Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 468 (2003) 21–25
www.elsevier.com/locate/ejphar

Taurine reduces ammonia- and *N*-methyl-D-aspartate-induced accumulation of cyclic GMP and hydroxyl radicals in microdialysates of the rat striatum

Wojciech Hilgier[a], Elmira Anderzhanova[b], Simo S. Oja[b,c], Pirjo Saransaari[b], Jan Albrecht[a,*]

[a] *Department of Neurotoxicology, Medical Research Centre, Polish Academy of Sciences, Pawińskiego St. 5, 02-106 Warsaw, Poland*
[b] *Brain Research Center, University of Tampere Medical School, Tampere, Finland*
[c] *Department of Clinical Physiology, Tampere University Hospital, Tampere, Finland*

Received 13 March 2003; accepted 25 March 2003

Abstract

Acute ammonia neurotoxicity caused by intraperitoneal administration of ammonium salts is mediated by overactivation of *N*-methyl-D-aspartate (NMDA) receptors, with ensuing generation of free radicals and extracellular accumulation of cyclic GMP (cGMP) arising from stimulation of nitric oxide (NO) synthesis. In this study, infusion of ammonium chloride or NMDA into the striata of rats via microdialysis probes increased the contents of cyclic GMP and hydroxyl radicals in the microdialysates. Co-infusion of taurine virtually abolished both the ammonia- and NMDA-induced accumulation of cGMP. Taurine also attenuated accumulation of hydroxyl radicals evoked by either treatment. This result is the first evidence of a potential of taurine to attenuate the effects of NMDA receptor overactivation by ammonia in vivo and points to the inhibition of the NMDA receptor-mediated NO synthesis as a possible mechanism of its neuroprotective action. Taurine or its blood–brain barrier penetrating analogues may be applicable in treatment of ammonia-induced neurological deficits.

Keywords: Ammonia neurotoxicity; NMDA receptor; cGMP; Hydroxyl radical; Microdialysis; Striatum; Taurine

1. Introduction

The nonproteinaceous sulfur-containing amino acid taurine protects cells in different tissues against oxidative injury. Taurine forestalls in cultured neurons (Boldyrev et al., 1999; El Idrissi and Trenkner, 1999) and brain slices (O'Byrne and Tipton, 2000; Zielińska et al., 2003) cell damage evoked by glutamate or excitotoxins that overactivate ionotropic glutamate receptors, mostly of the *N*-methyl-D-aspartate (NMDA) class (reviewed by Saransaari and Oja, 2000). However, the neuroprotective potential of taurine has not yet been proven in vivo.

Overactivation of NMDA receptors leads to mitochondrial damage associated with calcium influx which results in the generation of free radicals including superoxide. The synthesis of nitric oxide (NO) is also stimulated and NO reacts with superoxide anions to form peroxynitrite (Lafon-Cazal et al., 1993). Peroxynitrite has been ascribed a major role in nerve cell damage and death in a spectrum of acute and chronic neuropathological conditions (reviewed by Heales et al., 1999). In vivo, NO production resulting from activation of NMDA receptors can be reliably quantified by measuring the resulting increase in the cyclic GMP (cGMP) levels in brain microdialysates (Fedele et al., 2000 and references therein). Ammonia in vitro (Montoliu et al., 1999), or administered intraperitoneally to rats in vivo also activates excessively *N*-methyl-D-aspartate (NMDA) receptors (Hermenegildo et al., 2000). In vivo, the ensuing metabolic disturbances and neurological symptoms are related to the degree of oxidative stress and the rate of formation of free radicals (Kosenko et al., 1995, 1998, 1999). NO synthesis is likewise enhanced, as indexed by the extracellular accumulation of cGMP in brain microdialysates (Hermenegildo et al., 2000; Monfort et al., 2001). We now show that ammonia and NMDA alike, infused directly to the rat striatum via microdialysis probes, promote the extracellular accumulation of both cGMP and hydroxyl

* Corresponding author. Tel.: +48-22-668-5323; fax: +48-22-668-5532.
E-mail address: jalb@cmdik.pan.pl (J. Albrecht).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01667-4

radicals and that taurine co-administration with either stimulus counteracts both effects, being relatively more effective in reducing the cGMP than the hydroxyl radical content.

