Bull. Environ. Contam. Toxicol. (1994) 53:591–597

Environmental Contamination and Toxicology

Influence of Cadmium on Ketamine-Induced Anesthesia and Brain Microsomal Na^+, K^+-ATPase in Mice

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Received: 16 October 1993/Accepted: 1 May 1994

Cadmium is a rare metallic element and it is present in almost all types of food. Shellfish, wheat and rice accumulate very high amounts of cadmium. Occupational and environmental pollutants are the main sources of cadmium exposure. Cadmium has a very long biologic half-life. Exposure to Cadmium causes anemia, hypertension, hepatic, renal, pulmonary and cardiovascular disorders. It is considered as a possible mutagen, teratogen and carcinogen (Friberg and Elinder. 1992). Cadmium can enter the brain and may interact with other xenobiotics in the central nervous system. It has been shown that acute cadmium treatment increased the hexobarbital sleeping time and inhibited hepatic microsomal drug metabolism due to a decrease in cytochrome P_{450} content (Schnell et al. 1978, 1979). Cadmium potentiated ethanol-induced sleep in a dose-dependent manner (Magour et al. 1981). The prolongation of ethanol sleeping time is not due to inhibition of ethanol metabolism or to an elevation of ethanol level in the blood or brain. Cadmium has been shown to inhibit brain microsomal Na^+, K^+-ATPase activity in vitro and in vivo (Webb 1979). Cadmium and ethanol additively inhibited brain Na^+, K^+-ATPase. This might be a direct interaction between cadmium and ethanol in the central nervous system because the divalent cation calcium, which inhibits cerebral Na^+, K^+-ATPase, also potentiates ethanol-induced sleep (Hosie 1965).

Ketamine is an intravenous anesthetic agent. It acts on central nervous system and produces "dissociative anaesthesia". Ketamine provides adequate surgical anesthesia and is used alone in humans and/or combination with xylazine, an α_2-adrenergic agonist in animals. It produces CNS depression, analgesia, amnesia, immobility and a feeling of dissociation from the environment (White et al. 1982). Ketamine is a non-competitive antagonist of the NMDA subset of the glutamate receptor. This perhaps results in an increase in neuronal activity leading to disorganization of normal neurotransmission and produces dissociative

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anesthetic state (Davis et al. 1988). Because it is different from most other anesthetics, ketamine may be expected to have a unique effect on brain biochemical parameters and enzymes. The purpose of this study was to examine the interactions between cadmium and ketamine on the central nervous system and ATPase, in an attempt to further understand the mechanism of action.

MATERIALS AND METHODS

Male, CD-1 mice, weighing 35-40 g were used in all experiments. They were housed in groups of 5 mice/cage, kept at a constant room temperature and maintained in a controlled environment with a 12 hour light : 12 hour dark cycle. Food and water were provided *ad libitum*. The mice were divided into 2 groups of 10 mice each. One is treated with ketamine alone and another is treated with cadmium plus ketamine. Cadmium chloride was dissolved in saline and injected subcutaneously 3 mg/Kg 2 hours prior to intraperitoneal administration of a single dose of ketamine at 300 mg/Kg. Sleeping time was measured as the time from which righting reflex was lost after ketamine administration until it was regained. Attempts of the individual mice within a group to right itself within 1 minute were considered to constitute return of the righting reflex. The mean±standard error of the mean (S.E.) of the duration of anesthesia were compared using paired t-test between ketamine and ketamine plus cadmium treated groups. A 95% probability was considered significant.

Mice were killed by cervical dislocation. The brains were rapidly removed and were washed several times with ice-cooled isotonic solution containing 0.25 M sucrose, 5.0 mM L-histidine, 5.0 mM EDTA-Na_2 and 0.15% sodium deoxycholate, PH 6.8 with Trizma base. Microsomes were prepared as described by Chen et al. (1992). two grams of brain were minced with scissors and homogenized in a Polytron homogenizer twice for 1 minute with 20 ml of above ice-cooled isotonic sucrose solution. The homogenate was centrifuged for 30 minute at 12,000xg. Following centrifugation of the supernatant for 60 minutes at 100,000xg, the sediments were resuspended in 10 ml suspension solution containing 0.25 M sucrose, 5.0 mM L-histidine, and 1 mM EDTA-acid, PH 7.0 with Trizma base. A same volume of 2.0 M LiBr was added and stirred gently for 1 hour. The suspension was centrifuged in 20 ml suspension solution and recentrifuged. The microsomal pellets obtained were diluted in suspension solution and stored at -70 °C until used. All above procedures were carried out at 2 °C. Protein was determined by the method of Smith and Krohn. (1985).

