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Mini-Review

Roles of Neuroactive Amino Acids in Ammonia Neurotoxicity

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Many neurologic disorders are related to congenital or acquired hyperammonemia (HA). Advanced symptoms of HA range from seizures in acute stages to stupor and coma in more chronic conditions, manifesting variable imbalance between the inhibitory and excitatory neurotransmission. Evidence obtained with the use of experimental HA models suggests that acute neurotoxic effects of ammonia are mediated by overactivation of ionotropic glutamate (GLU) receptors, mainly the *N*-methyl-D-aspartate (NMDA) receptors, and to a lesser degree the KA/AMPA receptors. NMDA receptor–mediated neurotoxicity may be potentiated by impaired control of their function by metabotropic GLU receptors, which are inactivated by ammonia. Prolonged overactivation of the NMDA receptors upon extended ammonia exposure causes their downregulation. The GLU receptor changes may be related to their excessive exposure to extrasynaptic GLU. Ammonia promotes GLU accumulation in the extrasynaptic space by enhancing its release from neurons, and/or by decreasing its reuptake to the nerve endings and astrocytes, where the effect results from inactivation (downregulation) of the astrocytic glutamate transporter GLT1. Excitotoxic effects of ammonia are augmented by increased synthesis of nitric oxide (NO), which is associated with NMDA receptor activation and/or increased synaptic transport of arginine (ARG). A shift toward neural inhibition is promoted by positive modulation of the γ-aminobutyric acid (GABA)ergic tone resulting from excessive accumulation in the brain of endogenous central benzodiazepine receptor agonists, and from upregulation of astrocytic peripheral benzodiazepine receptors leading to elevated levels of prognenelone-derived neurosteroids, which positively modulate the GABA(A) receptor complex. Inhibitory neurotransmission may also be favored by enhanced release from astrocytes of an inhibitory amino acid, taurine. J. Neurosci. Res. 51:133–138, 1998.

Key words: ammonia; neurotoxicity; glutamate; receptors; GABA; taurine; benzodiazepines

INTRODUCTION

Ammonia is a well-documented neurotoxin and its increased entry to brain is a primary cause of neurological disorders associated with hyperammonemia (HA), such as hepatic encephalopathies (HE) including portal–systemic encephalopathy and fulminant hepatic failure, congenital deficiencies of urea cycle enzymes, Reye's syndrome, and also several metabolic or toxic encephalopathies (for reviews, see Szerb and Butterworth, 1992; Conn and Bircher, 1994). Neurophysiologic effects of experimentally induced HA in vivo mirror the major HA symptoms noted in neurologic patients. At high doses measured in the brains of patients with acute hyperammonemia (1–5 mM), ammonia produces seizures in animals resulting from its depolarizing action on nerve cell membranes (Iles and Jack, 1980); the seizures often lead to rapid death of the animals (Marcaida et al., 1992, and references therein). Upon prolonged exposure at lower doses, ammonia induces stupor and/or coma, consistent with its hyperpolarizing effects (Raabe, 1989). In vitro studies with brain tissue slices or cultured nerve cells have confirmed the depolarizing and hyperpolarizing effects of ammonia, with variable contribution of pre- and postsynaptic events (Fan et al., 1990; Raabe, 1992). In simple terms thus, the neurophysiological effects of ammonia may be viewed as reflecting variable shifts in balance between the inhibitory and excitatory neurotransmission.

The main focus of this review is on glutamate (GLU), which mediates excitatory transmission at a majority of CNS synapses. The role of the inhibitory γ-aminobutyric acid (GABA)ergic transmission is discussed with emphasis on the role of endogenous benzodiazepines and their interaction with the GABA(A)-benzodiazepine receptor complex. Taurine (TAU), a small sulfur amino acid postulated to serve as a glia-

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derived inhibitory neuromodulator, osmoregulator, and/or neuroprotectant, is also considered here in the context of its massive ammonia-induced release from astrocytes.

The functioning of neuroactive amino acids, i.e., their release, interaction with receptors, and reuptake, are discussed. Reviews of the effects of ammonia on the metabolism of amino acids are to be found elsewhere (Cooper and Plum, 1987; Albrecht, 1996; Norenberg, 1996).

