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Research Report

Enhancement of lactate metabolism in the basolateral amygdala by physical and psychological stress: Role of benzodiazepine receptors

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Abstract

Lactate is considered to play a significant role in energy metabolism and reflect neural activity in the brain. Using in vivo microdialysis technique, we measured extracellular lactate concentrations in the basolateral amygdaloid nucleus (BLA) of rats under electric footshock or psychological stress. We also attempted to determine whether the stress-induced changes of extracellular lactate concentrations in the BLA are attenuated by diazepam, an agonist at benzodiazepine receptors, and whether FG7142, an inverse agonist at benzodiazepine receptors, have a facilitative effect on energy metabolism in the BLA. Both footshock and psychological stress led to an increase in extracellular lactate concentrations in the BLA. Similar increment of extracellular lactate levels was observed by administration of FG7142. Pretreatment with diazepam attenuated the ability of FG7142, as well as physical or psychological burden, to increase lactate levels in the BLA. These results indicate that a variety of stressors enhances energy metabolism in the BLA, and suggest that some stress-induced changes in energy metabolism are regulated by benzodiazepine receptors.

Theme: Disorders of the nervous system
Topic: Neuropsychiatric disorders

Keywords: Footshock stress; Psychological stress; Lactate; Brain energy metabolism; Basolateral amygdaloid nucleus; Benzodiazepine receptor; Anxiety; Panic disorder

1. Introduction

Glucose has long been considered to be the substrate for neural energy metabolism in the brain. However, in vitro studies using hippocampal brain slices have shown that lactate supports the normal synaptic function in the face of glucose deprivation [12,38]. Moreover, even in the presence of glucose, brain tissues have been demonstrated to use lactate as an energy substrate in vitro [19–21,39]. On the other hand, extracellular lactate in the brain, as monitored with intracerebral microdialysis, has been shown to be of local origin and not influenced by plasma levels [18]. Local increases in extracellular lactate concentrations have been observed after neural stimulation (e.g., electroconvulsive shock, local administration of kainic acid) [16,17]. Local administration of tetrodotoxin (TTX), thought to block the electrical activity of neurons by inhibiting Na^+ channels [27], does not affect the basal levels of extracellular lactate concentrations, while TTX attenuates the electroconvulsive shock [17] or immobilization stress [45] induced increment of extracellular lactate levels. Moreover, inhibition of glycolysis by addition of 2-deoxyglucose to the dialysis perfusate causes an immediate decrease in lactate levels in extracellular fluid [17]. These observations suggest that extracellular lactate levels reflect the neural activity and glucose metabolism in the brain [17]. More recently, it was suggested that lactate is produced by astrocytes and released

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in the extracellular space to form a pool readily available for neurons in case of high energy demands [30].

Various types of stressors (e.g., tail pinch, immobilization) have been shown to increase extracellular lactate concentrations in the medial prefrontal cortex, hippocampus, and the striatum in the rat [5,7,16,37,40]. However, only a few studies have focused on cerebral blood flow or energy metabolism in the amygdala during exposure to stress. Thus, conditioned fear stress has been shown to increase local cerebral blood flow in the amygdala of rats, as measured by quantitative autoradiography with ^{14}C-labeled iodoantipyrine as a ligand [22]. We previously reported that immobilization stress increases extracellular lactate concentrations in the BLA, which is attenuated by stimulation of benzodiazepine receptors [45]. The amygdala has been implicated in the production of behaviors associated with fear and anxiety. Information from all sensory modalities reaches the amygdala via projections from the cortex and a variety of subcortical structures, most notably the thalamus and parabrachial complex, which converge on the basolateral amygdaloid complex, i.e., the lateral and basolateral subdivisions [23]. Sensory and cognitive information is relayed to adjacent amygdala subdivisions and other forebrain areas that ultimately mediate the physical and psychological manifestations of anxiety [4]. Various neurotransmitters are released in larger quantities in the BLA during emotional arousal than control conditions. These include noradrenaline [34,41,47], serotonin [2,14], dopamine [10,46,49], and acetylcholine [25].

