Protective Action of M- and N-Cholinoceptor Blockers in Acute Ammonium Intoxication

E. A. Kosenko, Yu. G. Kaminskii, V. N. Korneev, and L. D. Luk'yanova

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Atropine and d-tubocurarine are shown to prevent convulsions in rats and mice poisoned by ammonium acetate and to protect these animals from its toxic effects. Ammonium and lactate levels in their brain were found to correlate directly with ammonium toxicity.

Key Words: *atropine; d-tubocurarine; ammonium intoxication; cholinoceptors; brain*

Hyperammonemia accompanies many human and animal diseases including hepatoencephalopathy, cirrhosis, acute liver failure, and congenital deficiency of uric cycle enzymes, to mention only a few. When blood levels of ammonium ions increase 5-10 times or more, severe central nervous system disorders, convulsions, and coma develop and death rapidly ensues [8]. The molecular basis underlying the pathogenesis of hyperammonemia-associated diseases remains, however, unknown.

Toxic doses of ammonium salts induce convulsions in rats and alter acetylcholine levels in their brain [14]. Administration of ammonium chloride or acetate to animals leads to a significant fall of acetylcholinesterase activity in the brain *in vivo*, while high concentrations of ammonium salts reduce this activity in brain homogenates *in vitro*. Acetylcholinesterase inhibitors (e.g., sulfonyl halides and carbamates) are neurotoxins and cause central nervous system disorders similar to those seen after high doses of ammonium salts [13]. In view of the fact that acetylcholinesterase is an integral component of the cholinergic system, the present study aimed at clarifying the role of acetylcholine receptors in ammonium toxicity. To this end, the influence of specific M- and N-cholinoceptor blockers on the survival of animals acutely poisoned with ammonium acetate was examined, as was the influence of the blockers on ammonium and lactate levels in their brain, since the brain content of these metabolites has been shown to be greatly increased in acute ammonium intoxication [9].

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences; Laboratory of Biophysical Instrumentation, Institute of Cell Biophysics, Russian Academy of Sciences; Laboratory of Bioenergetics, Institute of Pharmacology, Russian Academy of Medical Sciences, Mosco

MATERIALS AND METHODS

Male Wistar rats (body weight 200-240 g) and male Swiss mice (body weight 16-20 g) were used. Acute ammonium poisoning was produced by administering ammonium acetate intraperitoneally at 7 mmol/kg (LD_{50}) to rats and at 7 or 12 mmol/kg to mice. To evaluate the role acetylcholine receptors may play in the mechanism of ammonium acetate toxicity, test animals received, 15 min prior to ammonium acetate, an intraperitoneal injection of a) the muscarine cholinoceptor blocker atropine (rats at 0.045-1.35 mg/kg, mice at 0.065-1.35 mg/kg); b) and/or the nicotine cholinoceptor antagonist d-tubocurarine (d-TC) (rats at 0.1 mg/kg, mice at 0.11 mg/kg); or c) benzohexonium (both rats and mice at 2.5 mg/kg), which is a nonspecific blocker of cholinergic receptors on autonomic ganglia. These doses of cholinergic receptor blockers were chosen because they do not affect the behavior of animals

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TABLE 1. Effects of Atropine and d−Tubocurarine (d−TC) on Convulsions and Survival in Rats and Mice Poisoned with Ammonium Acetate

Group	No. of animals	No. of animals with convulsions	No. and % (in parentheses) of survivors
Rats			
Control	16	16 (100)	6 (38)
Atropine, 0.045 mg/kg	21	12 (57)	18 (86)
d-TC, 0.1 mg/kg	22	8 (36)	19 (86)
Atropine+d-TC	20	6 (30)	18 (90)
Mice			
Control	12	12 (100)	0 (0)
Atropine, 0.13 mg/kg	20	16 (80)	4 (20)
d-TC, 0.11 mg/kg	12	9 (75)	3 (25)
Atropine+d-TC	15	11 (73)	4 (27)
Atropine, 0.26 mg/kg+d-TC, 0.2 mg/kg	14	9 (64)	3 (36)

Note. Figures in parentheses are percentages of survivors relative to the control group. Ammonium acetate was administered intraperitoneally at 7 mmol/kg to all rats and at 12 mmol/kg to all mice.

and are close to those used in pharmacological practice [1,2,5]. Rats and mice administered ammonium acetate alone or saline served as controls.

Brain levels of ammonium and lactate levels were measured before the administration of cholinoceptor blockers and ammonium acetate (control group) and 15 min after ammonium acetate injection into animals preinjected or not preinjected with the blockers. All rats were decapitated and their brains were removed, frozen in liquid nitrogen, extracted with perchloric acid [4], and assayed for the metabolites ammonium and lactate enzymatically [6,11] using microfluorimetry [3]. The results were statistically analyzed by Student's *t* test.

