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# The changes in activities of some ammonia metabolizing enzymes in liver and brain of rats intoxicated by chronic administration of acetaldehyde

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Acetaldehyde (AcH) is a main natural metabolite of ethanol oxidation *in vivo*. Its ability to initiate hepatotoxicity has been well documented [1]. AcH is considered to be 10 to 30 times more toxic than ethanol [2]. It is capable of interacting with cellular constituents like proteins [3], glutathione [4] or many enzymes, and of modifying in this way metabolic functions of liver and other organs. AcH inhibits protein synthesis in the liver [5] and in the pancreas [6] as well as secretion of glycoproteins [7].

In rats treated with AcH for 4 weeks the increase of aspartate aminotransferase, alanine aminotransferase and γ-glutamylotranspeptidase activities was observed in the serum [8]. Histomorphological studies revealed a marked steatosis, necrosis of single cells and microthrombosis in the liver [9]. Some alterations caused by AcH have also been found in liver cell mitochondria resulting in impairment of citric acid cycle [10]. The changes in protein and nonprotein sulfhydryl compounds of rat plasma, liver and brain after chronic AcH administration has been also observed by several authors [4, 11, 12].

In rats chronically intoxicated with ethanol the increase of liver ammonia concentration and changes in the activities of ammonia metabolizing enzymes in the liver and brain were found [10, 13].

The aim of the present study was to examine the effect of chronic acetaldehyde administration on the activities of ammonia metabolizing enzymes in liver and brain, i.e. glutamine synthetase, glutamate dehydrogenase and glutaminase, and on the blood ammonia concentration in rats.

## *Materials and methods*

*Animals*. The experiments were carried out on male Wistar rats weighing about 300 g. The animals were fed the standard commercial rodent diet *ad lib*. The rats were divided into two groups. The first group of 12 rats was given intragastrically 1.5% (w/v) solution of AcH in an isotonic NaCl in a daily dose of 0.3 ml/kg of body weight, every day for a period of 4 weeks. The second (control) group of 12 rats received an equivalent volume of 0.15 M NaCl. All rats were weighed before and after the experiment to determine the differences between mean initial and final body weight of each group. Mean daily food intake in AcH and control rats were compared and analysed by the *t*-test.

*Preparation of tissues*. After the blood had been taken, the liver and brain tissues were quickly removed and placed into 0.15 M NaCl in an ice bath. The tissues were subsequently blotted on filter paper in order to remove the physiological salt, then weighed and homogenized in 9 vol. of ice-cold 0.25 M sacharose. The homogenates were centrifuged at 600 *g* for 10 min at 4° and supernatant was kept in ice until assayed. Protein was determined in diluted aliquots of the tissue homogenates by the method of Lowry *et al.* [14], using crystalline bovine serum albumin as a standard.

The liver and brain glutamine synthetase activity was estimated according to the method of Rowe *et al.* [15]. The glutamate dehydrogenase activity was determined by the method of Schmidt [16], and glutaminase activity was measured by the method described by Mardashev *et al.* [17]. All enzyme activities were determined at 25°. One unit of enzymatic activity was defined as the amount of enzyme which catalysed the synthesis of μM of γ-glutamylhydroxamate, the amount of oxidized NAD and ammonia formed per mg of protein per hr for glutamine synthetase, glutamate dehydrogenase and glutaminase, respectively.

*Blood ammonia concentration*. Blood was taken by cardiac puncture into 2 ml heparinized tubes and analysed for ammonia concentration according to the method described by Hilgier and Albrecht [18].

*Statistical analysis*. Data were expressed as mean ± SD. The results were elaborated statistically using Student's *t*-test.

## *Results*

No differences were found in final body weight between AcH and control rats groups. A marked increase of blood ammonia concentration in AcH treated group was observed, from 41.1 to 82.8 μmol/l. The differences were statistically significant ($P < 0.05$) (Table 1).

Table 2 shows the effect of AcH on the liver and brain glutamate dehydrogenase, glutamine synthetase and glutaminase activities in the rats. In AcH-treated groups the liver activities of glutamate dehydrogenase and glutaminase were higher than in the control group and the differences were statistically significant. However, the differences in the liver activity of glutamine synthetase were not significant ($P = 0.12978$). In the brain tissue of the AcH-treated group the activities of all analysed enzymes were significantly higher than in control group.

## *Discussion*

It has been widely demonstrated that 4 weeks intoxication with AcH does not lead to the loss of body weight in the rats, while both studied organs—liver and brain—

Table 1. Effect of 4 weeks administration of AcH on body weight of rats and blood ammonia concentration

| | Initial body weight (g) | Final body weight (g) | Ammonia concentration (μmol/l) |
|---|---|---|---|
| Control | 297.3 ± 9.1 | 366.6 ± 29.5 | 41.1 ± 14.3 |
| AcH | 294.2 ± 11.7 | 350.6 ± 29.1 | 82.8 ± 40.9* |

Values are mean ± SD. N = 12, * – $P < 0.05$.

