Differential Effects of Ammonia and β-Methylene-DL-Aspartate on Metabolism of Glutamate and Related Amino Acids by Astrocytes and Neurons in Primary Culture*

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The effects of ammonium chloride (3 mM) and β-methylene-DL-aspartate (BMA; 5 mM) (an inhibitor of aspartate aminotransferase, a key enzyme of the malate-aspartate shuttle (MAS)) on the metabolism of glutamate and related amino acids were studied in primary cultures of astrocytes and neurons. Both ammonia and BMA inhibited 14CO2 production from [U-14C]- and [1-14C]glutamate by astrocytes and neurons and their effects were partially additive. Acute treatment of astrocytes with ammonia (but not BMA) increased astrocytic glutamine. Acute treatment of astrocytes with ammonia or BMA decreased astrocytic glutamate and aspartate (both are key components of the MAS). Acute treatment of neurons with ammonia decreased neuronal aspartate and glutamine and did not apparently affect the efflux of aspartate from neurons. However, acute BMA treatment of neurons led to decreased neuronal glutamate and glutamine and apparently reduced the efflux of aspartate and glutamine from neurons. The data are consistent with the notion that both ammonia and BMA may inhibit the MAS although BMA may also directly inhibit cellular glutamate uptake. Additionally, these results also suggest that ammonia and BMA exert differential effects on astroglial and neuronal glutamate metabolism.

KEY WORDS: Glutamate metabolism; astrocytes; neurons; effects of ammonia and β-methylene-DL-aspartate; aspartate aminotransferase; malate-aspartate shuttle; aspartate; glutamine.

INTRODUCTION

Ammonia toxicity has been implicated in a number of diseases (including hepatic encephalopathy, inborn errors of the urea cycle, Reye’s syndrome, and valproate encephalopathy) in which hyperammonemia is a persistent feature (see: Cooper and Plum (1) for a full discussion and references). Much evidence suggests that therapeutic practices directed towards decreasing the hyperammonemia exert beneficial effects on the clinical outcome (see: Cooper and Plum (1) and Cooper and Lai (2) for discussions). However, the pathogenetic role(s) of ammonia has (have) not been fully elucidated.

Diverse mechanisms have been proposed to account for the neurotoxic effects of ammonia (1-8). These include: (i) alterations in the structure and function of the blood-brain barrier (4,5), (ii) disturbances in the physiology of blood vessels and blood flow (see Refs. cited in Ref. 1), (iii) changes in the electrophysiological properties of neurons (see: Raabe (7) for a full discussion and references), (iv) morphological changes in neurons and astrocytes (8), (v) interferences with energy metab-
olism, including inhibition of astrocytic glutamate (9) and pyruvate (10) oxidation and of brain mitochondrial α-ketoglutarate dehydrogenase complex (KGDHC) activity (11) (see Refs. 1-3 for a full discussion and additional Refs.), and (vi) interference with key metabolic pathways (e.g., oxidation of branched chain amino acids (12-14); also see Ref. 1). Hindfelt et al. (16), and more recently Ratna Kumari et al. (17), proposed that one of the mechanisms whereby ammonia interferes with brain energy metabolism is through the disruption of the transfer of reducing equivalents across the inner mitochondrial membrane. Hindfelt et al. (16) further speculated that the hyperammonemia-induced depletion of glutamate and aspartate, and inhibition of the malate-aspartate shuttle (MAS) may be the key neurochemical lesions.

Our research has focused on two of these proposed mechanism(s) (namely (v) and (vi)); the studies reported are part of an ongoing effort (2, 9-15) in our laboratories to understand the mechanism(s) underlying the disturbances of brain energy metabolism in ammonia toxicity.

Evidence is accumulating that, in brain (as in several peripheral organs), the MAS is one of the most important mechanisms that mediate the transfer of reducing equivalents across the inner mitochondrial membrane (18-24). β-Methylene-DL-aspartate (BMA) is an irreversible and reasonably selective inhibitor of aspartate aminotransferase (AAT) (24), a key component of the MAS. Fitzpatrick et al. (24) observed that BMA inhibited glucose- and pyruvate-supported oxygen uptake by cerebro-cortical slices. Oxygen consumption in slices respiring on glucose noticeably declined in parallel with the BMA-induced inhibition of AAT activities. Furthermore, in BMA-treated cerebro-cortical slices, the contents of citrate, malate, and aspartate were decreased but the content of lactate was increased. Thus, Fitzpatrick et al. (24) suggested that BMA would be a useful tool for elucidating the role(s) of AAT and MAS in the regulation of cerebral oxidative metabolism. Additionally, Fitzpatrick et al. (15) recently noted that, when primary cultures of cerebro-cortical neurons and astrocytes were acutely exposed to BMA, the contents of ATP and glutamate were decreased in neurons (but not in astrocytes) and the content of phosphocreatine was decreased in astrocytes (but not in neurons). Acute exposure of cultured astrocytes or neurons to BMA (but not ammonia) decreased 14CO2 production from [U-14C]glucose (15).

The present study was undertaken to (i) further investigate the effects of acute exposure to ammonia on the metabolism of glutamate (cf. 9) and metabolically related amino acids in astrocytes and neurons in primary culture, (ii) examine the effects of BMA on additional aspects of intermediary metabolism in cerebral neurons and astrocytes in primary culture in order to further elucidate the metabolic role(s) of MAS in neuronal and astroglial metabolism, and (iii) determine whether the effects of BMA and ammonia on glutamate and glutamine metabolism in neurons and astrocytes in primary culture are similar. In addition, in some experiments, the effects of BMA and ammonia are compared to those of the more classical (but much less selective) inhibitor of AAT, namely, aminoxyxacetate (AOAA). Preliminary reports of some of the results of the present study have appeared (25-27).

**EXPERIMENTAL PROCEDURE**

**Primary Cultures of Neurons and Astrocytes.** Neuronal cultures were prepared from the neocortex of newborn (0-24 hours) mice as described previously (28-32). The procedure involved removal of the meninges from the cerebral hemispheres, dissociation of the cells by vortexting and serial passage through sterile Nitex nylon sieves (pore sizes 80 and 10 μm respectively), and incubation of the cells in a manner similar to that for the culture of neurons with the exception of a lower glucose concentration (7.5 mM) and a higher serum concentration (initially 20% (v/v) but subsequently reduced to 7% (v/v)) in the medium. The medium was changed initially at day 3 and thereafter twice weekly. The cultures attained confluency by 2 weeks. Of the cells present in the cultures, more than 95% were astrocytes; the remainder were mainly macrophages, and neurons were absent (see Hertz et al. (23) for discussion). Since there is some evidence that "differentiated" astrocytes are less sensitive than "undifferentiated" astrocytes to certain toxic effects of ammonia (33), the astrocytic cultures used in the present studies were not treated with the "differentiating" agent, dibutyryl cyclic AMP. Astrocytes that had been cultured for 4 weeks in vitro were employed for the biochemical studies (see below).

**Determination of 14CO2 production from L-[1-14C]- and L-[1-14C]glutamate.** The culture medium was removed from the culture dish with a Pasteur pipette and the cells on the dish were rinsed 3 times with 2 ml of glutamine-free MEM. The incubation