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Short communication

Immobilization stress-induced increment of lactate metabolism in the basolateral amygdaloid nucleus is attenuated by diazepam in the rat

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Abstract

Using in vivo microdialysis technique, extracellular lactate levels were measured in the basolateral amygdaloid nucleus of the rat under immobilization stress. Immobilization stress (40 min) led to a tetrodotoxin-reversible increase in dialysate lactate levels. Diazepam (1.0 mg/kg, i.p.) reduced the ability of immobilization stress to increase lactate levels. Furthermore, the attenuation of the immobilization stress-induced increase of lactate levels by diazepam was antagonized by pretreatment with flumazenil (15 mg/kg, i.p.), a selective antagonist at benzodiazepine receptors. These findings suggest that immobilization stress increases lactate levels in rat basolateral amygdaloid nuclei, which is attenuated by stimulation of benzodiazepine receptors.

Keywords: Microdialysis; Immobilization stress; Amygdaloid nucleus, basolateral; Lactate; Benzodiazepine

1. Introduction

Regional cerebral activity is associated with an increase in local glucose utilization (Fox et al., 1988). Lactate has been considered to be a major metabolite of the anaerobic glucose metabolism. The increase in the tissue content of lactate in the brain has been used as an indicator of the anaerobic metabolism during hypoxia and ischemia in the brain (Hillered et al., 1985). This measure has also been associated with chemically induced seizures (Evans and Meldrum, 1984) and electroconvulsive shock (Miller et al., 1982). Moreover, an in vitro study using hippocampal brain slices has shown that lactate, as the sole energy substrate, supports the normal synaptic function in the face of glucose deprivation (Schurr et al., 1988). Recently, the combination of in vivo microdialysis technique with an enzyme reactor/fluorometric detector has enabled on-line measurements of extracellular lactate levels (Korf, 1989; Kuhr and Korf, 1988b). A transient increase in the extracellular level of lactate was found after neuronal stimulation (e.g., electroconvulsive shock or local administration of kainic acid) (Kuhr and Korf, 1988a; Krugers et al., 1992), whereas inhibition of glycolysis with 2-deoxyglucose decreases lactate levels in the extracellular fluid (Kuhr and Korf, 1988a). These changes in the concentrations of lactate may be related to alterations in the glucose metabolism and neural activity. Thus, lactate may be a useful alternative as a substrate for the energy metabolism. Kuhr and Korf (1988a) have suggested that the extracellular lactate level, as measured by a modified brain microdialysis method, is a useful indicator of the local brain glucose metabolism and neural activity.

Various types of stress (e.g., tail pinch, immobilization) have been shown to increase extracellular lactate levels in the medial prefrontal cortex, hippocampus, and the striatum in the rat (De Bruin et al., 1990; Fellows et al., 1993; Krugers et al., 1992; Schasfoot et al., 1988; Takita et al., 1992). However, little is known about the exact nature of the stress-induced changes in the lactate level in the limbic regions. The amygdala is involved in both the anxiolytic effects of benzodiazepines and the expression of anxiety itself (Davis et al., 1994; Ledoux et al., 1990; McCabe and Wamsley, 1986; Mohler and Okada, 1978; Treit et al., 1993). The benzodiazepine receptor density is high in the amygdala (Nagy et al., 1979), especially in the basolateral nucleus (Niehoff and Kuhar, 1983). Recently, some studies have shown that the benzodiazepine receptors in the

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basolateral amygdaloid nucleus may play a significant role in the regulation of anxiety (Harris and Westbrook, 1995; Petersen et al., 1985; Sanders and Shekhar, 1995).

In the present study, we used the microdialysis technique, according to the method of Korf et al. (Korf, 1989; Korf and DeBoer, 1990) with minor modification, to measure the degree and time course of the lactate metabolism in the basolateral amygdaloid nucleus of rats under immobilization stress. To investigate the role of benzodiazepine receptors in the lactate metabolism in the stressed rats, the effects of diazepam and flumazenil, agonist and antagonist at these receptors, respectively, on the extracellular concentration of lactate, were also determined.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 300–350 g (Japan SLC, Japan) were used. All rats were housed at 24 ± 2 °C under a cycle of 12 h of light (7:00–19:00 h)/12 h of dark. All experimental procedures were performed according to the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

