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An astrocyte toxin influences the pattern of breathing and the ventilatory response to hypercapnia in neonatal rats

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Abstract

Recent in vitro data suggest that astrocytes may modulate respiration. To examine this question in vivo, we treated 5-day-old rat pups with methionine sulfoximine (MS), a compound that alters carbohydrate and glutamate metabolism in astrocytes, but not neurons. MS-treated pups displayed a reduced breathing frequency (*f*) in baseline conditions relative to saline-treated pups. Hypercapnia (5% CO_2) increased *f* in both groups, but *f* still remained significantly lower in the MS-treated group. No differences between treatment groups in the responses to hypoxia (8% O_2) were observed. Also, MS-treated rats showed an enhanced accumulation of glycogen in neurons of the facial nucleus, the nucleus ambiguus, and the hypoglossal nucleus, structures that regulate respiratory activity and airway patency. An altered transfer of nutrient molecules from astrocytes to neurons may underlie these effects of MS, although direct effects of MS upon neurons or upon peripheral structures that regulate respiration cannot be completely ruled out as an explanation.

Keywords: Glia; Ventrolateral medulla; Glycogen; Respiratory rate; Methionine sulfoximine

1. Introduction

Hypercapnia is thought to exert its effects on ventilation mainly through activation of discrete chemosensitive cell groups within the medulla oblongata (Coates et al., 1993; Richerson, 2004). These effects are mediated partly via alterations in extracellular and intracellular pH (Ballantyne and Scheid, 2001). Proteins that regulate pH are present on both astrocytes and neurons, and include Na/HCO_3 co-transporters and a family of Na/H exchanger proteins (Baird et al., 1999; Makara et al., 2001; Schmitt et al., 2000). Thus, glia cells may participate in the regulation of the ventilatory response to hypercapnia.

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Recently, brainstem infusions of a glia-specific toxin, fluorocitrate, were reported to alter tissue pH and respiratory output (Erlichman et al., 1998; Holleran et al., 2001). Such an effect could perhaps arise from an altered regulation of intracellular and extracellular pH by astrocytes (Deitmer and Rose, 1996). Furthermore, there is considerable evidence that astrocytes can influence neuronal firing rates and neuronal metabolism, perhaps via release from astrocytes of lactate or ATP (Alvarez-Maubecin et al., 2000; Haydon, 2001; Magistretti et al., 1999; Swanson and Choi, 1993). Recently, it has been shown that astrocytes respond to increased neuronal activity by consuming more glucose and producing more lactate (Pellerin and Magistretti, 1994).Glucose consumption is tightly linked to neuronal activity in the brain. The traditional view that glucose is consumed directly and solely by neurons and that glucose consumption directly reflects neuronal activity is under challenge. In vitro, ex vivo, and in vivo experiments have shown that astrocytes respond metabolically to increased neuronal activity. In parallel, neurons preferentially oxidize lactate present in the extracellular space rather than glucose to meet their energy demands (Pellerin and Magistretti, 1994). More recently, two-photon fluorescence imaging of nicotinamide adenine dinucleotide was used to resolve metabolic signatures in processes of astrocytes and neurons in the brain tissue slices. This study revealed neuro-glial metabolic coupling in which early oxidative metabolism in neurons is eventually sustained by late activation of the astrocyte-neuron lactate shuttle (Kasischke et al., 2004). Accordingly, we hypothesized that astrocytes may play a role in the response of chemoreceptive brainstem regions to hypercapnia and/or hypoxia via neuro-astroglia metabolic pathways.

One approach to testing this hypothesis is the use of a compound, methionine sulfoximine (MS) that has metabolic effects largely restricted to astrocytes. MS is a glutamate analogue that inhibits an enzyme, glutamine synthetase, present only in astrocytes. Blockade of this enzyme prevents the synthesis of glutamine from glutamate and also results in the loading of astrocytes with glycogen (D'Amelio et al., 1987; Guttierez and Norenberg, 1977; Hevor et al., 1985). In contrast to effects on glial glutamine synthetase, MS has no effect on the activity of four other enzymes of glutamate metabolism that are present in both astrocytes and neurons (Rothstein and Tabakoff, 1985).

Available data suggest that direct effects of MS are restricted to astrocytes. After systemic administration of MS, brain astrocytes show cytoplasmic swelling, a doubling in numbers of mitochondria, and glycogen deposition, whereas neurons and oligodendroglia show no ultrastructural abnormalities (Guttierez and Norenberg, 1977). MS substantially increases astrocyte glutamate uptake, but leaves neuronal glutamate uptake and lactate dehydrogenase unaltered (Swanson and Choi, 1993). Similarly, MS does not affect neuronal levels of choline acetyltransferase or glutamate decarboxylase (Somers and Beckstead, 1990). Low doses of MS that alter glial glutamine content fail to affect brain levels of neurotransmitters such as dopamine, norepinephrine, serotonin or their metabolites (Chance et al., 1991). Higher doses of MS do appear to reduce brain serotonin, perhaps as a consequence of altered extracellular levels of glutamate (Hevor and Delorme, 1990).

Application of MS to medullary slices in vitro diminishes the respiratory-related rhythm in the rostral medulla oblongata (Hulsmann et al., 2000). No data are available on the effects of MS upon respiration in vivo. We resolved to utilize MS as a tool to examine whether or not a drug-induced alteration in astrocyte metabolism affects the ventilatory response to hypercapnia or hypoxia in vivo.

2. Methods

2.1. Testing of respiratory parameters

This experiment was performed in Sprague–Dawley rat pups weighing about 15 g. On day 5 of postnatal life, rat pups of four different litters were mixed and assigned either to the experimental group ($n = 14$) injected with DL-methionine DL-sulfoximine (MS, 100 μg/g) intraperitoneally once a day for 2 days, or to the control group ($n = 14$) injected with the same volume of saline (Young et al., 2000). Twenty-four hours after the second dose of MS, ventilatory responses to steady state hypoxia (8% O_2, balance N_2) and hyperoxic hypercapnia (5% CO_2, balance O_2) were tested. All animals survived the MS treatment and showed no signs of motor impairment or abnormalities in righting behavior. Respiratory responses were