THE GLUTAMATERGIC SYSTEM

Ionotropic Glutamate Receptors

The first direct evidence for the role of NMDA receptors in mediating ammonia toxicity was the demonstration that pretreatment of mice with a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor agonist, dizocilpine (MK-801), reduced the mortality of mice subsequently injected with a toxic dose of ammonium acetate from 73% to 5% (Marcaida et al., 1992). Later, a spectrum of competitive and noncompetitive NMDA receptor antagonists were demonstrated in the same experimental paradigm (Hermenegildo et al., 1996). One major metabolic effect of ammonia shown to be mediated by activation of NMDA receptors is the increase of Na^+/K^+ ATPase activity, which often accompanies acute or chronic hyperammonemia (Albrecht et al., 1985; Kosenko et al., 1994; Ratnakumari et al., 1995). Activation of Na^+/K^+ ATPase and depletion of ATP following injection of large ammonia doses to rats were both prevented by pretreatment with MK-801 (Kosenko et al., 1994). Stimulation of the Na^+/K^+ ATPase activity was found to be due to decreased protein kinase C–mediated phosphorylation of the enzyme (Kosenko et al., 1994) but could also be associated with calcineurin-mediated dephosphorylation of the enzyme molecule (Marcaida et al., 1996).

Ammonia neurotoxicity appears to involve enhanced generation of nitric oxide (NO), and this effect is partly due to stimulation of NMDA receptors: Ammonia toxicity in acutely hyperammonemic mice was attenuated by previous administration of an inhibitor of nitric oxide synthase (NOS), nitroarginine (Kosenko et al., 1995). However, the effect of nitroarginine was qualitatively different from MK-801, indicating the coexistence of NMDA-dependent and -independent mechanisms of NOS activation. The NMDA-independent component is probably associated with the increased availability of the NOS substrate L-arginine. Increased arginine uptake was measured in synaptosomes derived from rats with thioacetamide-induced HE (Albrecht et al., 1990) and in portacaval shunted rats, where the increased uptake was shown to be directly coupled to activation of NOS (Rao et al., 1995, 1997). In addition, stimulation of synaptosomal arginine uptake by ammonia has been suggested to promote increased arginine metabolism to GLU (Albrecht et al., 1990).

Some electrophysiological evidence supports the view of ammonia directly interacting with NMDA receptors. Superfusion of rat hippocampal slices with 1–4 mM ammonia enhanced the NMDA-induced currents (Fan and Szerb, 1993). By contrast, ammonia treatment reduced the NMDA-induced depolarization of mice cortical wedge preparations (Lombardi et al., 1994). The reasons for these discrepancies are unknown.

Prolonged exposure to ammonia downregulates the NMDA receptors. Chronic hyperammonemia in rats or long-term treatment with ammonia of cultured cerebellar neurons decreased the binding of [^{3}H]MK-801 to, respectively, synaptosomal membranes or neuronal homogenates (Marcaida et al., 1995). In cultured neurons, the treatment depressed NMDA receptor–mediated, glutamate-induced neuronal cell death or calcium influx, and the receptor functions could be restored by activation of protein kinase C (Marcaida et al., 1995). A delayed protection of neurons against NMDA-mediated ammonia toxicity was afforded by prenatal exposure of rats to ammonia (Miñana et al., 1995). The binding of MK-801 was found to be decreased in hippocampus in thioacetamide-treated rats (Saransaari et al., 1997) and in whole brain sections of mice with congenital ornithine transcarbamylase activity (Ratnakumari et al., 1995). Furthermore, NMDA-sensitive GLU binding was found to be decreased in selected brain regions in portacaval-shunted rats (Peterson et al., 1990), in cerebella of hyperammonemic rats (Rao and Murthy, 1991), and in striata of rats with thioacetamide-induced liver failure (Saransaari et al., 1997), pointing to the NMDA receptor inactivation in all these HA models. However, evidence from other receptor binding studies has been contradictory (reviewed by de Knegt et al., 1993).

A recent study by Vogels et al. (1997) provided, for the first time, evidence for the causal relation between the activation of NMDA receptors and sublethal HE. In this study, administration of a noncompetitive NMDA receptor antagonist, memantine, to rats with HE caused by portacaval shunting or hepatic ligation, brought about a significant improvement in clinical grading, electroencephalographic (EEG) activity, and a return to control of the extracellular GLU concentration, whose increase is a consistent symptom of hyperammonemia (see the following section).