2. Materials and methods

2.1. Animal preparation

Adult male Sprague–Dawley rats, 200–230 g, from Orion (Espoo, Finland) breeding colony were used in this study. The rats kept under controlled environmental conditions (temperature 22 °C, relative humidity 45–55%, 12 h light/dark cycle) and housed individually in separate cages were supplied with food and water ad libitum. The animals were randomly divided into the control and treatment groups. The experimental procedures were in accordance with the European Community Directive for the ethical use of experimental animals. All efforts were made to minimize both the suffering and the number of animals used.

The rats were anesthetized with 4% halothane in air within 2 min and then maintained under anesthesia with 1% halothane in air delivered at 1.2 l/min. They were placed in a stereotaxic frame with blunt ear bars and a small incision (3–5 mm) was made in the skin over the skull. Holes were drilled for the skull screws and the concentric microdialysis probes implanted in the left and right caudate putamen [coordinates from bregma, AP = 0.5, ML = ± 0.3, DV = – 5, 9–6.1. according to the atlas of Paxinos and Watson (1982)].

2.2. Microdialysis

Microdialysis probes of a concentric design (0.5 mm o.d., 3-mm dialyzing membrane) were used (CMA 12, CMA/Microdialysis, Sweden). The probes were perfused with artificial cerebrospinal fluid containing (in mM): Na^+ 150; K^+ 3.0; Ca^{2+} 1.2, Mg^{2+} 0.8; $H_2PO_4^-$ 31.0; Cl^- 155; pH 7.4, at a rate of 2.5 μl/min. Sixty mM ammonium chloride ("ammonia") or 1 mM NMDA, and/or 85 mM taurine were infused for 40 min. The extracellular concentrations of ammonia and taurine during infusions were 5 and 10 mM, respectively, when corrected for the probe efficiency (Zielińska et al., 2002). For cGMP assays, microdialysate fractions were collected to tubes containing 4 mM ethylenediaminetetra-acetate. In order to determine the level of hydroxyl radicals, 5 mM salicylic acid was added into the perfusion fluid.

A constant flow rate (2.5 μl/min) in the perfused probes was maintained with a microdialysis pump (CMA/Microdialysis, Sweden) throughout the experiment.

2.3. Determination of cGMP

cGMP was assayed with the BIOTRAK cGMP enzyme immunoassay kit (Hermenegildo et al., 2000).

2.4. Determination of hydroxyl radical generation

The generation of hydroxyl radicals was determined by quantifying the rate of formation of 2,3-dihydroxybenzoic acid from salicylic acid in the microdialysis perfusates (Yamamoto and Zhu, 1998). The acids were separated by high-performance liquid chromatography and detected in a system designed for monoamine assays (Sharp et al. 1986). The mobile phase was 0.1 M citrate–phosphate buffer (pH 3.0), 1.1 mM octanesulfonic acid, 0.1 mM ethylenediaminetetra-acetate and 9–13% acetonitrile. The flow rate with analytic PR-C18 column, 3 μm packing, 3 mm i.d. × 15 cm (ESA, USA), was 500 μl/min. The detection was electrochemical with a Coulochem II (ESA). Electrodes 1 and 2 were set at − 175 and + 200 mV, respectively.

