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Differential effects of allopregnanolone and GABA on kainate-induced lactate dehydrogenase release in cultured rat cerebral cortical cells

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KEY WORDS steroids; allopregnanolone; cell culture; lactate dehydrogenase; $GABA_A$ receptors

ABSTRACT

AIM: To exam the effects of allopregnanolone and γ-amino-butyric acid (GABA) on the excitotoxicity. **METHODS:** The excitotoxicity was evoked by kainate (KA) in the primary culture of rat cerebral cortical cells. Effect of allopregnanolone or GABA on the excitotoxicity was examined by the measurement of lactate dehydrogenase (LDH) activity released in the culture medium. **RESULTS:** Either acute (3 h) or chronic (24 h) treatment with KA (0.01–1 mmol/L) produced a concentration-dependent increase in LDH activity released. The EC_{50} values were (0.16±0.03) mmol/L and (0.257±0.015) mmol/L, respectively. Acute treatment with allopregnanolone (10–1000 nmol/L) for 3 h did not significantly affect the 0.2 mmol/L KA-induced LDH activity. On the other hand, chronic treatment with allopregnanolone (10–1000 nmol/L) for 24 h, produced inhibition on the KA-induced LDH activity in a concentration-dependent manner. The EC_{50} value was (436±19) nmol/L. Acute treatment with GABA (0.1–100 μmol/L) exacerbate the 0.2 mmol/L KA-induced LDH activity in a concentration-dependent manner, with an EC_{50} value of (2.7±1.0) μmol/L; while chronic treatment with GABA had no significant effect. **CONCLUSION:** There were differential patterns between the effects of allopregnanolone and GABA on the KA-induced excitotoxicity.

INTRODUCTION

Kainate mediate excitatory synaptic transmission in the mammalian central nervous system (CNS) through ligand-induced opening of transmembrane ion channels. Over-activation of kainate receptors has been shown to play an important role in the mechanisms underlying several neurodegenerative disorders[1]. Generally, the effect of kainate is thought to be indirect through innervations of glutamatergic neurons. It is the release of glutamate that leads to neuronal death through activation of post synaptic *N*-methyl-aspartic acid (NMDA) receptors[2]. However, conflicting observations regarding the roles of presynaptic kainate- and postsynaptic NMDA-receptors have also been reported[3].

The neuroprotective potential of neurosteroid has

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gained increasing attention during the last years. The neurosteroid allopregnanolone is synthesized *de novo* in the brain from cholesterol[4]. It is a potent γ-aminobutyric acid$_A$ ($GABA_A$) receptor modulator with anticonvulsant[5] and anxiolytic[6] effects. Recently, we noticed a protective effect of allopregnanolone against kainate-induced lethality in mice as observed in an earlier report[7]. Base upon the fact that allopregnanolone is the most efficacious endogenous compound to enhance $GABA_A$ receptor function *in vitro*[8], the role $GABA_A$ receptor in this potential protective pathway needs to be clarified. In this study, therefore, we examined the effects of allopregnanolone and GABA on the kainate (KA)-induced excitotoxicity by the measurement of lactate dehydrogenase (LDH) activity released in the culture medium of rat cerebral cortical cells.

MATERIALS AND METHODS

Animals Embryos were obtained from Sprague-Dawley rats (Experimental Animal Resource, Medical Center of Fudan University, Grade II, Certificate №2-2-22-2) between gestational d16 and 18.

Cultures The embryonic cerebral cortex was removed from the skull and placed into minimum essential medium (MEM) containing glucose 5 g/L and $NaHCO_3$ 1.2 g/L. Cells were mechanically dispersed by repeated triturations and filtered through a 200 μm metal mesh. Two mL aliquots of the cell suspension were plated at a final density of 1.5×10^6 cells per Petri dish (35 mm) previously coated with poly-*D*-lysine 0.25 g/L. Cells were then cultivated at 37 ℃ in a 5 % CO_2 atmosphere.

Drugs Allopregnanolone, GABA, and kainic acid were purchased from Sigma Chemical Co (St Louis, MO, USA).

LDH assay As a quantitative measure of cellular toxicity, the LDH activity was estimated in 0.1 mL aliquots of culture medium. The activity of LDH was determined spectrophotometrically[9].

Data analysis To characterize the excitotoxic effect of KA, the LDH activities released into the culture medium were expressed in percent of total LDH activities detected in culture medium and the cellular fraction. From the concentration-response curve, a suitable concentration was derived for the subsequent study. In the study of modulations of KA toxicity, changes in LDH activities were expressed as percent of the value obtained with KA treatment alone. Allopregnanolone or GABA was tested at 5 doses spanning the doses producing 50 % attenuation or exacerbation of KA toxicity. EC_{50} values and E_{max} values were the averages from three separate experiments, each in triplicate. Data were expressed as mean±SD.

