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Influence of adenosine receptor ligands on ketamine-induced anesthesia in mice

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ABSTRACT

The influence of adenosine receptor ligands on anesthetic activity of ketamine was studied in mice. Ketamine-induced anesthesia (100 mg/kg) was significantly increased by the higher doses of CPA (selective A_1 adenosine receptor agonist) and NECA (A_1/A_2 adenosine receptor agonist). CGS 21680 (selective A_{2A} receptor agonist) markedly but not significantly prolonged this ketamine effect. Administration of both selective adenosine receptors antagonists DPCPX (A_1) and DMPX (A_{2A}) resulted in significant decrease in the duration of ketamine-induced anesthesia. Caffeine (A_1/A_2 adenosine receptor antagonist) dose-dependently but not significantly diminished the anesthetic effect of ketamine. The results suggest that adenosinergic system is involved in ketamine anesthetic activity and seem to appear that the effects are connected with both type (A_1 and A_{2A}) of adenosine receptors.

Keywords: ketamine, adenosine, the loss of the righting reflex time, mice

INTRODUCTION

Ketamine, 2-(o-chlorophenyl)-2-methylaminocyclohexanone hydrochloride, is a useful anesthetic agent with a wide margin of safety. Characteristic for ketamine-induced anesthesia is rapid onset of action, deep analgesia and lack of cardiorespiratory depression [5]. However, ketamine produces posthypnotic emergency reactions such as prolonged hallucination, delirium and hyperlocomotion [21], which can limit its clinical use.

Ketamine exerts anesthetic effect mainly by binding to the phencyclidine site on the N-methyl-D-aspartate (NMDA) receptor protein [11, 25]. Some experimental data suggest that ketamine may also produce anesthesia by blocking central cholinergic neurotransmission [10,15].

Moreover, it was demonstrated that ketamine markedly increased noradrenaline release (in the medial prefrontal cortex), the central neurotransmitter with an important role in the regulation of sleep-awake cycle [13], which may play an important role in its anesthesia [14]. In addition, evidences suggest that ketamine has γ-aminobutyric $acid_A$ ($GABA_A$) receptor agonistic properties and that ketamine-induced anesthesia is mediated, at least in part, by $GABA_A$ receptors [12].

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Adenosine, acting as neuromodulator in the central nervous system (CNS), seems to play a critical role in the production of sleep. Enhance of sleep is induced by adenosine and adenosine receptor agonists, whereas antagonists of adenosine receptors increase wakefulness [2,17]. Some evidences suggest that sedative and sleep-promoting effects of adenosine are connected with the basal forebrain [22] and ventrolateral preoptic area of the hypothalamus, probably an important regions for somnogenic process [16]. Extracellular concentration of adenosine in the basal forebrain accumulated with prolonged sleep deprivation slowly fall during rebound sleep [17]. The role of the basal forebrain adenosine receptors, especially A1 in sleep homeostasis, was confirmed by Gass et al. [8]. Moreover, adenosine has been reported to reduce the release of acetylcholine [19, 24], noradrenaline [1], serotonin [6] and GABA [9], neurotransmitters, which have been implicated in the regulation of sleep.

The present experiments were undertaken to determine the role of adenosine system in the hypnotic effect of ketamine. In order to explore this possibility, the influence of adenosine receptors agonists and antagonists on ketamine-induced anesthesia were estimated in mice.

MATERIAL AND METHODS

Animals. The experiments were carried out on male albino Swiss mice (18-26 g). The animals were kept 8-10 to

a cage at room temperature of 20±1°C and under a 12 h light/dark cycle. Standard food (Murigran pellets, Bacutil, Motycz, Poland) and tap water were available ad libitum. All experiments were performed between 8 a.m. and 3 p.m.

The experiments were performed in accordance with the opinion of Local Ethics Committee.

Drugs

The following drugs were used: adenosine receptors agonists: N^6- cyclopentyladenosine (CPA) – A_1 receptor agonist, 2-p-(carboxyethyl) phenethyl amino-5'-N-ethylcarboxamidoadenosine (CGS 21680) – A_{2A} receptor agonist, 5'-N-ethylcarboxamidoadenosine (NECA) – A_2/A_1 adenosine receptor agonist (all from RBI, USA); adenosine receptor antagonists: 8-cyclopentyl-1, 3-dimethylxanthine (DPCPX) – A_1 receptor antagonist and 3, 7-dimethyl-1-propargylxanthine (DMPX) – A_2 receptor antagonist (both RBI, USA), caffeine – a nonselective adenosine receptor antagonist (Polfa, Poland), and NMDA receptor antagonist: ketamine (Ketanest, Parke-Davis, Germany).