ATPase activity in mouse brain microsomes was measured by the enzymatic method (Broekhuysen et al. 1972) with modifications. Total ATPase activity was determined in 0.1 ml of enzyme (30-50 μg of protein). Various concentrations of ketamine or cadmium in 0.1 ml were added to

the reaction medium. Mg^{2+}-ATPase activity was determined under the same experimental condition except 0.2 mM ouabain was added. The difference between the total ATPase and the Mg^{2+}-ATPase activity was considered to be Na^+, K^+-ATPase activity.

The reaction medium was preincubated with different concentrations of ketamine or cadmium at 37 °C for 5 minutes. The reaction was initiated by adding 3 mM of Tris-ATP. The reaction was then carried out for 10 minutes at 37 °C and was stopped by the addition of 1 ml of 15% TCA (w/v). After centrifugation, the inorganic phosphate liberated in 1 ml aliquot of supernatant was measured by adding 3 ml of 2 N H_2SO_4 : 2.5% ammonium molybdate : 10% L-ascorbic acid (3:1:1). The absorbance of reaction mixture was measured at 660 nm after 20 minutes at 37 °C. The concentrations necessary to inhibit 50% of the maximal enzyme activity was calculated using computer software (Tallarida and Murray. 1986). The activity of Na^+, K^+-ATPase was also assayed over a range of ATP concentration. ATP concentration from 0.25 mM to 3.0 mM. 65 μM cadmium, 6.0 mM ketamine or both added to incubation medium. ATPase activity was expressed as μg Pi/min/mg protein.

RESULTS AND DISCUSSION

The sleeping time of ketamine was investigated in male mice. The effects of subcutaneous dose of 3 mg/Kg cadmium significantly increased the duration of ketamine-induced sleeping time (Fig. 1). The duration of anesthesia with ketamine is 38.18 ± 3.36 minutes (mean ± S.E.). Sleeping time increased to 55.09 ± 4.20 minutes (mean ± S.E.) after pretreatment with 3 mg/kg cadmium.

Fig. 2 shows the effects of ketamine on ATPase in mouse brain microsomes. The results show a dose dependent decrease in the activities of Na^+, K^+-ATPase and Mg^{2+}-ATPase in mouse brain microsomes incubated with different concentrations of ketamine. Its IC_{50} value of Na^+, K^+-ATPase is 6.256 mM and the IC_{50} value of Mg^{2+}-ATPase is 21.185 mM for brain. Fig. 3a shows 65 μm cadmium or 6.0 mM ketamine inhibits brain microsomal Na+, K+-ATPase 41.58% and 34.51%, respectively. However, a combination of both ketamine and cadmium produces a 62.09% inhibition. It is shown in Fig. 3b that both ketamine and cadmium produce an uncompetive inhibition of brain microsomal Na^+, K^+-ATPase, indicating an additive action. In contrast, the ouabain-insensitive Mg^{2+}-ATPase was not significantly affected by either cadmium or ketamine.