RESULTS

Many of the rats injected with ammonium acetate (7 mmol/kg) developed a typical syndrome of ammonium intoxication characterized by repeated (2 to 5 times) convulsive episodes during the first 7-10 min postinjection, hyperventilation, clonic and tonic convulsions (the latter occurred at minutes 15-20), loss of the right-sided reflex, and coma followed by death. However, the condition of other rats (30-50%) returned to normal (after two or three convulsive episodes) by minutes 60-90 (Table 1). Mice proved to be more resistant to ammonium acetate than rats, a dose of 7 mmol/kg being quite ineffective: none of the mice died and none developed convulsions. For mice, the LD_{50} of ammonium acetate has been reported to be about 11 mmol/kg and its $LD_{99.9}$, 18 mmol/kg [15]; in our study, however, the ammonium acetate dose of 12 mmol/kg caused 100% mortality (Table 1).

As mentioned above, there is evidence that the cholinergic system is implicated in the mechanism of acute ammonium intoxication [13,14]. We therefore tested cholinoceptor blockers for their impact on the latter, after exploring, in preliminary tests, how they might affect the behavior of animals.

As found in these tests, atropine in doses of 3-7 mg/kg induced coma in the animals 20-30 min postinjection, but did not affect their general condition in doses of 0.1-1.35 mg/kg which are close to those used clinically (0.03-1.0 mg/kg [1,5]) to terminate convulsive seizures. For this reason, subthreshold atropine doses (0.045-1.35 mg/kg) were used for rats in the present study. The 0.045 mg/kg dose prevented convulsions in some of the acutely poisoned rats and cut the mortality rate among them more than 50% (Table 1). Higher atropine doses (0.09-0.5 mg/kg) proved less and less protective, while the highest dose used (1.35 mg/kg) failed to protect at all. In mice, atropine doses of 0.065-0.13 mg/kg afforded protection to

TABLE 2. Effects of Atropine and d−Tubocurarine (d−TC) on Ammonium and Lactate Levels in the Brain Tissue of Rats Poisoned with Ammonium Acetate

Group	No. of animals	Ammonium	Lactate
		μmol/g tissue	
Control	4	0.28±0.04	1.50±0.21
Ammonium acetate (7 mmol/kg)	6	4.06±0.28*	4.11±0.27*
Atropine (0.045 mg/kg)+d-TC (0.1 mg/kg) + ammonium acetate	6	1.80±0.18*	2.23±0.21*

Note. *$p<0.01$ as compared to any of the other groups.

20% of the animals (Table 1); these did not develop convulsions.

In the preliminary tests, d-TC caused instantaneous (upon injection) death of rats at 0.5-5 mg/kg but just elicited convulsions in some animals at 0.2 mg/kg. At the 0.1 mg/kg level used in this study, d-TC did not produce any changes in the general condition of rats and protected against the toxic action of ammonium (Table 1), lowering the incidence of convulsions 3-fold and the mortality rate 2-fold. Similar results were obtained by other authors in rats given d-TC at 0.2 mg/kg and ammonium chloride at 12 mmol/kg [2].

In mice, d-TC at 0.11 mg/kg produced similar beneficial effects as the 0.1 mg/kg dose in rats. Co-administration of 0.13 mg/kg atropine and 0.11 mg/kg d-TC did not alter the individual effects of these adrenoceptor blockers in the indicated doses. When the atropine and d-TC doses were doubled, the percentage of animals with convulsions and that of survivors both increased (Table 1). Benzohexonium, used at a dose rate (2.5 mg/kg) considered to be pharmacological for man [5], did not affect the behavior of animals and failed to protect them from the acute ammonium intoxication.

Thus, suppressing the activity of central M- and N-cholinoceptors afforded protection against hyperammonemia, which confirms that the cholinergic system, like the system of glutamate receptors [7,10,12], is implicated in the mechanism by which this pathological condition develops.

As demonstrated previously [13], administration of ammonium salts to animals results in concurrent rises of ammonium and lactate levels in the brain. In view of the protective action exerted by cholinoceptor blockers on the survival of animals acutely poisoned with ammonium acetate, the inhibition of cholinoceptor activity in hyperammonemia could be expected to reduce ammonium and lactate concentrations in the brain. Our assays for ammonium and lactate bore out this expectation (Table 2). As early as 15 min after ammonium acetate administration, ammonium and lactate levels in the brain were increased 14- and 2.7-fold, respectively ($p<0.001$). Much smaller increases in these metabolites occurred in rats preinjected with atropine and d-TC (Table 2 and Fig. 1). As shown in Fig. 1, the correlation between brain levels of ammonium and lactate is represented by a line that does not pass through the origin of the coordinates ($r=0.885$; $p<0.0005$). A direct correlation also appears to exist between ammonium and lactate levels in the brain, on the one hand, and the state of cholinoceptors, on the other.