All substances were dissolved in saline. Adenosine receptor ligands and ketamine were administered intraperitoneally (ip) at the injection volume of 10 mg/kg. Control animals received the same volumes of saline at the respective time before the test.

Procedure. The ketamine-induced anesthesia (100 mg/kg) was recorded as the time elapsing between the loss and recovery of the righting reflex (LORR test). CPA (0.0125, 0.025, 0.05 mg/kg), CGS 21680 (0.01, 0.05, 0.1 mg/kg), NECA (0.001, 0.005, 0.01 mg/kg), DPCPX (1 and 3 mg/kg), DMPX (3 and 6 mg/kg) and caffeine (10 and 20 mg/kg) were given 10 min prior to ketamine administration. Anesthetic dose of ketamine (100 mg/kg) was chosen during pilot experiments.

Statistics. The obtained data were analyzed statistically using Student's t-test. The results are presented as mean ± SEM (data in figures). A probability (p) value of 0.05 or less was taken to indicate statistical significance.

RESULTS

Influence of adenosine receptor agonists on ketamine-induced anesthesia in mice

Duration of anesthesia induced by the dose of 100 mg/kg of ketamine ranged from 17.3±1.56 to 25.2±2.98 min (Fig. 1 and 2).

Duration of anesthesia evoked by ketamine was significantly prolonged by higher doses of CPA (0.025 and 0.05 mg/kg, $p<0.01$, Fig. 1A) and NECA (0.01 mg/kg, $p<0.01$, Fig. 1C). CGS 21680 given at the dose of 0.01 mg/kg also increased the duration of ketamine-induced anesthesia but the result did not reach the level of significance (Fig 1B).

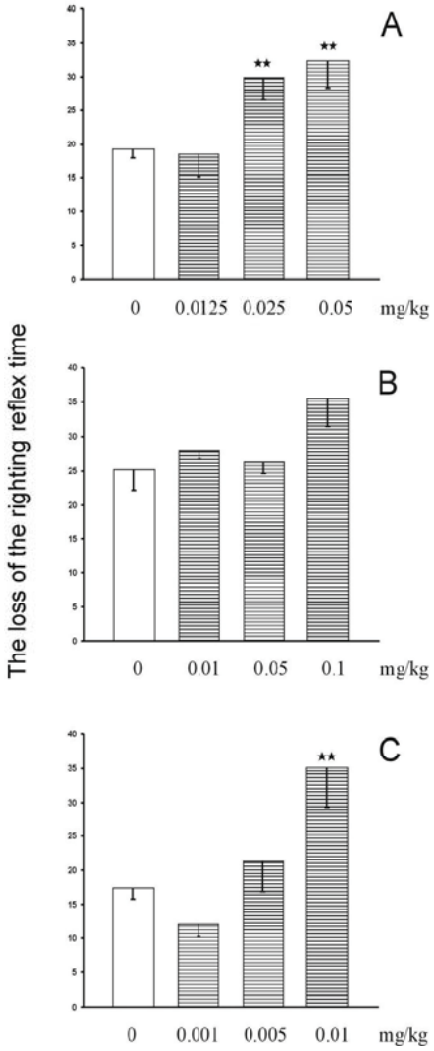

Fig. 1. The influence of adenosine receptor agonists: A. CPA, B.CGS 21680 and C.NECA on ketamine-induced anesthesia recorded as the loss of the righting reflex time in mice. The results are expressed as means SEM of the group consisting of 10 mice each. Open bars: ketamine 100 mg/kg, ruled bars (hatched bars): agonists + ketamine 100 mg/kg; ** $p<0.01$ vs. ketamine (Student's t-test)

Influence of adenosine receptor antagonists on ketamine-induced anesthesia in mice

Duration of anesthesia evoked by ketamine (100 mg/kg) was significantly shortened by DPCPX (1 and 3 mg/kg, $p<0.01$, Fig. 2A) and DMPX (3 and 6 mg/kg, $p<0.001$, Fig. 2B). Caffeine, given at the doses of 10 and 20 mg/kg, markedly and dose-dependently but not significantly diminished the ketamine-induced anesthesia (Fig 2C).

