about the metabolic potential of mitochondria, they may misinform about the actual metabolic rates.

We thank Ms Mirosława Poławska for skillful technical assistance.

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Received 10 January 1996, accepted 24 January 1996

This paper is dedicated to Professor Stella Niemierko on the occasion of her 90th birthday, with esteem and admiration