2.2. Surgery

The rats were anesthetized with pentobarbital (40 mg/kg, i.p.), and then mounted on a stereotaxic apparatus. A push–pull dialysis probe (molecular weight cutoff 10,000; 200 μm in outer diameter) was implanted into the right basolateral amygdaloid nucleus according to the atlas of Paxinos and Watson (1998), and was secured with skull screws and dental acrylate. The exposed tip length of the probe was 1.5 mm. Coordinates of the tip were 2.8 mm posterior to bregma, lateral 5.2 mm, ventral 9.6 mm relative to the surface of the skull. Following the surgery, the rats were housed in individual cages with free access to food and water.

2.3. Experimental conditions

Forty-two to forty-eight hours after surgery, the dialysis was carried out on the freely moving rats placed in cubic plastic home cages (30 × 30 × 30 cm). Artificial CSF (consisting of 147 mM NaCl, 3 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.4 mM NaH_2PO_2, pH 7.40) was perfused at a rate of 5.0 μl/min into the dialysis probe in the freely moving rats. The dialysates were mixed on-line with an enzyme solution containing L-lactate dehydrogenase and NAD^+ in a T-tube. The enzyme solution consisted of 5.0 μg/ml LDH (L-lactate: NAD oxdoreductase, E.C.1.1.1.27; isolated from pig heart, specific activity ca. 250 U/mg; Boeringer Mannheim, F.R.G.) and 0.5 mM NAD^+ (Boeringer Mannheim, F.R.G.) in a carbonate buffer (62.5 mM, adjusted to pH 7.4 with NaOH). The solution was pumped using a Model EP-50 microinfusion pump (Eicom, Kyoto, Japan) at the flow rate of 20 μl/min. The mixture from the T-tube was passed for 10 min reaction before reaching a fluorometer equipped with a 12 μl flow-cell (SHIMAZU RF-530, Kyoto, Japan). During transport to the fluorometer, lactate was enzymatically oxidized and the fluorescence of the nicotinamid adenosine dinucleotide diphosphate formed (NADH) was continuously measured at excitation at 340 nm and emission at 450 nm. A standard solution of 100 M lactate was used for calibration.

2.4. Experimental procedure

After a 3-h period of stabilization, the rats were restrained in a home-made cage (5 cm in height × 6 cm in width × 16 cm in length) made of plastic and wire for 40 min. The cage prevented any movement, but did not compromise respiration. Only minimal handling of the animal was involved at the start of the procedure. Diazepam (1.0 mg/kg i.p., in diazepam solution, 5 mg/ml, Takeda Chemical Industries, Osaka, Japan), and flumazenil (15 mg/kg i.p., in flumazenil solution, 1 mg/10 ml, Yamanouchi Pharmaceutical, Osaka, Japan) or saline as a vehicle, were administered at 10 and 20 min before the start of the immobilization stress, respectively. In control experiments, the same volume of vehicle (saline) was given. At the end of the experimental sessions, the position of the dialysis probe was verified macroscopically for all rats.

2.5. Presentation of the results and statistics

The average of the extracellular lactate concentration during the period preceding immobilization (10 measurements performed every 2 min) was used as the control value (100%). Data were converted into percent change from the mean baseline values for statistical evaluation by repeated measures analysis of variance (ANOVA). A probability (P) of less than 0.05 was considered to be significant.

3. Result

3.1. Responses to immobilization stress

The basal concentration of lactate in the dialysate was 41.3 ± 1.9 μM (mean ± S.E.M.) in the basolateral amygdaloid nucleus. Immobilization stress (40 min) caused an increase in the dialysate lactate levels to 149 ± 9% (mean ± S.E.M.) of the mean baseline value (Fig. 1). They reached the maximum levels at 4 min after the start of immobilization stress and immediately decreased despite continued immobilization stress. After termination of the immobilization stress, a transient significant increase was seen.