DISCUSSION

Adenosine, considered to be inhibiting neuromodulator, by A1 receptor activation inhibits synaptic transmission, primary by decreasing excitatory neurotransmitter release [3, 7]. Among others, the activation of A1 adenosine receptor in the striatal cholinergic interneurons

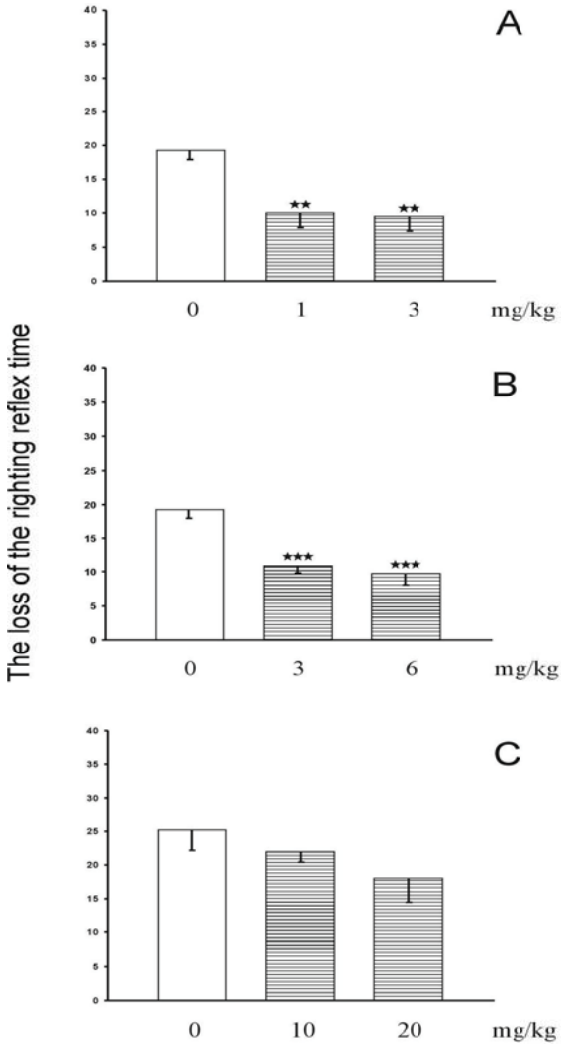

Fig. 2. The influence of adenosine receptor antagonists: A. DPCPX, B. DMPX and C. Caffeine on ketamine-induced anesthesia recorded as the loss of the righting reflex time in mice. The results are expressed as means SEM of the group consisting of 10 mice each. Open bars: ketamine 100 mg/kg, ruled bars (hatched bars): antagonists + ketamine 100 mg/kg; ** p <0.01, *** p <0.001 vs. ketamine (Student's t-test)

reduces N-type Ca2+ currents via a membrane and leads to reduce acetylcholine release [20]. Additionally, adenosine has been described to inhibit the release of GABA in the CNS [4]. Recently, it has been shown that adenosine may promote sleep by blocking inhibitory inputs on ventrolateral preoptic area of the hypothalamus sleep-active neurons [16]. It is hypothesized that sleep-active ventrolateral preoptic neurons are hyperpolarized during wakefulness through a combination of monoaminergic and GABA-ergic input. Well, it is possible that a rise of adenosine level may promote sleep, first, by blocking inhibitory GABA-ergic neurotransmission and, second, by directly inhibiting cholinergic system [for review 16].

It is known that ketamine-induced anesthesia, resulting mainly from inhibition of excitatory glutamatergic neurotransmission, is also connected with other systems e.g. cholinergic or GABA-ergic [see Introduction]. Because the mechanism by which ketamine produces anesthesia is still not well known we studied the influence of adenosine receptors' ligands on the duration of the loss of the righting reflex evoked by the anesthetic in mice.

The data presented here suggest that adenosine system is, in some part, involved in ketamine-induced anesthesia. We have shown that activation of adenosine receptors prolonged, whereas its inhibition shortened the time elapsing between the loss and recovery of the righting reflex evoked by ketamine. Although more distinct effects were observed after administration of selective adenosine A1 receptor ligands, CPA – an agonist, DPCPX – an antagonist, significant inhibition of the ketamine-induced anesthesia by DMPX – a selective adenosine A2A receptor antagonist, did not allow to link the effect only with A1 adenosine receptor. Our observations are in agreement with that of Radulovacki et al.[18] who has shown that activation of A1 rather than A2 receptors contributed to the sleep effects of adenosine and adenosine analogs because activation of A2 receptors requires the larger quantities of the compound than that of A1 receptors . On the other hand, Tung et al. [23] have found no significant differences between the adenosine A_1 receptor antagonist and adenosine A_{2A} receptor antagonist in respect to the sleeping time (LORR) induced by isoflurane in sleep-deprived rats. Then, our results are similar to that of Tung et al.[23].

Summing up, our results show that the adenosinergic system is involved in ketamine-induced anesthesia and indicate no significant differences in the involvement of adenosine A_1 and A_{2A} receptors in this effect. For a better understanding of the mechanism underlying the role of adenosine receptors in the hypnotic effects of ketamine, further studies are required.

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