Neuroscience Letters 453 (2009) 219–224

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Production of panic-like symptoms by lactate is associated with increased neural firing and oxidation of brain redox in the rat hippocampus

Peter J. Bergold[a,*], Valariya Pinkhasova[a], Maryam Syed[a], Hsin-Yi Kao[a], Anna Jozwicka[a], Ning Zhao[a], Jeremy D. Coplan[b], Diana Dow-Edwards[a], André A. Fenton[a]

[a] Department of Physiology and Pharmacology, SUNY-Downstate Medical Center, Brooklyn, NY 11203, United States
[b] Department of Psychiatry, SUNY-Downstate Medical Center, Brooklyn, NY 11203, United States

ARTICLE INFO

Article history:
Received 9 July 2008
Received in revised form 13 February 2009
Accepted 19 February 2009

Keywords:
Oxamate
NADH/NAD$^+$
Intrahippocampal injection
Single unit recording
Neural discharge

ABSTRACT

Lactate uses an unknown mechanism to induce panic attacks in people and panic-like symptoms in rodents. We tested whether intraperitoneal (IP) lactate injections act peripherally or centrally to induce panic-like symptoms in rats by examining whether IP lactate directly affects the CNS. In Long-Evans rats, IP lactate (2 mmol/kg) injection increased lactate levels in the plasma and the cerebrospinal fluid. IP lactate also induced tachycardia and behavioral freezing suggesting the production of panic-like behavior. To enter intermediate metabolism, lactate is oxidized by lactate dehydrogenase (LDH) to pyruvate with co-reduction of NAD$^+$ to NADH. Therefore, we measured the ratio of NADH/NAD$^+$ to test whether IP lactate altered lactate metabolism in the CNS. Lactate metabolism was studied in the hippocampus, a brain region believed to contribute to panic-like symptoms. IP lactate injection lowered the ratio of NADH/NAD$^+$ without altering the total amount of NADH and NAD$^+$ suggesting oxidation of hippocampal redox state. Lactate oxidized hippocampal redox since intrahippocampal injection of the LDH inhibitor, oxamate (50 mM) prevented the oxidation of NADH/NAD$^+$ by IP lactate. In addition to oxidizing hippocampal redox, IP lactate rapidly increased the firing rate of hippocampal neurons. Similar IP pyruvate injections had no effect. Neural discharge also increased following intrahippocampal lactate injection suggesting that increased discharge was a direct action of lactate on the hippocampus. These studies show that oxidation of brain redox and increased hippocampal firing are direct actions of lactate on the CNS that may contribute to the production of lactate-induced panic.

Introduction

Sodium lactate infusion induces not only the somatic symptoms that frequently accompany panic attacks, but also the cognitive symptoms, thereby closely resembling naturalistic panic attacks [19,21]. Lactate also induces panic-like effects in non-human primate and rodent experimental models [6,23,31–34,38,40]. Drugs used to treat panic disorder minimized the panic attack symptoms induced by sodium lactate [7,20,25,29]. Despite the insights gained by these studies, the mechanism(s) by which lactate induces panic remains largely unknown. It remains controversial whether lactate acts directly on the CNS to produce panic attack symptoms and/or exerts peripheral effects that trigger panic through activation of viscerosensory afferents [15].

Lactate is one of the diverse sets of compounds that induce experimental panic. The "respiratory" panicogens include sodium lactate, bicarbonate and CO_2 lack specific receptors and have ill-defined pharmacological actions [2,16]. They may work through a common mechanism involving lactate. A recent study, for instance, suggested that CO_2 may produce panic attacks by increasing brain lactate [11].