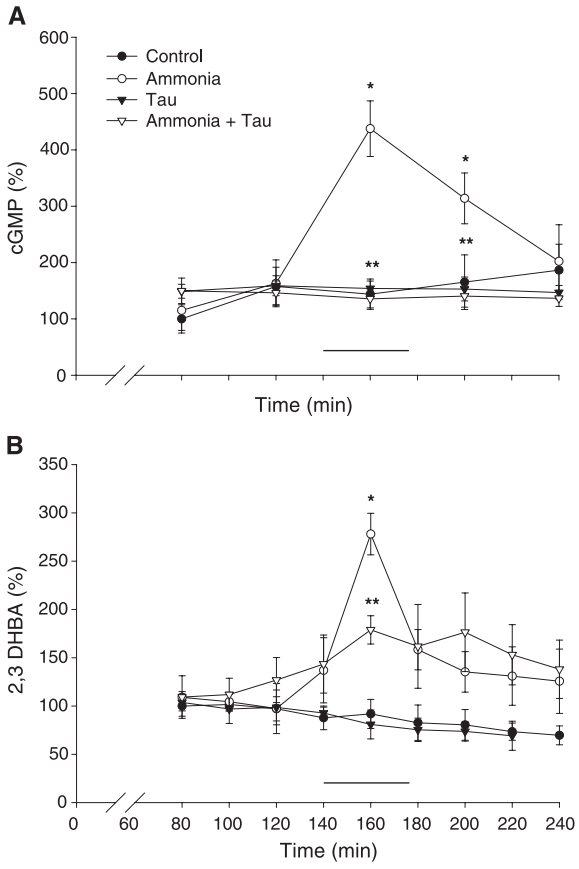

Fig. 1. Accumulation of cGMP (A) and hydroxyl radicals (B) in microdialysates of the rat striatum upon superfusion with artificial cerebrospinal fluid ("Control"), 60 mM ammonium chloride ("Ammonia"), 85 mM taurine ("Tau"), and a combination of ammonia and taurine ("Ammonia + Tau"). The absolute values for control samples collected at 80 min were: 157 ± 39 pmol/l for cGMP and 102 ± 12 nmol/l for 2,3-DHBA. The bar indicates the period in which ammonia and/or taurine were present in the superfusion medium. Results are mean ± S.D. of five to six experiments: *$P < 0.05$ "Ammonia" vs. "Control", **$P < 0.05$ "Ammonia + Tau" vs. "ammonia" (one-way ANOVA followed by Dunnet's test).

3. Results

In the absence of ammonia, the contents of hydroxyl radicals and cGMP in microdialysates remained at approximately constant low levels throughout the whole infusion period, both in the presence (“Tau”) and absence (“control”) of taurine (Fig. 1A and B). Ammonia alone increased the cGMP and hydroxyl radical contents in microdialysates by about 3-fold (Fig. 1A) and 2.5-fold (Fig. 1B), respectively. After ammonia withdrawal, cGMP returned to the control level within the next 1 h, whereas the hydroxyl radical content remained at about 150% of the control level throughout the observation period. Taurine co-administration (“ammonia + Tau”) completely prevented the ammonia-induced increase in the cGMP content (Fig. 1A) and reduced the hydroxyl radical content to about 1.7-fold of the control level (Fig. 1B). NMDA increased the cGMP content by about 2-fold with a subsequent return to the control level (Fig. 2A). A biphasic response to NMDA was noted with regard to the hydroxyl radical content: two peaks, each amounting to a 2-fold increase above the control level were noted after the first 20 min of treatment and 40 min after NMDA withdrawal, respectively. Similar to ammonia, taurine co-administration completely prevented the ammonia-induced increase in the cGMP content (Fig. 2A, “NMDA + Tau”). Taurine was relatively ineffective in reducing the NMDA-induced increase of free radical content in its first phase, but almost completely abolished free radical accumulation in the second phase (Fig. 2B, “NMDA + Tau”).

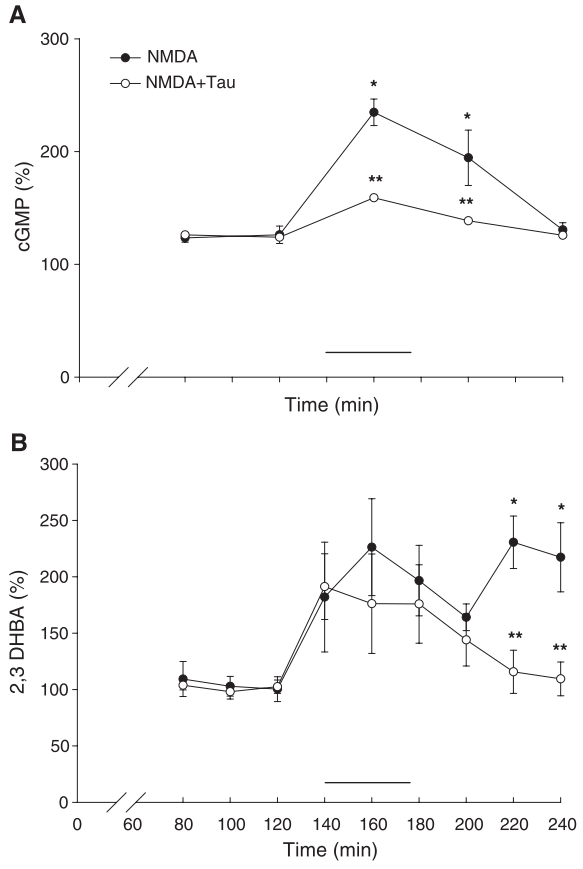

Fig. 2. Accumulation of cGMP (A) and hydroxyl radicals (B) in microdialysates of the rat striatum upon superfusion with 1 mM *N*-methyl-D-aspartate (“NMDA”) alone or in combination with 85 mM taurine (“NMDA + Tau”). Results are mean ± S.D. of five to six experiments: $*P<0.05$ “NMDA” vs. “control” of Fig. 1, $**P<0.05$ “NMDA + Tau” vs. “NMDA” (one-way ANOVA followed by Dunnet’s test). For further details, see the legend in Fig. 1.