Only a few studies have dealt with the effect of ammonia or hyperammonemic conditions on the kainate (KA) or α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor function. Ammonia reduced the AMPA-induced depolarization of mice cortical wedge preparations (Lombardi et al., 1994). A selective loss of

KA and AMPA binding sites was noted in the cerebral cortex of dogs with spontaneous chronic HE (Maddison et al., 1991) and in different brain areas of rats with acute liver failure. This selective loss of non-NMDA receptors has been speculated to promote NMDA-mediated neurotransmission (Michalak and Butterworth, 1997).

Metabotropic Glutamate Receptors

One preliminary report has indicated that ammonia downregulates metabotropic glutamate receptors (mGluRs) in cultured astrocytes as evidenced by decreased formation of inositol phosphates after glutamate stimulation and decreased binding of a radiolabeled ligand, AP3 (Bruce et al., 1995). Ammonia also reduced the 1S,3R-ACPD–induced formation of inositol phosphates and the degree of depolarization in mice cortical wedge preparations (Lombardi et al., 1994). It must be recalled that activation of mGluR by its selective agonist, trans-ACPD, attenuated NMDA neurotoxicity (Koh et al., 1991), and carnitine, an activator of mGluR, prevented ammonia neurotoxicity in mice (Miñana et al., 1996). Inactivation of mGluR by ammonia may thus potentiate NMDA-mediated neurotoxicity. Whether and to what degree the above-described effects involved the astrocytic or neuronal mGluRs remains to be envisaged.

Mechanisms Underlying Overactivation of Glutamate Receptors by Ammonia

Permanently or transiently enhanced extracellular accumulation of GLU has been found to accompany hyperammonemia in most of the experimental models (Tossman et al., 1987; Michalak et al., 1996) and is considered to be the major cause of overstimulation of GLU receptors. The stimulation is thought to be additionally enhanced by the increase of extracellular glycine, a positive modulator of the NMDA receptor complex (Michalak et al., 1996), by the loss of GABAB receptors which negatively control GLU release (Oja et al., 1993), and possibly also by increased accumulation in the brain of an endogenous NMDA receptor agonist, quinolinic acid (Moroni et al., 1986a,b).

Hyperammonemia in vivo (Hilgier et al., 1991) or treatment with ammonia in vitro (Moroni et al., 1983) have been shown to increase the calcium-dependent release of GLU from various brain regions. The release may be due to the depolarizing action of ammonia on the nerve cell membranes, but also to its stimulatory effect on NO production: NO is a potent releaser of vesicular synaptic glutamate (Katchman and Hershkowitz, 1997). Ammonia also impairs GLU clearance from the extrasynaptic cleft. A decrease of high-affinity uptake of GLU was measured in nerve endings (synaptosomes) treated with ammonia in vitro (Mena and Cotman, 1985) or isolated from rats with acute liver failure (Oppong et al., 1995). Decreased uptake of GLU and/or its nonmetabolizable analogue D-aspartate was observed in cultured cerebral astrocytes treated with 5 mM ammonia for 24 hr (Bender and Norenberg, 1996) and in bulk isolated astrocytes derived from rats with acute liver failure (Albrecht et al., 1988) or acute HA (Rao and Murphy, 1991). The inhibition of astrocytic GLU transport is consistent with the diminished expression of the astroglia-specific GLU transporter, GLT 1, as measured in three different models of acute liver failure (Knecht et al., 1996; Norenberg et al., 1997).

THE GABA(A)ERGIC SYSTEM: FOCUS ON ENDOGENOUS BENZODIAZEPINES AND BENZODIAZEPINE RECEPTORS

The contribution of the GABAergic system to ammonia-induced neural inhibition has long been a subject of hot debate (for a review and references, see Norenberg, 1996). One aspect of the GABA hypothesis that has successfully gone through experimental verification is that ammonia enhances the GABAergic tone by overactivating benzodiazepine receptors (BZDRs). Elevated levels of endogenous ligands of the central benzodiazepine receptor have been measured in hyperammonemic patients (Mullen et al., 1990; Basile et al., 1994) and experimental animals (Basile et al., 1989; Basile, 1991), and treatment with a specific BZDR antagonist, flumazenil, improved neurological status of patients with hepatic failure in a number of clinical trials (Pomier-Layrargues et al., 1994; for a review see Rothstein, 1994).

