Impact of Ammonium Chloride in a Toxic Dose on the Bioelectrical Activity of Rat Brain

I. A. Zhuravin, E. F. Derkachev, A. K. Dobrylko, I. V. Mindukshev, and A. I. Krivchenko

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Ammonium has been shown to be toxic to the CNS when present in elevated concentrations in the body [4]. In rats, for example, an ammonium salt administered in a dose of >12 mmol/kg body weight leads to coma, convulsions and death. Judging by the clinical picture of acute poisoning, ammonium exerts differential toxic effects on different parts of the CNS. An indirect indication of this is intensified synthesis of tubulin (a possible marker of adaptation to ammonium) in the reticular formation (RF), hippocampus, and septum, but not elsewhere in the brain [10]. Glutamate levels are reported to be altered to unequal degrees in animals with portocaval anastomosis [6]. Antagonists of the NMDA receptor are known to afford protection against lethal ammonium poisoning [9]. However, the existing electrophysiological data on the magnitude of changes in the activity of various brain systems in ammonium poisoning are fragmentary and contradictory. In rats with implanted electrodes, paroxysmal bursts and high-voltage activity were observed in the EEG following administration of ammonium chloride in doses up to 7.4 mmol/kg [12]. Ammonium may impair postsynaptic inhibition and modify the EEG, and there is evidence that the ascending activating system of the RF does not contribute significantly to the EEG modification [11]. Ammonium has also been reported to alter the EEG without affecting RF neurons [7]. These findings, however, do not shed much light on the mechanisms of encephalopathy developing in ammonium poisoning.

Assuming that ammonium acts selectively on particular brain structures, we tried to evaluate the activity and characteristics of cortical regulation in brain structures of various hierarchical levels in rats with increased ammonium content in the body with a view to finding possible ways of compensating for the consequences of ammonium toxicity.

MATERIALS AND METHODS

As the test animals we used 28 male Wistar rats (200-250 g in weight) made immobile with D-tubocurarine (0.2 mg/kg intravenously) either under local anesthesia with artificial ventilation or under general Nembutal anesthesia (40 mg/kg intraperitoneally). The operation of trephining for placing electrodes was performed under ether anesthesia in a stereotaxic apparatus. Ball-type silver electrodes were placed on the dura mater at the site of the primary response to cutaneous electrical stimulation of the forepaws and also 4 mm caudal to that site. Glass microelectrodes with a diameter

Laboratory for Comparative Physiology and Pathology of the CNS and Laboratory for Comparative Physiology of Respiration, Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg (Presented by B. I. Tkachenko, Member of the Russian Academy of Medical Sciences)

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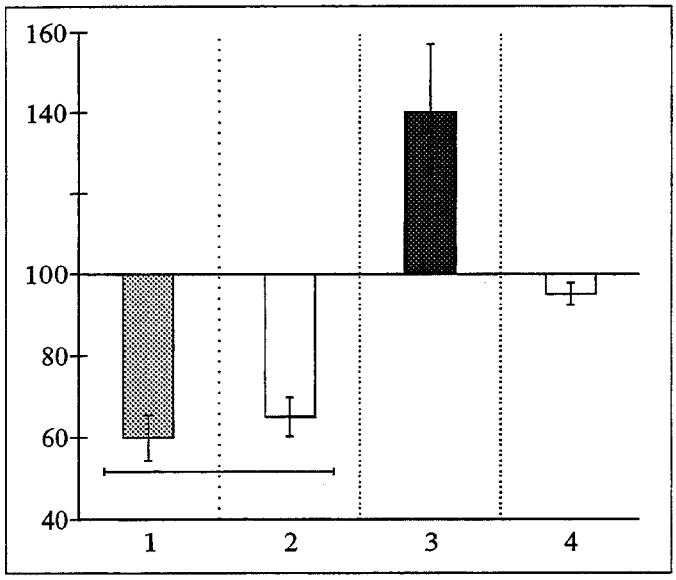

Fig. 1. Amplitudes of biopotentials 40 min after NH_4Cl injection of a curarized rat. *Ordinate*, 100 A/A_0, in %, where A_0 and A are the maximal amplitudes of total bioelectrical activity before and after NH_4Cl administration, respectively (averaged for all tests) in the first somatosensory (C1) and parietal (P) regions of the cortex, and in the reticular formation (RF) and amygdala (AM).

of about 10 μ at the tip and filled with sodium chloride solution (2 mol/liter) were inserted in subcortical structures. The coordinates for electrode placement were defined using an atlas of rat brain [5] and were verified by examining histological sections.