4. Discussion

The here observed two downstream effects of intrastriatal administration of ammonia via the microdialysis probe, the accumulation of cGMP and free radicals in the microdialysates, are fully consistent with previous observations in rats treated intraperitoneally with toxic doses of ammonium salts (Kosenko et al., 1995, 1998, 1999; Hermenegildo et al., 2000; Monfort et al., 2001). The similarity of the responses to ammonia and NMDA bespeaks the role of NMDA receptor activation in the ammonia-induced impairment of the nerve cell metabolism and function. With both stimuli alike, co-administration of taurine fully prevented the generation of cGMP and partially decreased the formation of hydroxyl radicals. The results point, to our knowledge for the first time, to the potential of exogenous taurine to counteract the downstream effects of overexcitation of NMDA receptors in general, and the NMDA-mediated aspect of the excitatory activity of ammonia in vivo in particular. It must be noted that short-term exposure to high ammonia concentrations in vivo causes neuronal dysfunction without profound changes in nerve cell morphology: these become apparent only in subchronic or chronic hyperammonemic conditions (Hilgier et al., 1999 and references therein). The major manifestation of acute hyperammonemia in vivo is astrocytic swelling (see Albrecht and Jones, 1999 for a review), which is held responsible for shrinkage of, and excessive glutamate (Glu) accumulation in the extracellular space (Butterworth, 1997; Vogels et al., 1997). Intrastriatal administration of ammonia in the present model does not increase Glu accumulation in the microdialysates (Zielińska et al., 2002). Whether and in what degree taurine is effective in preventing authentic ammonia-induced nerve cell damage will have to be tested using a model incorporating prolonged treatment of rats with ammonia.

The exact mechanism of the antiexcitatory action of taurine remains to be unraveled. There are reasons to suggest the involvement of the $GABA_A$ receptor complex. In vitro, taurine interacts with the ligand recognition site in $GABA_A$ receptors (Malminen and Kontro, 1986) and activates Cl^- conductance via these receptors, producing long-term changes in corticostriatal transmission (Oja et al., 1990; Chapkova et al. 2002). In vivo, infusion of agonists of the $GABA_A$–benzodiazepine receptor complex by micro-

dialysis counteracts the NMDA receptor-mediated accumulation of cGMP in different brain regions (Fedele et al., 2000 and references therein). The antiexcitotoxic action of taurine in brain slices treated with MPP^+ (O'Byrne and Tipton, 2000) and reduction of NMDA receptor-mediated cell swelling in brain slices exposed to ammonium ions (Zielińska et al., 2003) is partly ameliorated by the $GABA_A$ receptor antagonist bicuculline. It will be of interest to see whether interference with the $GABA_A$ receptor function in vivo will affect the response of the NMDA/NO/cGMP pathway to ammonia. One other possible target of taurine action worth testing is the central glycine receptor: recent evidence suggest that taurine rather than glycine is the endogenous mediator of the inhibitory signal at the glycine receptor (Sergeeva and Haas, 2001; Martin and Siggins, 2002).

Of note, in vitro studies have shown that the MPP+- or ammonia-induced cellular impairments in brain slices are prevented both in the presence and absence of a taurine uptake inhibitor, guanidinoethane sulfonate (GES) (O'Byrne and Tipton, 2000; Zielińska et al., 2003). These observations further support the receptor-mediated rather than intracellular mechanism of the antiexcitotoxic action of taurine. Consistent with these observations, taurine in the present in vivo model turned out to be more effective in counteracting the accumulation of cGMP than in preventing the generation of free radicals. Of note, taurine has been relatively ineffective in scavenging peroxynitrite in cultured neurons treated with NO donors (Mehta and Dawson, 2001). The antiexcitotoxic activity of taurine may bypass its ability to scavenge free radicals: in cultured cerebellar neurons, taurine has prevented the kainate-induced cell death without reducing the free radical levels (Boldyrev et al., 1999).