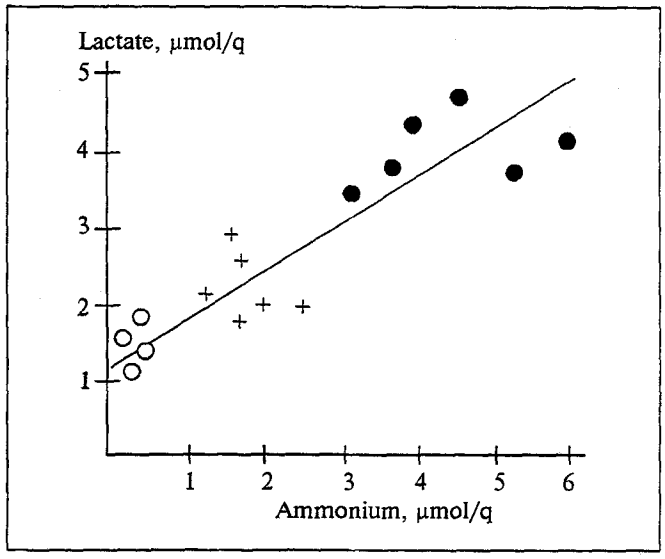

Fig. 1. Correlation between ammonium and lactate concentrations in the brain of rats acutely poisoned with ammonium acetate. White circles: control; black circles: 15 min after ammonium acetate administration (7 mmol/kg) to rats preinjected with atropine (0.045 mg/kg) and d-tubocurarine (0.1 mg/kg).

Atropine and d-TC, unlike benzohexonium, thus prevent convulsions in some rats and mice given ammonium acetate in toxic doses and reduce the associated mortality among the poisoned animals, in addition to which they reduce the elevations of ammonium and lactate in the brain of such animals. All this suggests that M- and N-cholinoceptors of the brain (but not those of the autonomic nervous system) are implicated in ammonium toxicity. These brain receptors appear to be involved in the regulatory mechanism controlling the development of ammonium intoxication. Moreover, the results presented for control animals in Table 1 point to differential sensitivities of rats and mice to high doses of ammonium salts. Indeed, the mice exhibiting convulsions after a toxic dose of ammonium acetate all died, whereas some of the rats survived after similar convulsions. The protective effect of cholinoceptor blocking agents was also more strongly marked in rats. The cholinergic system probably modulates ammonium toxicity to different extents in different animal species.

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Enkephalinase Activity in Various Brain Structures of Naloxone-Treated and Morphine-Insensitive Rats

L. F. Panchenko, V. Yu. Baronets, N. N. Terebilina, V. V. Aristova,* S. V. Litvinova,* and L. V. Kalyuzhnyi**

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Morphine injected subcutaneously in a dose of 2 mg/kg body weight exerted an analgesic effect in some Wistar rats (morphine-sensitive animals), as was indicated by a significantly prolonged latency of the tail-flick response, but failed to produce analgesia in others (morphine-insensitive animals). In morphine-sensitive rats, the striatum had the highest enkephalinase A activity, followed in decreasing order by the mesencephalon, hippocampus, pons, cortex, medulla oblongata, and hypothalamus. Thirty minutes after intraperitoneal administration of naloxone (0.3 mg/kg body weight) to morphine-sensitive rats, enkephalinase activity fell significantly in the hippocampus, striatum, and cortex, remained unchanged in the pons and medulla oblongata, and rose significantly in the mesencephalon and insignificantly in the hypothalamus; generally similar differences in enkephalinase activity from naloxone-untreated morphine-sensitive rats were recorded in the brain structures of morphine-insensitive rats given saline instead of naloxone.

Key Words: *morphine; sensitivity; enkephalinase; naloxone; nociception; analgesia*

Naloxone inhibits the analgesic effect of morphine [7] mediated by increased release of endogenous opioids [4]. However, individual animals, and up to 30% of rats in particular, are insensitive to morphine and to acupuncture [5,6]. Such rats were found to contain lowered levels of endogenous opioids [6], as were morphine-sensitive (MS) rats following administration of naloxone alone [3]. These findings may be accounted for either by reduced release of endogenous opioids or by heightened activity of the endopeptidases that inactivate them [3]. d-Phenylalanine, which inhibits enkephalinase A, wes shown to restore the analgesic effect of morphine in morphine-insensitive (MI) and acupuncture-insensitive animals [2,6].

It was therefore decided to compare enkephalinase A activity in different parts of the brain in MI and MS rats and in naloxone-treated and -untreated MS rats.

State Center of Addiction, Ministry of Health and Medical Industry of the Russian Federation, Moscow; *Department of Higher Nervous Activity, Moscow State University; **P. K. Anokhin Institute of Normal Physiology, Moscow (Presented by K. V. Sudakov, Member of the Russian Academy of Medical Sciences)

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