Ketamine reduces lethality on the acute ammonia intoxication in mice.

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Abstract. Injection of large doses of ammonia (1.2 g/kg, i.p.) was used to induce acute toxicity in mice which was characterized by hyperresponsiveness, taquipnea, clonic and tonic seizures and death. Pretreatment with 20, 40, or 80 mg/Kg, i.p., of ketamine increased 30 to 55% survival rate. This pretreatment significantly retarded the beginning of the first tonic convulsion attenuating its intensity and delayed the time of the animal death; but did not alter the onset of the first clonic seizures. These experiments may be an evidence that support the hypothesis that seizures due to hyperammonemia involve activation of excitatory amino acid receptors.

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Palabras claves: toxicidad por amonio, hiperamonemia, ketamina.

Resumen. La inyección de dosis altas de amonio (1,2 g/kg, i.p.) fue usada para inducir una toxicidad aguda en ratones la cual se caracterizó por excitabilidad, taquipnea, convulsiones clónicas y tónicas y muerte. El tratamiento previo con 20, 40, o 80 mg/kg, i.p., de ketamina aumentó el porcentaje de sobrevivencia de 30 a 55%. Este pretratamiento retardó significativamente el comienzo de la primera convulsion tónica atenuando su intensidad y retrasó el tiempo en el cual ocurrió la muerte, pero no alteró el comienzo de la primera convulsión clónica. Estos experimentos podrían ser una evidencia que apoya la hipótesis de que las convulsiones debidas a hiperamonemia involucran activación en receptores de aminoácidos excitatorios

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INTRODUCTION

The effect of acute ammonia toxicity in human is commonly seen in hepatic encephalopathy resulting from acute liver failure, portal-systemic shunting of blood, amino acid metabolism disorders and urea intoxication in ruminants; in all cases, it may lead to seizures and coma (14). Although, this type of seizures has been recognized for more than a century, there is no fully supportive theory to explain it.

Some direct excitatory activity of ammonium ions are known; for instance, it depolarizes distant dendrites, which additionally decreases the Mg^{2+} block of the N-methyl-d-aspartate (NMDA) receptors (1, 10), increasing the capability of the neurons to be depolarized. However, more complex effects of ammonium ions are also known. In hyperammonemia an increase in brain content of quinolinic acid occurs (9) and the latter has agonistic effect at NMDA receptors eliciting a similar effect that glutamate (13).

Recently, indirect evidences were reported by using MK-801 (a noncompetitive NMDA receptor antagonist) that acute ammonia toxicity may involve activation of NMDA receptors, a type of glutamate receptors in the central nervous system (CNS) (7).

Ketamine is another potent noncompetitive NMDA antagonist (8), which elicits anticonvulsant properties in several convulsive models (3) including the spinal seizures evoked by sudden cooling reported in our laboratory (5). In the present work, this dissociative anesthetic agent was used to further assess the hypothesis that acute ammonia toxicity was mediated by NMDA-receptors. Preliminary accounts of some of this work have been reported (2).

MATERIALS AND METHODS

Adult male mice, strain NMRI, weighing 30 to 35 g, raised in the Animal Facility of our Veterinary School, were used.. They were kept in groups of 10 in standard metal cages at a 12 h light, 12 h dark period and had access to standard diet and water *ad libitum*. Intraperitoneal (i.p.) injection of ammonium acetate was utilized to induced the acute neurotoxicity as previous studies have demonstrated (15). The latency of the onset of first clonic convulsion, the first tonic convulsion, as well as the latency to either death or recovery was noted. Ketamine hydrochloride (Ketaset®, Aveco Co, Inc., Fort Dodge, Iowa) dissolved in 0.85% saline was injected, i.p., 15 min before intoxication. Control mice were treated with sodium acetate.

The data were analyzed with a statistical package using one way analysis of variance (ANOVA) followed by Wilcoxon's test. All values are reported as mean ± S.E.M. In most cases a level of significance was established at $p< 0.01$.

RESULTS

To determine the concentration of ammonium acetate that produced the typical symptoms of neurotoxicity and 90% rate of mortality, mice separated in groups of 10 were injected i.p. at increasing doses of ammonium acetate. A dose of 1.2 g/kg was found to produce the required neurotoxicity in our study. Thus, all the subsequent experiments were performed with this dose of ammonium acetate. Behaviorally, ammonium neurotoxicity was initially characterized by hyperresponsiveness, especially to any sound stimuli, followed by taquipnea, depression and coma. The second phase of toxicity began within several minutes during the comatose state consisting of clonic-convulsions lasting approximately 1 to 4 min in a popcorn appearance. The last state was the clonic-tonic convulsion with strong muscle contractions and extension in both limbs ending in the animals death. Control mice treated with equal concentration (0.8M) and volume of sodium acetate did not show any significant changes in their behavior.