Cadmium potentiated ketamine-induced sleeping time. It is possible that cadmium may inhibit the metabolism of ketamine in vivo and decreases the elimination rate of ketamine. But there might be a direct interaction between cadmium and ketamine in brain. Because Na^+, K^+-ATPase in the

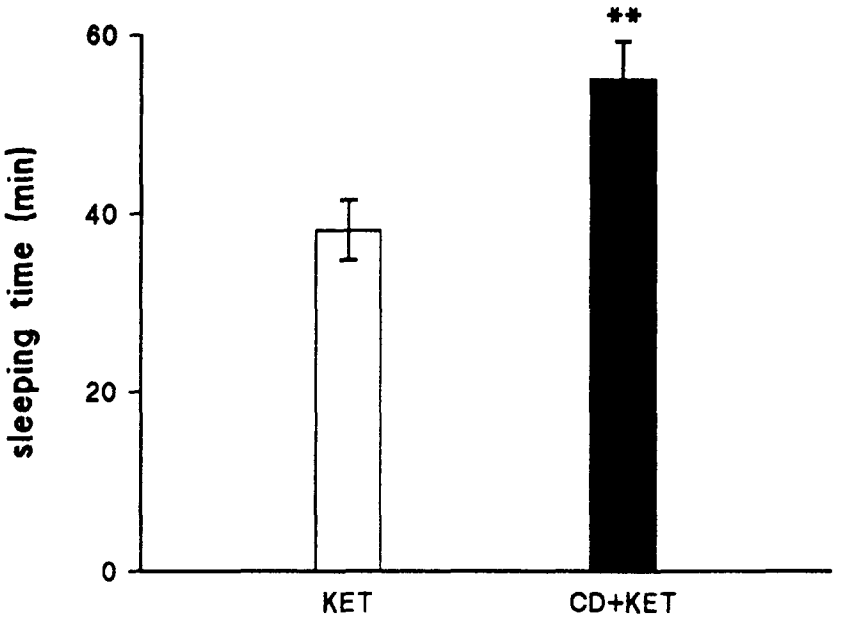

Figure 1: The effect of pretreatment with cadmium on ketamine-induced sleeping time in mice. ** P<0.01. Each bar represents the Mean±S.E. of 10 mice.

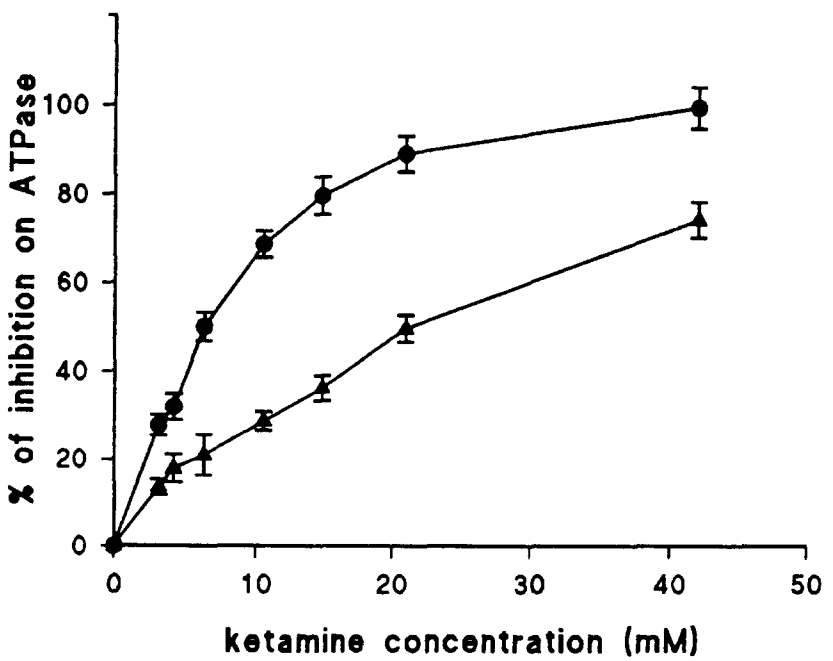

Figure 2: Effects of ketamine on mouse brain microsomal Na+, K+-ATPase and Mg2+-ATPase activity. ● Na+, K+-ATPase; ▲ Mg2+-ATPase.