RESULTS

Characterization of KA-induced LDH release in cultured rat cerebral cortical cells Either acute (3 h) or chronic (24 h) treatment with KA (0.01, 0.05, 0.1, 0.5, 1 mmol/L) produced a concentration-dependent increase LDH activity released (Fig 1). The EC_{50} values were (0.16±0.03) mmol/L and (0.257±0.015) mmol/L, respectively. The E_{max} values were (16.0±1.8) % and (71±13) %, respectively. Apparently, the concentration of 0.20 mmol/L was suitable for KA in the subsequent modulating studies, either acute or chronic.

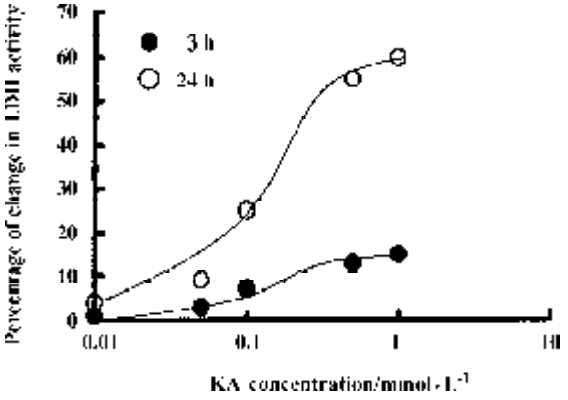

Fig 1. Representative concentration-effect curve of KA on the excitotoxic release of LDH in the culture medium of rat cerebral cortical cells. Similar results were repeated three times, each in triplicate.

Effect of allopregnanolone on KA-induced LDH release Acute (3 h) treatment with allopregnanolone (10–1000 nmol/L) and KA (0.20 mmol/L) produced no significant changes in the KA-induced LDH release.

Chronic treatment (24 h) with allopregnanolone (10–1000 nmol/L) inhibited KA-induced LDH activity in a concentration-dependent manner (Fig 2). The EC_{50} value was (436±19) nmol/L. The E_{max} values were

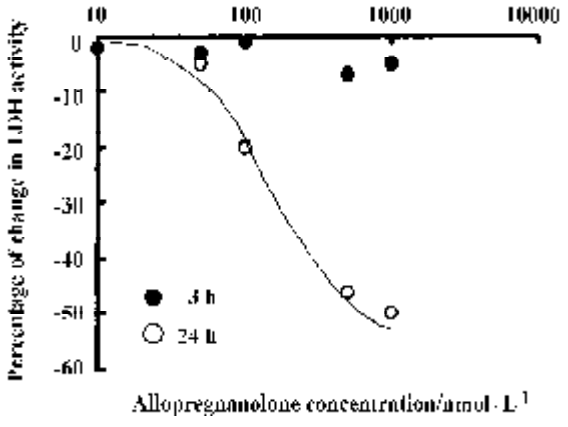

Fig 2. Representative concentration-effect curve of allopregnanolone on LDH activity in the culture medium of rat cerebral cortical cells induced by KA (0.2 mmol/L). Similar results were repeated three times, each in triplicate.

(73.6±0.8) %.

Effect of GABA on KA-induced LDH release

Acute (3 h) treatment with GABA (0.1–100 μmol/L) for 3 h exacerbate the kainate (0.2 mmol/L)-induced LDH activity in a concentration-dependent manner, with an EC_{50} value of (2.7±1.0) μmol/L (Fig 3). The E_{max} values were (75±15) %. Chronic (24 h) treatment with

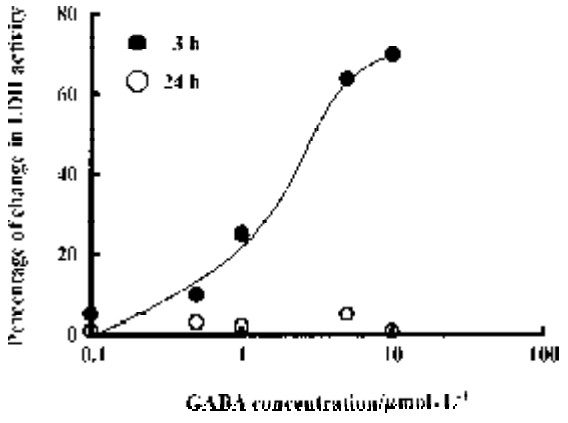

Fig 3. Representative concentration-effect curves of GABA on LDH activity in the culture medium of rat cerebral cortical cells induced by KA (0.2 mmol/L). Similar results were repeated three times, each in triplicate.