To understand its panicogenic properties, one needs to review lactate metabolism. Lactate dehydrogenase (LDH), a major oxidoreductase in the brain converts lactate to pyruvate by the following reaction: Lactate + NAD$^+$ ↔ pyruvate + NADH + H$^+$ [14,22]. LDH activity thereby contributes to the NADH/NAD$^+$ ratio, a major determinant of the brain redox state [5,22]. Changes in lactate metabolism may potentially alter brain redox. In addition to potential redox effects, lactate is an important fuel for the brain in a process referred to as the "lactate shuttle hypothesis". In that hypothesis, glucose is metabolized to lactate by astrocytes, and astrocytic lactate is transferred to neurons where it is oxidized to CO_2 and H_2O [22]. A variant of the lactate shuttle is the redox switch/redox coupling hypothesis that suggests that the brain generates energy by continuously switching between glucose and

* Corresponding author at: Box 29, Department of Physiology and Pharmacology, SUNY-Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203, United States. Tel.: +1 718 270 3927; fax: +1 718 270 2241.
E-mail address: pbergold@downstate.edu (P.J. Bergold).

0304-3940/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2009.02.041

lactate [5,22]. Rapid elevation of lactate during experimental panic may switch the energy substrate preference of the brain to lactate over glucose. This hypothesis received support from a recent finding that elevated lactate lowered glucose utilization in the human CNS [35].

As a measure of the potential central effects of lactate administration, we recorded from hippocampal neurons in rodents exposed to sodium lactate. Electrophysiological measures are a reliable real-time indicators of neuronal activity. A lactate effect on hippocampal neuron firing would provide the first evidence, to our knowledge, for a central effect of lactate. Hippocampal activation has been implicated by many, but not all, studies as either preceding lactate-induced panic or involved in the actual panic attack [10,17,30,37]. Recent imaging studies also show the importance of the hippocampus in anxiety disorders [3]. Evidence also suggests that the hippocampus has the capacity to modulate autonomic activity via subicular efferents to the lateral hypothalamus and/or amygdala [18]. Moreover, the dorsal location, laminar structure and relatively large size of the rat hippocampus facilitated the electrophysiological recordings, stereotaxic injections and biochemical measurements used in this study. We report that panic-like symptoms induced by sodium lactate administration in rats are accompanied by both an increased firing of hippocampal neurons and oxidation of the ratio of NADH/NAD^+. These hippocampal effects of lactate appear to be direct rather than an activation of an intermediary process.

Materials and methods

Animals

All experiments were performed using 3-month old Long-Evans rats (400–450 g, Taconic, Hudson, NY). All experiments, other than those assaying rat behavior, were performed under urethane anesthesia (1–1.25 g/kg). SUNY-Downstate Medical Center is licensed by the U.S. Department of Agriculture and abides by all laws regarding laboratory animal use.

Assay of blood lactate, heart rate and freezing behavior

Blood was obtained from tail veins and cerebrospinal fluid obtained at the level of the cervical vertebrae. Lactate concentration was measured using the Accutrend Lactate assay (Roche Diagnostics, Manheim, Germany). For heart rate and freezing behavior, rats were cannulated through the carotid vein. After one day, the catheter was flushed with 0.5 ml heparin–saline solution (100 units/ml) and attached to a blood pressure analyzer (BPA 400, Micro-Med Inc., Louisville, KY). The rats were placed in an open Plexiglas box (45.7 cm × 45.7 cm × 30.5 cm (w × d × h) for 15 min. Digitized heart rate from the analyzer was acquired by Dig-Med System Integrator (DMSI-400) at 5-s intervals. After rats received an IP injection of lactate (2 mmol/kg) or saline (all chemicals unless indicated were from Sigma (St. Louis, MO)), rats were returned for recording of heart rate and freezing behavior. The sodium salts of lactate and pyruvate were used for all injections. The pyruvate injection provides a control for any hypertonic effect of the sodium lactate. A blinded observer analyzed videotapes of the rats at 8, 15, 22, 29, 35, 55, and 65 min after injection (Observer 2.0, Noldus Information Technology, Leesburg, VA.).

