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TWO RECEPTORS ARE INVOLVED IN THE CENTRAL FUNCTIONS OF KYNURENIC ACID UNDER AN ACUTE STRESS IN NEONATAL CHICKS

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Abstract—Intracerebroventricular (i.c.v.) injection of kynurenic acid (KYNA) had sedative and hypnotic effects during stress in neonatal chicks. However, its mechanism has not been clarified. KYNA is an endogenous antagonist of the α7 nicotinic acetylcholine (α7nACh) receptor and N-methyl-D-aspartate (NMDA) receptor. Therefore, this study clarified the mechanism of sedative and hypnotic effects of KYNA in the brain during an acute stress. In Experiment 1, to investigate the relationship between KYNA and the α7nACh receptor, KYNA was injected i.c.v. with galantamine, an agonist of the allosteric potentiating site of the α7nACh receptor. Galantamine did not attenuate the effect of KYNA, but higher levels of galantamine caused harmful effects. In Experiment 2, the role of the NMDA receptor was investigated using the NMDA receptor antagonist (+)-MK-801, D-serine which has high affinity to a co-agonist glycine site at the NMDA receptors, NMDA as the NMDA receptor agonist, and 2,3-pyridinedicarboxylic acid (QUIN), an agonist of the NMDA receptor subgroup containing the subunits NR2A and NR2B. The behavioral changes following KYNA were partially attenuated by QUIN alone. In conclusion, we suggest that KYNA functioned via the simultaneous inhibition of the α7nACh receptor and NMDA receptor subgroup containing the subunits NR2A and NR2B.

Key words: stress, kynurenic acid, α7nACh receptor, NMDA receptors, chick.

INTRODUCTION

Kynurenic acid (KYNA) is one of the tryptophan (TRP) metabolites in the kynurenine (KYN) pathway (Stone, 2001). KYNA is present in the mammalian brain in low (rodents) to high (human) nanomolar concentrations, and is an endogenous antagonist of two receptors that are closely linked to cognition and psychosis, i.e. the α7 nicotinic acetylcholine (α7nACh) receptor (Hilmas et al., 2001) and the N-methyl-D-aspartate (NMDA) receptor (Kessler et al., 1989). Two nACh receptor subtypes are found in abundance in the mammalian central nervous system. One binds nicotine with high affinity and is composed of α4 and β2 subunits; the other binds α-bungarotoxin and is most probably a homomeric α7nACh receptor (Lindstrom, 1997). KYNA, a non-competitive blocker for the presynaptic α7nACh receptor (Hilmas et al., 2001), regulates glutamate release and acts as a competitive antagonist of several types of glutamate receptors with a particularly high affinity to the strychnine-resistant glycine-co-agonist site of the NMDA receptor (Füvesi et al., 2012).

In previous studies, intracerebroventricular (i.c.v.) injection of some amino acids caused sedative and hypnotic effects under an acute stressful condition. For instance, L-proline induced sedative and hypnotic effects acting at the NMDA receptor (Hamasu et al., 2010), as did L-serine (Shigemi et al., 2008) and L-ornithine (Kurata et al., 2011) acting through a gamma aminobutyric acid A ($GABA_A$) receptor under a social isolation stress. We also found that i.c.v. injection of KYNA induced sedative and hypnotic effects in neonatal chicks (Yoshida et al., 2012), but the precise mechanism has not been studied. Therefore, this study investigates the mechanism of sedative and hypnotic effects of KYNA in the brain under an acute stress. As described above, because KYNA may be associated with two primary receptors, we examined the relationships between the function of KYNA and the α7nACh receptor in Experiment 1 and the NMDA receptor in Experiment 2.

In Experiment 1, galantamine hydrobromide, with modification of the levels applied by Wu et al. (2007), was used to investigate the role of the α7nACh receptor. Galantamine is a nicotinic allosteric potentiating ligand effectively increasing α7nACh receptor activation at sub-saturating agonist concentrations (Pereira et al., 2002). Additionally, galantamine is a weak reversible cholinesterase inhibitor. However, its nicotinic allosteric potentiating ligand action seems to be an important determinant of its clinical effectiveness (Lopes et al., 2007). Acting primarily as a nicotinic allosteric potentiating ligand, galantamine improves synaptic transmission and decreases neurodegeneration, two effects essential for its cognition-enhancing properties (Santos et al., 2002; Dajas-Bailador et al., 2003; Arias et al., 2004; Kihara et al., 2004; Zhang et al., 2004).

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Abbreviations: α7nACh, α7 nicotinic acetylcholine; ANOVA, analysis of variance; KYNA, kynurenic acid; NMDA, N-methyl-D-aspartate; QUIN, 2,3-pyridinedicarboxylic acid; TRP, tryptophan.