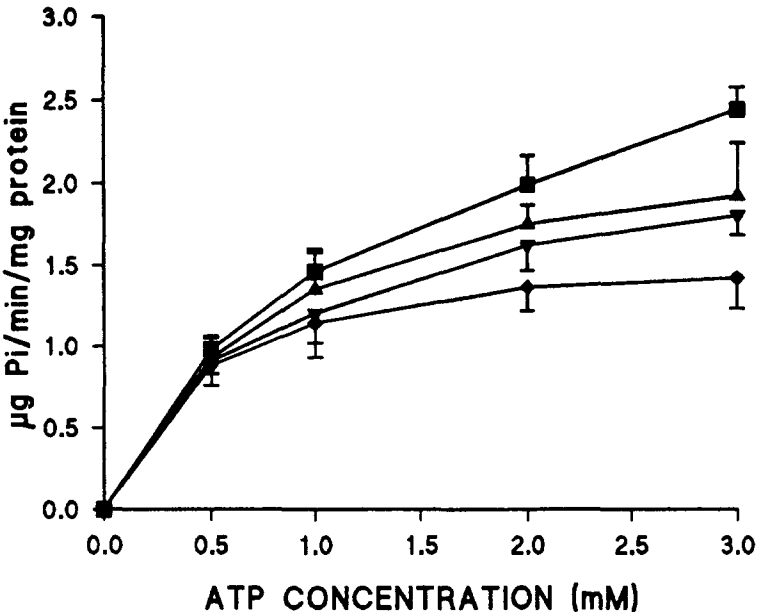

Figure 3a. Inhibition of brain microsomal Na+, K+-ATPase by cadmium or ketamine and by a combination of both chemicals in the presence of various concentration of ATP. ■ control ▲ cadmium (65 um); ▼ ketamine (6.0 mM); ◆ cadmium (65 um) +ketamine (6.0 mM).

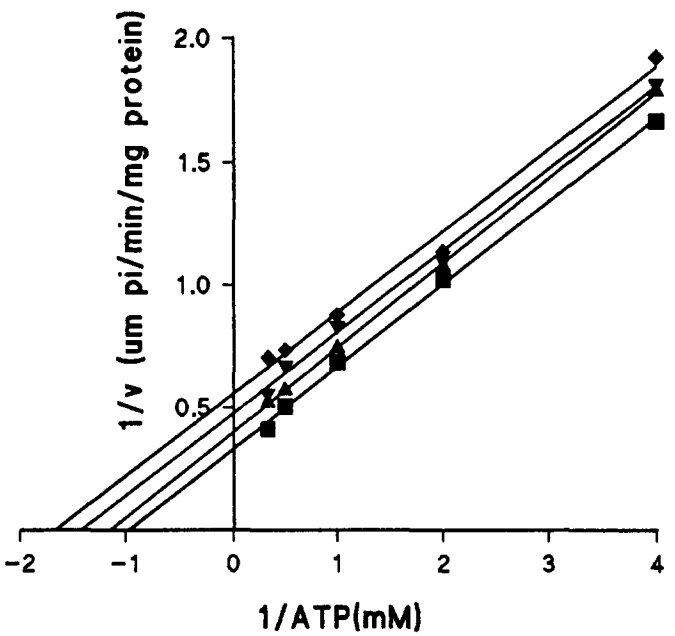

Figure 3b: Lineweaver-Burk plot of the figure 3a data. ■ control; ▲ cadmium (65 um); ▼ ketamine (6.0 mM); ◆ cadmium (65 um)+ketamine (6.0 mM).

central nervous system is responsible for the regulation of membrane polarization, alteration of the enzyme activity may affect excitation and subsequently the response of neurons to electric impulses (Skou 1965). The Na^+, K^+-ATPase in the brain plays an important role in the active transport of cation across cell membrane. The observed effects of ketamine on this important enzyme indicate that ketamine may alter sodium pump in CNS membrane and contribute to its CNS effects.

In summary, Our results have demonstrated that pretreatment with cadmium produced a significant increase on ketamine-induced anesthesia. Ketamine and cadmium additively inhibited brain microsomal Na^+, K^+-ATPase in mice. Therefore, it is likely that the observed prolongation of ketamine sleeping time is, at least in part, mediated via inhibition of brain microsomal Na^+, K^+-ATPase.

Acknowledgments. This work was supported by personal funds of Dr. Frederick Radd of Ponca City, Oklahoma.

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