Table 2. Glutamate dehydrogenase, glutamine synthetase and glutaminase activities in liver and brain of rats treated with AcH for 4 weeks

| | Glutamate dehydrogenase | Glutamine synthetase | Glutaminase |
|---|---|---|---|
| Control *Liver* | 3.53 ± 1.20 | 0.19 ± 0.07 | 0.87 ± 0.09 |
| AcH | 5.36 ± 1.17* | 0.25 ± 0.09 | 1.49 ± 0.43* |
| Control *Brain* | 1.70 ± 0.23 | 0.39 ± 0.06 | 4.66 ± 1.13 |
| AcH | 3.35 ± 0.42* | 0.60 ± 0.15* | 6.73 ± 0.99* |

Values are mean ± SD. N = 10, * – $P < 0.05$.

indicate distinct injury [8, 9]. In our previous experiments, the higher activities of aspartate and alanine aminotransferases were found in the serum of rats treated with ethanol or acetaldehyde than in control groups [8]. These findings clearly indicate that exogenous AcH may impair liver function in rats.

Nitrogen metabolism in liver and brain depends on the activities of ammonia metabolizing enzymes and it may be changed in many acute and chronic liver diseases. Recently, a peculiar distribution of enzymes involved in liver ammonia metabolism has been discovered [19, 20]. Urea cycle enzymes carbamoylphosphate synthetase and argininosuccinate synthetase and also glutaminase are localized in periportal region of liver acinus, whereas glutamine synthetase and glutamate dehydrogenase have a common (subacinar) perivenous localization [19–22]. It is well known that ethanol produces periportal cell necrosis and inhibits urea synthesis in rat liver [23, 24]. On the other hand, the hepatotoxicity of ethanol have been often attributed to its major metabolite—acetaldehyde. Mitochondrial low $K_m$-aldehyde dehydrogenase which is responsible for the oxidation of acetaldehyde is predominantly localized in the perivenous area [22]. It is possible that after AcH ingestion the hepatocytes of periportal area are bathed in higher concentration of AcH than in perivenous area resulting in localized injury.

Our results indicate that 4 weeks exposure to AcH affects ammonia detoxication in rat liver. The observed hyperammonemia seems to be caused by inhibition of urea cycle enzymes and activation of glutaminase in rat treated with AcH, however, the activities of urea cycle enzymes in AcH-damaged liver has not been generally examined. Additionally, AcH has no effect on the activity of glutamine synthetase catalysing amidation of glutamate in liver tissue. This enzyme is responsible for detoxication of ammonia which escapes periportal urea synthesis. i.e. 10–30% of the portal ammonia load *in vivo* and *in vitro* [25, 26]. Cascales *et al.* [10] have shown that AcH consumption induces a significant decrease of 2-oxoglutarate and pyruvate concentration in rat liver and raises glutamate dehydrogenase activity. It is possible that impairment of citric acid cycle and energy supply does not permit the increase of the glutamine synthesis.

Since brain lacks carbamoyl-phosphate synthetase and ornithine transkarbamylase, this tissue normally removes ammonia by only one major enzymatic mechanism: the glutamate–glutamine cycle [27]. Brain glutamate dehydrogenase and glutamine synthetase are localized mainly in astrocytea, and glutaminase—in neurons [27, 28]. In our experiment the increase of all analysed enzyme activities was observed in the brain tissue of AcH-treated rats. It seems that observed changes represents the metabolic adaptation to increased brain ammonia load. This is especially important in the light of evidence that the glutamate–glutamine cycle plays a significant role in neuronal–astroglial amino acid neurotransmitter interaction. The changes in the activities of these enzymes may impair glutamatergic and GABA-ergic neurotransmission [29].

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# Comparative *ex vivo* inhibitory effects of (*E*)-2-(3,4-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) on amine oxidase activities in the rat

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Monoamine oxidase (MAO) is a mitochondrial enzyme found in two molecular forms (A and B), having different substrate and inhibitor specificities, and existing in varying proportions in different animal tissues. Recent interest in the therapeutic potential of drugs which act as selective inhibitors of the B-form of MAO, has arisen from favourable clinical experience with L-deprenyl, which is believed to potentiate the benefits of L-dihydroxyphenylalanine (L-DOPA) therapy in Parkinsonian patients by blocking the striatal degradation of dopamine by MAO-B. Also, by sparing MAO-A activity in peripheral tissues, deprenyl administration does not precipitate the occurrence of hypertensive episodes after dietary ingestion of tyramine (reviewed in Ref. 1).

Experimental studies have shown that (*E*)-2-(3,4-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) is another selective irreversible inhibitor of MAO-B in rat and mouse brain [2, 3]. Like deprenyl, the administration of MDL 72145 can protect certain laboratory species from the neurodegenerative effects of *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) upon the nigrostriatal dopamine pathway (e.g Ref. 4) and *in vitro* MDL 72145 blocks the oxidation of MPTP by MAO-B to putative neurotoxic compounds [5], that are believed to be responsible for the MPTP-induced Parkinsonian-like state which can occur in man. Thus, MDL 72145 is another potential anti-Parkinsonian agent with MAO-B inhibitory properties, without however, the additional amphetamine-like actions exhibited by deprenyl and its metabolites in rodents [3, 6].

We showed recently that MDL 72145 and some related analogues are also potent irreversible inhibitors *in vitro* of a semicarbazide-sensitive amine oxidase (SSAO) in the rat aorta [7, 8]. This enzyme is particularly active in vascular smooth muscle cells [9], probably as a plasmalemmal component [10], and it is distinguishable from MAO activities