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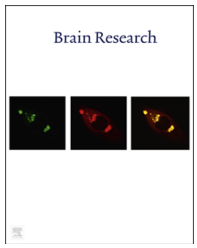

Research Report

Reduced cerebral monocarboxylate transporters and lactate levels by ethanol and normobaric oxygen therapy in severe transient and permanent ischemic stroke

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ABSTRACT

Objectives: Neuroprotective benefits of ethanol (EtOH) and normobaric oxygenation (NBO) were previously demonstrated in transient and permanent ischemic stroke. Here we sought to identify whether the enhanced lactic acidosis and increased expression of monocarboxylate transporters (MCTs) observed after stroke might be attenuated by single and/or combined EtOH and NBO therapies.

Methods: Sprague-Dawley rats ($n=96$) were subjected to right middle cerebral artery occlusion (MCAO) for 2 or 4 h (transient ischemia), or 28 h (permanent ischemia) followed by 3, 24 h, or no reperfusion. Rats received: (1) either an intraperitoneal injection of saline (sham treatment), one dose of EtOH (1.5 g/kg), two doses of EtOH (1.5 g/kg at 2 h of MCAO, followed by 1.0 g/kg 2 h after 1st dose), or (2) EtOH+95% NBO (at 2 h of MCAO for 6 h in permanent ischemia). Lactate levels were detected at 3 and 24 h of reperfusion. Gene and protein expressions of MCT-1, -2, -4 were assessed by real-time PCR and western blotting.

Results: A dose-dependent EtOH neuroprotection was found in transient ischemia. Following transient ischemia, a single dose of EtOH (in 2 h-MCAO) or a double dose (in 4 h-MCAO), significantly attenuated lactate levels, as well as the mRNAs and protein expressions of MCT-1, MCT-2, and MCT-4. However, while two doses of EtOH alone was ineffective in

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permanent stroke, the combined therapy (EtOH+95% NBO) resulted in a more significant attenuation in all the above levels and expressions.

Conclusions: Our study demonstrates that acute EtOH administration attenuated lactic acidosis in transient or permanent ischemic stroke. This EtOH-induced beneficial effect was potentiated by NBO therapy in permanent ischemia. Because both EtOH and NBO are readily available, inexpensive and easy to administer, their combination could be implemented in the clinics shortly after stroke.

1. Introduction

Due to a sudden lack of oxygen, ischemic tissues switch from aerobic respiration to much less efficient anaerobic processes. Instead of shuttling pyruvate, the product of glycolysis into the tricarboxylic acid (TCA) cycle, it is instead funneled into the production of lactic acid. Accumulation of lactate in tissue can decrease pH below that which is physiologically safe and stable (Oliva, 1970). Acutely stressful events such as stroke can precipitate these metabolic shifts, leading to increased production of lactate and modulation of the monocarboxylate transporters (MCTs) that regulate lactate levels (Martinov et al., 2009; Moreira et al., 2009). Of the 14 known isoforms in the MCT family, only three are currently known to be prominently expressed in the central nervous system: MCT1 in endothelial cells and astrocytes, MCT2 in neurons, and MCT4 in astrocytes only (Pierre and Pellerin, 2005). These proton-dependent symporters are responsible for facilitating the exchange of lactate (as well as other monocarboxylates) in, out, and between cellular compartments. During increased, non-pathologic neuronal activity, the expression of MCTs has been found to be increased (Simpson et al., 2007). The high glycolytic capacity of astrocytes coupled with their increased expression of MCT1 and MCT4 allow these cells the potential to act as a high-output source of lactate-derived energy (Dienel and Cruz, 2003). Concurrently neurons participating in synaptic activity have been noted to increase their expression of MCT2, possibly to take advantage of this relationship (Magistretti, 2006). Designated as the astrocyte–neuron lactate shuttle (ANLS) by some, the proposed system has recently garnered attention in studies on stroke pathophysiology (Genc et al., 2011; Tarczyluk et al., 2013). Although the ANLS may potentially act as a functional energy reserve in this regard, recent research suggests that its contributions are relatively minor (Hall et al., 2012). Overall, any benefits of increased lactate are speculative at best. Conversely, it has long been known, with little opposing evidence, that prolonged high levels of lactate are detrimental mainly by inducing acidosis (Kreisberg, 1980; Oliva, 1970).

In rat models of stroke, ethanol (EtOH), as well as normobaric oxygenation (NBO), were previously shown by our group to be neuroprotective during periods of cerebral ischemia (Geng et al., 2013a; Geng et al., 2013b; Geng et al., 2015; Kochanski et al., 2013). This neuroprotection occurred at least partially, from amelioration of aberrant glucose metabolism concurrent with a decrease in the production of reactive oxygen species (ROS) after stroke (Kochanski et al., 2013). Due to the finely tuned relationship between lactate, glucose, and how this relationship can be disturbed after stroke, the current study was designed to further elucidate the putative beneficial effects of EtOH and/or NBO. Specifically, we aimed at investigating whether EtOH and/or NBO might ameliorate the overproduction of both lactate and MCT that occurs as a result of stroke. Since an early reperfusion strategy is often not available for most stroke patients, and since the proposed treatments could be easily administered and disseminated to ischemic regions through circulatory collaterals (Bang et al., 2011a; Bang et al., 2011b; Liebeskind, 2003; Liebeskind, 2012; Liebeskind, 2014), we designed therapeutic strategies along more clinically relevant stroke models by including the use of longer ischemia periods (2 vs. 4 h) and, in certain groups, by eliminating reperfusion altogether (simulating permanent ischemia).

2. Results

2.1. Cerebral lactate levels

In ischemic rats, cerebral lactate levels were significantly increased by 28.2% as early as 3 h and remained elevated by 24.3% at 24 h of reperfusion (Fig. 1A), when compared with controls. Administration of 1.5 g/kg EtOH, 2 h post-stroke onset, significantly decreases expression of lactate at both 3 ($p<0.01$) and 24 h ($p<0.05$) of reperfusion. After 4 h ischemia, significant ($p<0.01$) increase in expression of lactate was observed at 3 and 24 h reperfusion. The increased levels were significantly diminished to ~35% above control levels by EtOH, which was administered at the two reperfusion time points (Fig. 1B). In comparison to control values, cerebral lactate levels in rats with PMCAO were nearly doubled ($p<0.01$) (Fig. 1C). When EtOH was applied, a significant decrease ($p<0.05$) was observed. Combination of EtOH and NBO induced a more potent and additive decrease in lactate levels ($p<0.01$).

2.2. MCT-1 expression

After 2 h ischemia and subsequent reperfusion at 3 and 24 h, MCT-1 mRNA expression was elevated ($p<0.05$) as compared to sham operation control, which was set at a value of 1 for reference (Fig. 2A). Following EtOH administration, mRNA expression was reduced at the time points after reperfusion although this reduction did not reach significant levels. In ischemic rats with 4 h MCAO, MCT-1 mRNA was significantly ($p<0.01$) increased by about 110% at 3 and 24 h reperfusion (Fig. 2B). In addition the increased mRNA expressions were attenuated to