Pretreatment with 20, 40, or 80 mg/Kg of ketamine increased the rate of survival when it was injected before the ammonia (Table I)

The characteristic symptoms of toxicity were either attenuated or delayed as shown in Fig 1. The time of death, as well as the beginning of the first tonic convulsion was significantly retarded, but not the beginning of the first clonic seizures. However, when higher ketamine doses of 120 mg/kg were used, a further delay in the tonic phase onset was observed, in spite of no recovery of the animal. The death in this group of mice was probably due to toxic effect of the anesthetic agent because they showed marked ataxia, absence of the righting reflexes and lack of responses to sound stimuli. Lack of co-ordination

TABLE I
RATE OF SURVIVAL AFTER PRETREATMENT WITH KETAMINE

Ketamine doses mg/Kg	Number animals used	Number of deaths	% of survival
Control	30	27	10
20	20	14	30
40	20	13	35
80	27	12	55

Mice were injected in groups with different doses of ketamine, 15 minutes before the administration of 1.2 g/kg of ammonium acetate.

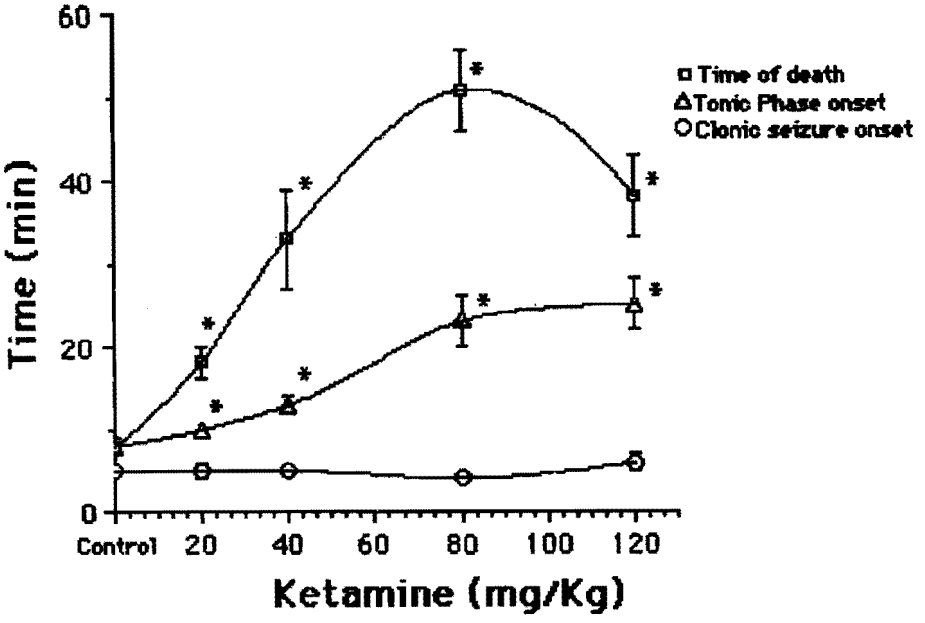

Fig. 1. Pretreatment with ketamine retarded the time of animals death, delayed the beginning of the first tonic seizure but not the onset of the first clonic convulsion. Data were plotted as mean ± SEM. *P < 0.01 when compared to control group.

and ataxia were also seen in mice pretreated at smaller ketamine doses.

In animals pretreated with ketamine the intensity of clonic convulsion was attenuated, and the accumulative time in which the animal was observed having this clonic seizure increased in a dose dependent manner as shown in Fig. 2A. In ketamine pretreated mice the first tonic phase was not severe enough to induce death. In this experimental group , in contrast to animals injected only with ammonium acetate, more than two tonic phases were needed to induced death, while those mice not protected by ketamine died after a few minutes and usually after the first tonic convulsion. The accumulative time between the observation of the first tonic phase and the animal death is plotted in Fig 2B.