Assay of NADH/NAD^+

Hippocampi were rapidly removed 30 min after IP injection of lactate (2 mmol/kg) or saline (0.9% (w/v) NaCl) and homogenized in perchloric acid (0.5N, 1 ml) at 4 °C. After 15 min, 1 ml of 0.33 M KPO_4, pH 7.5 was added, incubated for 1 h at 4 °C and centrifuged at 12,000 × *g* for 15 min at 4 °C. The supernatant contained both NAD^+ and NADH. Protein in the pellet was assayed by bicinchoninic acid assay following solubilization in 0.1 M NaOH (Pierce Chemical, Rockford, IL). A 60 °C 30-min treatment destroyed NAD^+ in an aliquot of the supernatant. Either heated or unheated supernatants (50 μl) were added to Tris–HCl, pH 8.0 (100 mM, 400 μl), EDTA (5 mM, 20 μl), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 0.5 mM, 40 μl) and phenazine ethosulfate (200 mM, 5 μl) at 25 °C. Alcohol dehydrogenase (3.2 units, from *S. cerevisiae*, 300–500 units/mg) was added followed by the addition of ethanol (6 M, 50 μl) 5 min later [39]. $OD_{570\,nm}$ was measured and compared to a standard curve of NAD^+. NAD^+ was determined as the difference between the unheated and heated supernatants.

Single unit recordings from hippocampus

Spontaneous discharge of hippocampal single units (relative to bregma: AP −3.5 mm; ML ±2.6 mm) was recorded [24]. At the end of the recording session, the tetrode placement was verified in brains that were sectioned and stained with cresyl violet. Action potential waveforms were discriminated by clustering of unitary waveforms (Wclust, A.A. Fenton). Pyramidal cells were defined as having: multiple action potential durations of greater than 350 μs, firing rates of <1 spike/s, and a peak probability of an interspike interval of ≤10 ms [26]. Recordings were analyzed if action potential waveforms remained stable for more than 1 h. For intrahippocampal injections, a micromanipulator lowered a 30-guage cannula to 1 mm lateral to the recording site. Lactate (1 M, 1 μl) or saline (0.9% (w/v), 1 μl) was delivered in an injection needle that was removed after completion of the injection.

Results

IP lactate injection produces panic-like symptoms in Long-Evans rats

Lactate has been previously shown to produce panic-like symptoms in Wistar rats or in Sprague–Dawley rats made panic-prone by pharmacologically disinhibiting the amygdala [23,32]. An effect of IP lactate injection has not been examined in Long-Evans rats. To ensure that IP lactate injection increased plasma lactate, lactate levels were measured in plasma following an intraperitoneal (IP) lactate injection (2 mmol/kg) in urethane-anesthetized Long-Evans rats (Fig. 1A). Lactate was 1.275 ± 0.025 mM in the cerebrospinal fluid of two urethane-anesthetized Long-Evans rats. Within 10 min after injection, baseline plasma lactate of 2.16 ± 0.09 mM (n = 5) significantly increased to 2.65 ± 0.12 mM. Lactate returned to baseline values at 60–70 min after the injection. Ten minutes following an injection of IP lactate (2 mmol/kg), CSF lactate increased to 1.6 ± 0.1 mM, n = 2) suggesting that CSF lactate was elevated at a similar time as plasma lactate. These CSF lactate levels are slightly lower than CSF lactate levels previously measured in unanesthetized Wistar rats [4]. The K_m for lactate uptake has been previously shown to be 0.2 mM in young adult hippocampal slice cultures [12]. These data strongly suggest substantial lactate uptake from the CSF into the hippocampus at a CSF lactate level in the 1–2 mM range seen in this study.

Two behaviors associated with panic in rats, tachycardia and behavioral freezing, were monitored following an IP injection of lactate (2 mmol/kg) or an equivalent volume of saline (0.9% (w/v)). Heart rate significantly increased 70% within 5 min after lactate injections, with no change observed following saline (Fig. 1A). Lactate-injected rats (n = 6) also spent a significantly greater time freezing than saline-injected rats (n = 3; Fig. 1B). Induction of