Kosenko et al. (1995) have reported the coexistence of the NMDA-dependent and NMDA-independent mechanisms of NO synthase activation by ammonia, but the nature of the latter is not known. Ammonia administered at the 60-mM concentration via the microdialysis probes to the rat striatum activates kainate receptors, as revealed by the kainate antagonist-sensitive release of neuroactive amino acids (Zielińska et al., 2002). However, direct involvement of ionotropic glutamate receptors other than NMDA receptors appears unlikely: activation of striatal kainate or α-amino-2,3-dihydro-5-methyl-3-oxo-4-isoxazolepropanoate (AMPA) receptors does not trigger NO-mediated cGMP accumulation (East et al., 1996).

In conclusion, the present results are the first evidence of an antiexcitotoxic potential of exogenous taurine in vivo, and the NMDA/NO/cGMP pathway appears to be the primary target of taurine activity. Since taurine poorly crosses the blood–brain barrier both under control and hyperammonemic conditions (Hilgier et al., 1996), its therapeutic use is problematic. However, taurine derivatives that penetrate the barrier (Oja et al., 1983; Van Gelder and Bowers, 2001) are worth of testing for this purpose.

Acknowledgements

We thank Ms. Irma Rantamaa for excellent technical assistance. The study was supported by the Medical Research Fund of Tampere University Hospital, the Academy of Finland and a statutory grant to the Medical Research Centre.

References

Albrecht, J., Jones, E.A., 1999. Hepatic encephalopathy: molecular mechanisms underlying the clinical syndrome. J. Neurol. Sci. 170, 138–146.

Boldyrev, A.A., Johnson, P., Wei, Y., Tan, Y., Carpenter, D.O., 1999. Carnosine and taurine protect rat cerebellar granular cells from free radical damage. Neurosci. Lett. 263, 169–172.

Butterworth, R.F., 1997. Hepatic encephalopathy and brain edema in acute hepatic failure: does glutamate play a role? Hepatology 25, 1032–1033.

Chapkova, A.N., Dreulee, N., Yanovsky, Y., Mukhopadhyay, D., Haas, H.L., Sergeeva, O.A., 2002. Long-lasting enhancement of cortocostriatal neurotransmission by taurine. Eur. J. Neurosci. 16, 1523–1530.

East, S.J., Parry-Jones, A., Brotchie, J.M., 1996. Ionotropic glutamate receptors and nitric oxide synthesis in the rat striatum. NeuroReport 20, 71–75.

El Idrissi, A., Trenkner, E., 1999. Growth factors and taurine protect against excitotoxicity by stabilizing calcium homeostasis and energy metabolism. J. Neurosci. 19, 9459–9468.

Fedele, E., Ansaldo, M.A., Varnier, G., Raiteri, M., 2000. Benzodiazepine-sensitive GABAA receptors limit the activity of the NMDA/NO/cyclic GMP pathway: a microdialysis study in the cerebellum of freely moving rats. J. Neurochem. 75, 782–787.

Heales, S.J., Bolaños, J.P., Stewart, V.C., Brookes, P.S., Land, J.M., Clark, J.B., 1999. Nitric oxide, mitochondria and neurological disease. Biochim. Biophys. Acta 1410, 215–228.

Hermenegildo, C., Monfort, P., Felipo, V., 2000. Activation of *N*-methyl-D-aspartate receptors in rat brain in vivo following acute ammonia intoxication: characterization by in vivo brain microdialysis. Hepatology 31, 709–715.

Hilgier, W., Olson, J.E., Albrecht, J., 1996. Relation of taurine transport and brain edema in rats with simple hyperammonemia or liver failure. J. Neurosci. Res. 45, 69–74.

Hilgier, W., Zielińska, M., Borkowska, H.D., Gadamski, R., Walski, M., Oja, S.S., Saransaari, P., Albrecht, J., 1999. Changes in the extracellular profiles of neuroactive amino acids in the rat striatum at the asymptomatic stage of hepatic failure. J. Neurosci. Res. 56, 76–84.

Kosenko, E., Kaminsky, Y., Grau, E., Miñana, M.D., Grisolia, S., Felipo, V., 1995. Nitroarginine, an inhibitor of nitric oxide synthetase, attenuates ammonia toxicity and ammonia-induced alterations in brain metabolism. Neurochem. Res. 20, 451–456.

Kosenko, E., Kaminsky, Y., Lopata, O., Muravyov, N., Kaminsky, A., Hermenegildo, C., Felipo, V., 1998. Nitroarginine, an inhibitor of nitric oxide synthase, prevents changes in superoxide radical and antioxidant enzymes induced by ammonia intoxication. Metab. Brain Dis. 13, 29–41.