DISCUSSION

In blood, under physiological conditions, 98-99% of ammonia is present as ammonium ions or NH_4+. Ammonia has a dissociation constant of 9.13-9.15 at 37°C. The unprotonated ammonia (NH_3) is lipid soluble and quickly passes through the plasma membranes; in contrast, ammonium ions, being hydrophilic,

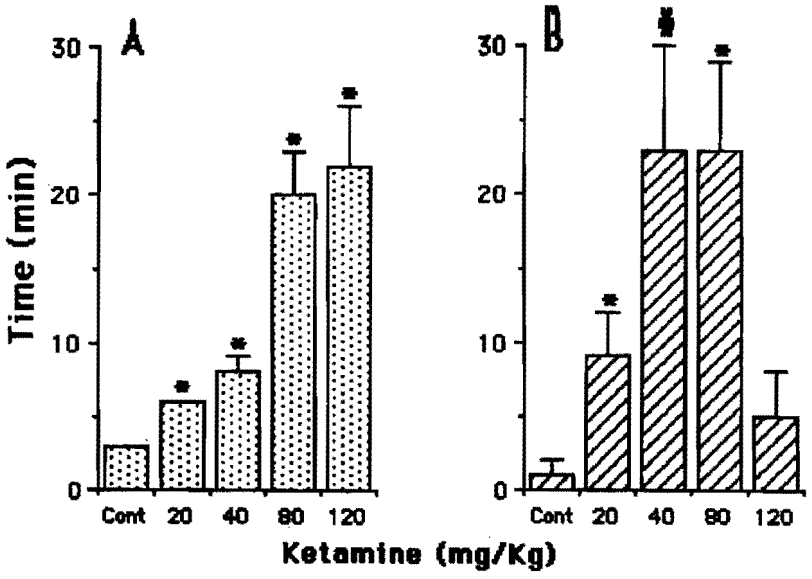

Fig. 2. Ketamine delayed the appearance of the first tonic seizure and also enhanced the time between this phase and the death of animals. A) Dose-response relationship between the time elapsed from the beginning of the first clonic seizure until onset of the tonic-phase. Data were obtained after the subtraction of the time of clonic convulsion onset from the time of tonic phase onset. B) Time elapsed from the observation of the first tonic seizure until the time of animal death. Data were obtained after subtraction of the time of tonic phase onset from the time of death.

* $P < 0.01$ in A; $P < 0.05$ in B, when compared with control. In both, data were plotted as mean ± SEM.

enter mostly through Na^+ and K^+ channels (14).

Ammonia has a profound influence on neuron-astrocytic metabolic interactions and more precisely on the integrity of the so-called "glutamate-glutamine cycle". Although it has generally been assumed that increased brain glutamine results from increased glutamine synthesis in hyperammonemic syndromes, experimental evidence for this is lacking (14).

The first agent reported to be effective against ammonia toxicity was ethanol (12). Ethanol, now well known as capable of inhibiting NMDA-activated ion current on neurons (6), reduces ammonium acetate lethality to only 7% when compared to a not protected group with 100% lethality. In a previous report, we confirmed the effectiveness of this agent (2). Similarly, butanol which is a more potent inhibitor of the NMDA receptor than ethanol, has a greater protective effect against ammonium toxicity (12).

Dizocilpine (MK-801), a potent noncompetitive NMDA antagonist,

is also effective against ammonia toxicity and only 5% of intoxicated animals died when they were protected by this agent. We found a modest 55% of protection with ketamine at the highest and safe dose used. However, we found a significant delay in the beginning of the tonic phase of seizures, a clear increase in the time of death and a significant attenuation of intensity of the clonic and tonic phase of convulsion that led us to support that, to some extent, NMDA receptor activation may be involved in ammonia lethality. Moreover, the possibility that ketamine protects against ammonia toxicity by acting on other systems may not be ruled out because it is not a selective drug (5, 16)

The use of ketamine or MK-801 as potentially useful drugs for preventing neurological disorders, such as stroke, have been questioned. These agents have psychotomimetic properties in humans and produce, by themselves morphological changes in neurons of the cingulate and retrosplenial cerebral cortices in rats, and high doses even cause irreversible neuronal necrosis (11). However, neurologic damage induced by ketamine may be prevented by diazepam and barbiturates as it was also demonstrated (11). Although, the use of ketamine as a neuroprotector is a matter of debate (4) and it has recognized side effects (16), the drug is still an useful and readily available tool to investigate the role of glutamate receptor over stimulation in the CNS.

Ammonium ions have a complex action on synaptic transmission in the mammalian CNS (14). Undoubtedly, in clinical cases of hyperammonemia, surviving of untreated patient may result in mental retardation and some CNS damage. Therefore, in clinical pharmacology, the investigation of novel competitive as well as noncompetitive NMDA receptor antagonists for treatment of hyperammonemia, in conjunction with agents that decrease their side effect, is an area of research that remains to be properly explored.

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