GABA did not significantly affect the KA-induced LDH activity.

DISCUSSION

Several recent investigations have shown that the neurosteroid allopregnanolone is neuroprotective in a variety of experimental paradigms. In kainate model of epilepsy, allopregnanolone was found to have a protective effect[10]. We also noticed a protective effect of allopregnanolone against kainate-induced lethality in mice. Since allopregnanolone allosterically modulate GABAergic transmission through a unique binding site on the $GABA_A$ receptor Cl^- channel complex, it is possible that allopregnanolone protect the KA-induced excitotoxcity via the enhancement of $GABA_A$ receptor responses. In the acute study, we found that GABA exacerbated the KA-induced LDH activity release in a concentration-dependent manner. This result contradicted to the fact that GABA is protective against excitototoxic brain damage *in vivo*[11]. Since the excitotoxic effect of kainate is thought to be mediated indirectly through activation of post synaptic NMDA receptors[2], this seeming conflicting result could be due to an indirect trans-synaptic action, which is negligible in this culture setting.

On the other hand, allopregnanolone exhibited a neuroprotective effect in a later stage. So far, there has been no report regarding the chronic effect of allopregnanolone on the KA-induced excitotoxicity in the cultured cortical cells. There was a report showing both positive and negative effects of allopregnanolone on neuronal survival following anoxia[12]. It has been proposed that glutamate excitotoxicity can contribute to the neurodegeneration associated with cerebral hypoxia-ischemia[13]. Therefore, their data represent the situation in glutamate excitotoxicity to certain extent. Our results of the chronic effect of allopregnanolone on the KA-induced excitotoxicity were consistent with their findings of the positive effect. The negative effect of allopregnanolone was only observed at lower concentration (1×10^{-10} mol/L) and could be due to a more complicated effect of allopregnanolone on the $GABA_A$ receptor. Our results, as discussed above, were

more likely produced through sites other than the $GABA_A$ receptor. Furthermore, we noticed insignificant effect of allopregnanolone after acute treatment, indicating the decreased excitatory neurotransmission could be developed only after chronic treatment of allopregnanolone.

Further support for the speculation of non-GABA mechanism involved was from the observed difference between allopregnanolone and GABA. Different time profile indicated different mechanism involved in their effects on KA-induced excitotoxicity. Allopregnanolone and pregnanolone sulfate manifested opposite effects of on the $GABA_A$ receptor complex, and pregnanolone sulfate can exacerbate NMDA-induced death of hippocampal neurons[14]. It was reasonable to expect the protective property of allopregnanolone. However, the difference of these nuerosteroids on the excitotoxicity can not simply explained by their bidirectional effects on the $GABA_A$ receptor, since the GABA itself did not protect the KA-induced excitotoxicity.

Therefore, the differential pattern between the effect of allopregnanolone and GABA on the KA-induced excitotoxicity revealed anon-GABA mechanism involved in the allopregnanolone protection along the pathway of KA-induced excitotoxicity.

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Allopregnanolone与GABA在大鼠大脑的皮层细胞中对kainate诱导乳酸脱氢酶释放的不同作用

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关键词 甾类；allopregnanolone；细胞培养；乳酸脱氢酶；$GABA_A$ 受体

目的：观察allopregnanolone与γ氨基丁酸(GABA)对兴奋性毒性的作用. 方法：对原代培养大鼠大脑的皮层细胞，采用kainate (KA)处理，诱发兴奋性毒性. 通过测定释放至细胞培养基中的乳酸盐脱氢酶（LDH）活性，观察allopregna-

nolone与GABA对兴奋性毒性的作用．结果：短期或长期KA处理，均增加LDH活性的释放，其作用呈剂量依赖性，EC_{50}值分别为(0.16±0.03) mmol/L和(0.257±0.015)mmol/L．短期(3 h) allopregnanolone（10-1000 nmol/L)处理对0.2 mmol/L KA诱导的LDH活性的释放无明显影响.而长期(24 h) allopregnanolone（10-1000 nmol/L)处理抑制KA诱导的LDH活性的释放，其作用呈剂量依赖性，EC_{50}值为(436±19) nmol/L．短期GABA（0.1-100 μmol/L)处理，加剧KA（0.2 mmol/L)诱导的LDH活性的释放，其作用呈剂量依赖性，EC_{50}值为(2.7±1.0) μmol/L．长期GABA处理，对KA诱导的LDH活性的释放无明显影响．结论：allopregnanolone和GABA对KA诱导的兴奋性毒性有不同作用．

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