3.2. Effect of local tetrodotoxin

Local infusion of 10 μM tetrodotoxin into the basolateral amygdaloid nucleus slightly decreased basal lactate

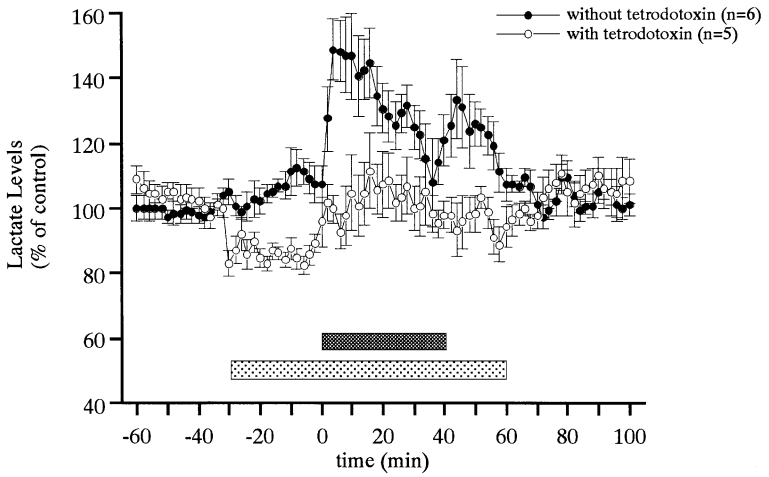

Fig. 1. Time course of the effects of immobilization stress on the extracellular lactate concentration in the basolateral amygdaloid nucleus of rats with (○) or without (●) tetrodotoxin (10^{-5} M). Extracellular lactate concentrations were evaluated every 2 min. For each time point, means are expressed as percent of the respective basal lactate level (average of 10 samples collected before immobilization stress or tetrodotoxin infusion). The immobilization stress (▩) and tetrodotoxin infusion (⬚) are indicated by the solid bars. Data are means ± S.E.M. at the corresponding time points.

levels, which did not reach statistical significance. Repeated measures ANOVA revealed a significant effect of tetrodotoxin ($F(1,10) = 6.232$, $P = 0.031$) and an interaction between tetrodotoxin infusion and time ($F(1,50) = 6.501$, $P < 0.0001$) on the immobilization-stress induced increase in extracellular lactate (Fig. 1).

3.3. Effects of diazepam and flumazenil on the immobilization stress-induced lactate increase

Injection of saline (i.p.) produced a small transient increase (about 110%) in the extracellular lactate concentration. Neither diazepam nor flumazenil alone affected basal lactate levels (data not shown). Repeated measures ANOVA (treatment × time) demonstrated that pretreatment of rats with diazepam (1.0 mg/kg, i.p.) resulted in an attenuation in the immobilization stress-induced lactate increment ($F(1,9) = 5.321$, $P = 0.0465$). Lactate levels increased transiently (about 130% at 2 ~ 8 min) and returned to the baseline within 16 min after initiation of the immobilization stress, and remained at basal levels thereafter. Coadministration of flumazenil (10 min before diazepam) and diazepam had no significant effect on the increase in lactate levels induced by immobilization stress ($F(1,9) = 0.011$, $P = 0.9192$). Immobilization stress resulted in a maximum lactate increase over the basal levels of 149 ± 7% (mean ± S.E.M.) at 4 min in animals treated with flumazenil and diazepam, followed by a gradual decrease to the basal levels (Fig. 2).