In addition, ammonia is thought to enhance the GABAergic transmission indirectly, by interacting with benzodiazepine receptors of the peripheral type (PBZDRs). Increased concentrations of PZBDRs have been measured in the brains of HE patients (Lavoie et al., 1990) and chronic hyperammonemic animals (Giguere et al., 1992) and in ammonia-treated cultured astrocytes (Ducis et al., 1989; Itzhak and Norenberg, 1994). PZBDRs, which in the CNS are located in the outer mitochondrial membranes of astrocytes (Anholt et al., 1986; Itzhak et al., 1993), appear to control the synthesis of prognenelone-derived neurosteroids, some of which are positive modulators of the GABA(A)-benzodiazepine receptor complex (Majewska, 1992). In two models of hyperammonemia in mice, the increase of PBZDR binding was coupled to increased prognenelone synthesis (Itzhak et al., 1995). An increased expression of PZBDR protein mRNA was measured in the brains of portacaval-shunted rats (Desjardins et al., 1997). Pretreatment of mice with a PBZDR antagonist substantially reduced their lethal response to ammonium acetate injection (Itzhak and Norenberg, 1994).

ASTROCYTIC TAURINE: A PUTATIVE CONTRIBUTOR TO AMMONIA-INDUCED NEURAL INHIBITION

The inhibitory sulfur amino acid taurine (TAU) is the most abundant amino acid in astrocytes (Holopainen et al., 1986). TAU is actively released from astrocytes in response to cell volume–increasing stimuli (reviewed in Martin, 1992). An increased spontaneous efflux of newly loaded radiolabeled TAU, possibly manifesting cell membrane leakage, was measured in slices derived from cerebral regions of hyperammonemic rats, and the increase was correlated with the tissue water content (Hilgier et al., 1996). Likewise, excessive spontanous TAU leakage was noted in cultured cerebellar astrocytes (Wysmyk et al., 1994) or rabbit Müller cells treated for 1 day with 1 mM ammonium chloride (Faff et al., 1997).

A short-term (10-min) treatment with 1–5 mM ammonia, which did not produce cell swelling, also stimulated TAU release from cultured rabbit Müller cells (Faff-Michalak et al., 1994) or cortical astrocytes in culture (Albrecht et al., 1994). The release from Müller cells was osmoresistant, calcium independent, and coupled to cAMP synthesis (Faff et al., 1996). It may be hypothesized that, in line with its inhibitory properties, TAU released from astrocytes counteracts ammonia-induced excitation of neurons in the acute stage of HA or augments neural inhibition accompanying chronic IIA. The neuronal targets of astrocytic TAU remain to be identified to prove this hypothesis.

CONCLUSIONS

The data discussed in this review support the view that changes in the functioning of neuroactive amino acids largely contribute to neurophysiological manifestations of HA. Particularly strong evidence has accumulated with regard to the role of GLUergic neurotransmission. Seizures, but also nerve cell death resulting from acute exposure to large ammonia doses, can be attributed to overactivation of ionotropic GLU receptors, mainly the NMDA receptors. In turn, stupor and coma, but also increased resistance to ammonia neurotoxicity at chronic stages of HA, may be causally related to downregulation of the NMDA receptors. It is also clear that changes in the GLUergic tone are contributed by excessive GLU accumulation in the extrasynaptic space, which can be ascribed to both its enhanced release and its decreased reuptake to astrocytes or nerve endings.

Inhibition of neural transmission promoted by downregulation of GLU receptors in chronic HA stages is further augmented by an increased GABAergic tone resulting from benzodiazepine receptor overstimulation by endogenous benzodiazepines and neurosteroids. Neural inhibition may in some degree be due to TAU which is excessively released from astrocytes by ammonia: However, the specific neuronal targets of TAU remain to be identified.

The reader should not be left with the impression that neuroactive amino acids are the only compounds implicated in the neurophysiological effects of ammonia: A large body of evidence suggests the involvement of catecholamines (dopamine, serotonin) and their derivatives (for reviews and recent references, see Norenberg, 1996; Mousseau et al., 1997). In this context, it will be of interest to establish how ammonia affects mutual interactions between the amino acid and non-amino acid neurotransmitter systems. A step in this direction is the demonstration that HA modulates GLU- and GLU agonist–dependent dopamine release from various brain regions in vitro (Borkowska et al., 1997).

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