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In addition to the α7nACh receptor, the NMDA receptor may also be involved in the function of KYNA since KYNA antagonizes the response to NMDA via an action at the strychnine-insensitive glycine receptor (Birch et al., 1988). Accordingly, in Experiment 2, we administered a variety of agonists or antagonists to the NMDA receptor to further investigate the mechanism of KYNA in the chick brain. Four pharmacological reagents were used including (+)-MK-801, D-serine, NMDA, and 2,3-pyridinedicarboxylic acid (QUIN). KYNA was co-injected with (+)-MK-801 (Hamasu et al., 2010), a NMDA receptor antagonist. While KYNA is a confirmed antagonist of the NMDA receptor in mammals, it had to be confirmed that KYNA acts as an agonist of the NMDA receptor in chicks. This idea came from the fact that i.c.v. injection of NMDA had a similar effect in chicks to that induced by KYNA (Yamane et al., 2009b). Secondly, we co-administered KYNA with D-serine (Danysz and Parsons, 1998) to confirm KYNA antagonized at the glycine site on the NMDA receptors, since D-serine has high affinity to a co-agonist glycine site at the NMDA receptors. Third, KYNA was co-administered with NMDA (Yamane et al., 2009b) to determine if KYNA has an agonist or antagonistic effect compared to NMDA. Finally, KYNA was co-administered with QUIN to confirm the contribution of NMDA receptor subgroup, since QUIN acts as the agonist of the NMDA receptor subgroup containing the subunits NR2A and NR2B (de Carvalho et al., 1996; Brown et al., 1998).

EXPERIMENTAL PROCEDURES

Animals

One-day-old male layer chicks (Julia) were purchased from a local hatchery (Murata Hatchery, Fukuoka, Japan) and housed in a windowless room at a constant temperature of 30 ± 1 °C. Continuous lighting was provided. Food (AX, Toyohashi Feed and Mills Co., Ltd., Aichi, Japan) and water were freely accessible. Chicks were reared in a group (20–25 per cage) until the start of the experiment. On the day of the experiment, chicks (5 days old) were assigned to treatment groups based on their body weight in order to produce uniform treatment groups, and the number of animals used in each group was kept to a minimum while still ensuring adequate statistical power. Experimental procedures followed the guide for animal experiments of the Faculty of Agriculture, the Graduate Course of the Kyushu University, as well as the Law (No. 105) and Notification (No. 6) of the Government.

Preparation of drugs

KYNA and NMDA were purchased from Sigma (St. Louis, MO, USA). Galantamine hydrobromide, D-serine, and QUIN were purchased from Wako (Osaka, Japan) and (+)-MK-801 maleate was purchased from Funakoshi (Tokyo, Japan). Drugs were dissolved in 0.85% saline containing 0.1% Evans Blue. In Experiments 1 and 2, the negative control group was given the saline solution mentioned above and the positive control was injected with KYNA alone.

I.c.v injection and experimental design

The i.c.v. injections were made using a microsyringe according to the method of Davis et al. (1979) and Koutoku et al. (2005). The stress and pain associated with this method are minimal, as described elsewhere (Koutoku et al., 2005). The injected volume was 10 μl. In Experiment 1, chicks were injected i.c.v. with either saline, KYNA (100 nmol) or KYNA (100 nmol) plus galantamine hydrobromide (0.25, 0.5, or 1 μmol). The dose of galantamine hydrobromide was modified from the doses reported by Wu et al. (2007). In Experiment 2, chicks were injected i.c.v. with either saline, KYNA (100 nmol), KYNA (100 nmol) plus (+)-MK-801 maleate (0.5 nmol), KYNA (100 nmol) plus D-serine (0.84 μmol), KYNA (100 nmol) plus NMDA (1 nmol) or KYNA (100 nmol) plus QUIN (100 nmol). After injection, chicks were immediately placed in an acrylic monitoring cage (40 cm × 30 cm × 20 cm), and behavioral observations were made for 10 min. During this period, chicks were deprived of water and diet. Chick vocalizations were simultaneously recorded and the number of distress vocalizations was counted using Sound Engine (Coderium Inc., Sapporo, Japan). Video cameras were positioned to record the behavior of chicks from three different directions on DVD. Based on the method reported by van Luijtelaar et al. (1987), chick behaviors were classified into four categories: (1) active wakefulness; (2) standing/sitting motionless with eyes opened; (3) standing motionless with eyes closed; and (4) sitting motionless with drooped head (sleeping posture). In addition, (5) an abnormal posture was added in Experiment 1. The monitoring systems were set in a separate room to avoid disturbing the animals as reported by Yamane et al. (2009a). At the conclusion of the experiments, the birds were decapitated following anesthetization with isoflurane (Mylan Inc., Tokyo, Japan). The brains were removed and the location of the Evans Blue dye was confirmed. Data from chicks without dye in the lateral ventricle were excluded from the analysis.

Statistical analysis

In all experiments, data were statistically analyzed by one-way analysis of variance (ANOVA). When significant ($P < 0.05$) effects were detected, the Tukey–Kramer test was used as a post hoc test. Data were analyzed using StatView Version 5.0 software (SAS Institute, Cary, NC, USA). Values are presented as means ± standard error of mean (SEM). All data were first subjected to the Thompson rejection test to eliminate outliers ($P < 0.01$), after which the remaining data were used.

RESULTS

Experiment 1: Effects of i.c.v. injection of KYNA and galantamine on social isolation-induced behaviors in chicks

Fig. 1a shows the effect of i.c.v. injection of KYNA and KYNA plus several doses of galantamine on distress