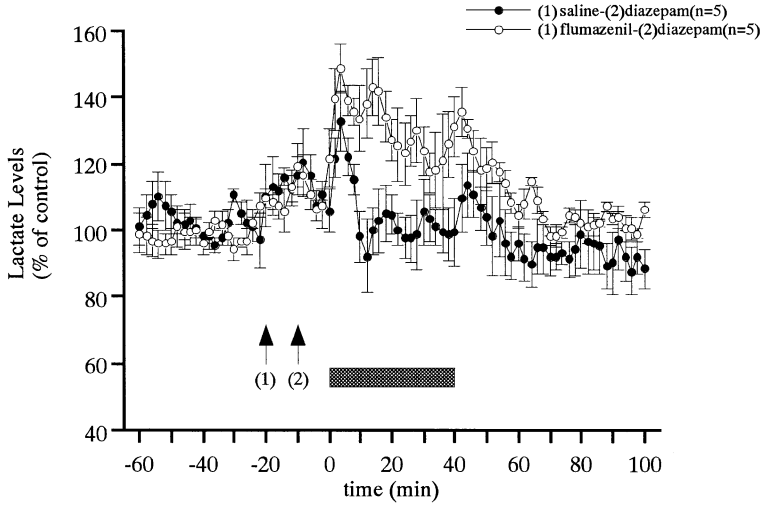

Fig. 2. Time course of the effects of diazepam and flumazenil on stress-induced lactate increment. Diazepam was administered at 10 min before immobilization stress, and flumazenil (○) or saline (●) at 20 min before. For each time point, means are expressed as percent of the respective basal lactate level (average of 10 samples collected before the first administration of drugs). Arrows indicate the drug injection time. The immobilization stress (▩) is indicated by the solid bar. Data are means ± S.E.M. at the corresponding time points.

4. Discussion

The present data indicates that immobilization stress led to an increase in extracellular lactate levels in the basolateral amygdaloid nucleus of rats. Local administration of tetrodotoxin totally eliminated the immobilization stress-induced increase in the lactate concentration in the amygdaloid dialysates. These observations indicate that immobilization stress augments the extracellular lactate release by increasing an impulse flow in the basolateral amygdaloid nucleus. Immobilization stress has been shown to increase the noradrenaline release in the basolateral amygdaloid nucleus (Tanaka et al., 1991). Therefore, the immobilization stress-induced increase in lactate release may be associated with the activation of noradrenaline or other neurotransmitter neurons in the basolateral amygdaloid nucleus.

Pretreatment of rats with diazepam resulted in an attenuation in the lactate increase, which was blocked by coadministration of flumazenil. These results suggest that the lactate-increase induced by immobilization stress in the basolateral amygdala is regulated by the $GABA_A$/benzodiazepine receptor complex. Benzodiazepines (e.g., diazepam) are the widely prescribed class of anxiolytic drugs, whose central effects are thought to be mediated through the benzodiazepine receptor sites allosterically coupled to the $GABA_A$ receptors (McCabe and Wamsley, 1986; Mohler and Okada, 1978). The amygdala has a high concentration of benzodiazepine receptors (Nagy et al., 1979), with the highest density being localized to the anterior basolateral amygdaloid nucleus, which may be one of the important sites of action of these anxiolytic drugs (Niehoff and Kuhar, 1983). Specifically, it is claimed that benzodiazepine receptors in the basolateral amygdaloid nucleus are involved in passive avoidance of nonpainful fear stimuli (Pesold and Treit, 1995). On the basis of these data, facilitation of the $GABA_A$/benzodiazepine receptor complex function may be involved in the attenuation of immobilization-induced lactate increase. In other words, anxiety induced by immobilization may elicit lactate increments in the basolateral amygdaloid nucleus.

The immobilization stress-induced lactate increment was transient despite continued administration of the stress. This finding is consistent with previous studies showing that immobilization for 90 min induced an immediate increase of lactate release in the early stage followed by a gradual decrease to basal levels in the medial prefrontal cortex of rats during a prolonged period of immobilization (Takita et al., 1992). These findings suggest that increment of the neural activity induced by immobilization stress disappears gradually during the continuous stress, which may represent an adaptation response. The rate of lactate removal from the extracellular space is greater when local energy requirements are increased (Fellows et al., 1993). Various forms of physiological stimulation, including immobilization stress, lead to closely coupled increases in regional blood flow (Bryan, 1990), leading to a gradual increase in the oxidative metabolism of glucose. Accordingly, lactate response, which indicates the degree of local nonoxidative glucose metabolism, may return to baseline levels during the relatively long exposure to immobilization stress. Transient increases in the lactate levels were also seen after the termination of immobilization stress. Hence, it appears that the termination of prolonged immobilization stress affects the release of lactate in the same manner as the start of a new stimulus. We are interested in whether a similar adaptation response occurs during a continuous stress in the other brain regions, e.g., the striatum and hippocampus. This issue should be a subject of future studies.

The results of our study may have relevance to the treatment of anxiety states in clinical subjects. Because the increment of extracellular lactate levels in the basolateral amygdaloid nucleus is supposed to be an indicator of stress, the technique of lactography may be useful in the development of new anxiolytics. Further investigations are needed to clarify the exact mechanism underlying the stress-induced increment of extracellular lactate levels in the basolateral amygdaloid nucleus.

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