Kosenko, E., Kaminski, Y., Lopata, O., Muravyov, N., Felipo, V., 1999. Blocking NMDA receptors prevents the oxidative stress induced by acute ammonia intoxication. Free Radic. Biol. Med. 26, 1369–1374.

Lafon-Cazal, M., Culcasi, M., Gaven, F., Pietri, S., Bockaert, J., 1993. Nitric oxide, superoxide and peroxynitrite: putative mediators of NMDA-induced cell death in cerebellar granule cells. Neuropharmacology 32, 1259–1266.

Malminen, O., Kontro, P., 1986. Modulation of the GABA–benzodiazepine receptor complex by taurine in rat brain membranes. Neurochem. Res. 11, 85–94.

Martin, G., Siggins, G.R., 2002. Electrophysiological evidence for expression of glycine receptors in freshly isolated neurons from nucleus accumbens. J. Pharmacol. Exp. Ther. 302, 1135–1145.

Mehta, T.R., Dawson Jr., R., 2001. Taurine is a weak scavenger of peroxynitrite and does not attenuate sodium nitroprusside toxicity to cells in culture. Amino Acids 20, 419–433.

Monfort, P., Corbalan, R., Martinez, L., Lopez-Talavera, J., Cordoba, J., Felipo, V., 2001. Altered content and modulation of soluble guanylate cyclase in the cerebellum of rats with portacaval anastomosis. Neuroscience 104, 1119–1125.

Montoliu, C., Llansola, M., Kosenko, E., Corbalan, R., Felipo, V., 1999. Role of cyclic GMP in glutamate neurotoxicity in primary cultures of cerebellar neurons. Neuropharmacology 12, 1883–1891.

O'Byrne, M.B., Tipton, K.F., 2000. Taurine-induced attenuation of MPP^+ neurotoxicity in vitro: a possible role for the $GABA_A$ subclass of GABA receptors. J. Neurochem. 74, 2087–2093.

Oja, S.S., Kontro, P., Lindén, I.-B., Gothóni, G., 1983. Anticonvulsant activity of some 2-aminoethanesulphonic acid (taurine) derivatives. Eur. J. Pharmacol. 87, 191–198.

Oja, S.S., Korpi, E.R., Saransaari, P., 1990. Modification of chloride flux across brain membranes by inhibitory amino acids in developing and adult mice. Neurochem. Res. 15, 797–804.

Paxinos, G., Watson, C., 1982. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Saransaari, P., Oja, S.S., 2000. Taurine and neural cell damage. Amino Acids 19, 509–526.

Sergeeva, O.A., Haas, H.L., 2001. Expression and function of glycine receptors in striatal cholinergic interneurons from rat and mouse. Neuroscience 104, 1043–1055.

Sharp, T., Zetterstrom, T., Ljungberg, T., Ungerstedt, U., 1986. Effect of sulpiride on the amphetamine-induced behavior in relation to changes in striatal dopamine release in vivo. Eur. J. Pharmacol. 129, 411–415.

Van Gelder, N.M., Bowers, R.J., 2001. Synthesis and characterization of *N*,*N*-dichlorinated amino acids: taurine, homotaurine, GABA and L-leucine. Neurochem. Res. 26, 575–578.

Vogels, B.A.P.M., Maas, M.A.W., Daalhuisen, J., Quack, G., Chamuleau, R.A.F.M., 1997. Memantine, a noncompetitive NMDA receptor antagonist improves hyperammonemia-induced encephalopathy and acute hepatic encephalopathy in rats. Hepatology 25, 820–827.

Yamamoto, B.K., Zhu, W., 1998. The effect of methamphetamine on the production of free radicals and oxidative stress. J. Pharmacol. Exp. Ther. 287, 107–114.

Zielińska, M., Hilgier, W., Borkowska, H.D., Oja, S.S., Saransaari, P., Goryński, P., Albrecht, J., 2002. Ammonia-induced extracellular accumulation of taurine in the rat striatum in vivo: role of ionotropic glutamate receptors. Neurochem. Res. 27, 37–42.

Zielińska, M., Law, R.O., Albrecht, J., 2003. Excitotoxic mechanism of cell swelling in rat cerebral cortical slices treated acutely with ammonia. Neurochem. Int. 43, 299–303.