Benzodiazepines are among the most widely prescribed therapeutic agents in the psychiatric practice and have anxiolytic, anticonvulsant, sedative/hypnotic, and amnestic properties. The amygdala, especially lateral and basolateral nuclei, is responsible for the manifestation of anxiolytic effects of benzodiazepines and expression of anxiety itself [4,23,43]. The pharmacological effects of benzodiazepines are mediated through allosteric modulation of $GABA_A$ receptors. Benzodiazepine-binding site resides on the alpha subunit of these receptors. Recent studies using genetically modified mice found that the alpha1 subunit of $GABA_A$ receptors may mediate the sedative effects [35], while the alpha2 subunit may be responsible for the anxiolytic effects of benzodiazepines [24]. Immunohistochemical localization of alpha1 subunit shows moderate staining in the BLA, while alpha2 subunits are localized heavily in the BLA [8].

In this study, we used a microdialysis technique coupled with an enzymatic/fluorometric detector for the measurement of lactate, according to the method of Korf et al. [15] with minor modifications [45]. We were particularly interested in the effect of electric footshock or psychological stress on extracellular lactate concentrations in the BLA of rats [46]. Especially, we sought to determine whether physical or emotional stress affects energy metabolism in the BLA. To examine the role of benzodiazepine receptors in the lactate metabolism, we also attempted to determine whether the stress-induced changes of extracellular lactate concentrations are attenuated by diazepam, an agonist at these receptors, and whether administration of FG7142 (*N*-methyl-beta-carboline-3-carboxamide), an inverse agonist at these receptors, has a facilitative effect on extracellular lactate release in the BLA.

2. Results

2.1. Basal extracellular lactate concentrations

The basal concentration of lactate in the dialysate was 42.8 ± 1.4 μmol/l (mean ± S.E.M.). No group differences in basal extracellular lactate levels were found ($F(6,35) = 2.05$, NS).

2.2. Effect of FG7142 on extracellular lactate concentrations

FG7142 (10 mg/kg, i.p.) caused a significant increase in lactate levels at 0–60 min after the injection. They reached the maximum level (129.4 ± 3.7% of the basal levels) after 8 min of injection and gradually decreased. In the rats pretreated with diazepam, extracellular lactate concentrations reached the maximum (111.4 ± 2.5% of basal levels) after 4 min of FG7142 injection and immediately returned to the basal levels. Repeated measures ANOVA revealed a significant effect of diazepam treatment ($F(1,30) = 15.07$, $P = 0.003$) and an interaction between Status (saline, diazepam) and time ($F(1,30) = 3.68$, $P < 0.0001$) on the FG7142-induced increase in extracellular lactate concentrations (Fig. 1).

2.3. Effect of stress on lactate levels

Footshock and psychological stress caused a significant increase in lactate levels at 0–60 min and 2–42 min, respectively, after the start of stress exposure. Extracellular lactate concentrations increased to 167.4 ± 4.8% of the mean baseline value at 10 min after the start of footshock stress and

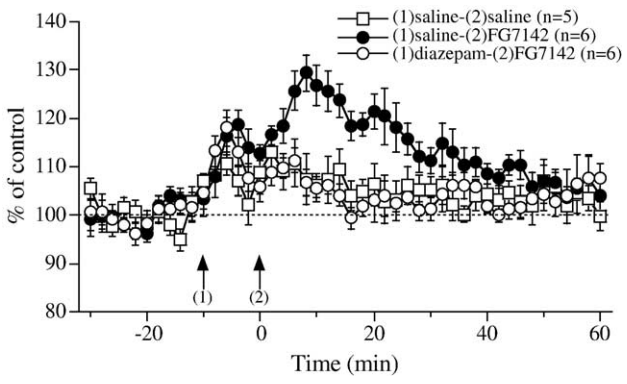

Fig. 1. Time course of the effects of diazepam on the FG7142-induced lactate increment. Diazepam or saline was injected (arrow 1) for 10 min, followed by administration of FG7142 (arrow 2). Saline + saline (open square), saline + FG7142 (closed circle), diazepam + FG7142 (open circle). Arrows indicate the point of drug injection. Data are mean ± SEM.