Evoked potentials (EP) were amplified with an UBF4-03 amplifier and recorded concurrently in various memory sections of an F37 analyzer. The right and left paws were stimulated alternately with 1.5-3 mA current pulses of 0.5 msec in duration at a frequency of 0.4 Hz. Total bioelectrical activity, led off from the same electrodes, was registered, together with respiratory frequency, on an automatic recorder and a magnetograph, followed by computer analysis. The results were treated statistically by standard methods.

Rats were poisoned with an ammonium chloride solution (2 mol/liter) injected intraperitoneally in a dose of 12 mmol/kg. Parameters of the acid-base balance in venous blood were analyzed in an IL-1306 pH/Blood Gas Analyzer and an IL-482 Co-Oximeter. As controls, six intact (nonoperated) rats were used; these were given an intraperitoneal injection of ammonium chloride and then observed for behavior.

RESULTS

The six intact rats given the toxic dose of NH_4Cl exhibited a clinical picture of poisoning with signs and symptoms described in the literature (hyperventilation, coma with clonic and tonic convulsions, appearance of froth at the nose, and death 10 min postinjection). In contrast, of the 28 rats made immobile by curare and artificially ventilated or under Nembutal anesthesia, only 3 died (11%), although the experiments lasted more than 4 h. Curare and Nembutal can therefore protect against the lethal effect of ammonium poisoning.

The greatest changes in the total activity of brain structures were observed toward the 40th minute after NH_4Cl injection. In curarized rats, the maximal amplitude of EP on the electrocorticogram (ECoG) decreased to 62.5±3.6% and 69.9±1.5% of its initial (preinjection) values in the C1 region and parietal cortex, respectively (Fig. 1), while increasing to 139.6±21.4% of its initial value in the RF and remaining almost unchanged (95.9±4.5% of its initial value) in the amygdala. Recovery of the biopotentials occurred 2.5-3 h postinjection. In rats under Nembutal anesthesia, biopotentials decreased both in the cortex and in the RF.

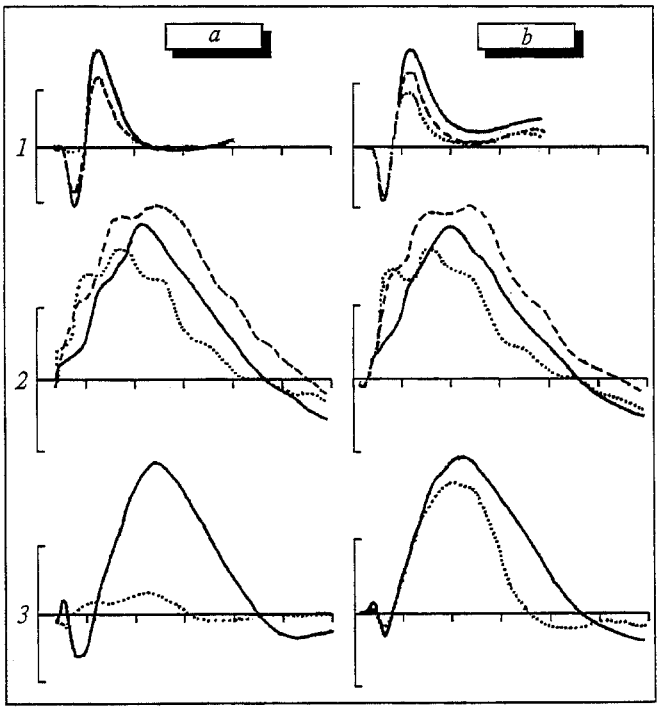

Fig. 2. Loss of specific evoked potentials (EP) in the reticular formation (RF) in curarized rats injected with NH_4Cl. Primary responses (PR) (*1*) in the first somatosensory cortical region (C1) of a rat at the site of the maximal amplitude and EP (*2*) in the RF elicited by electrical stimulation of the right (*a*) and left (*b*) forepaws. Solid line: initial state; dashed line: 40 min postinjection; *dotted line*: after removal of the area of the PR site in the C1 region of the left hemisphere. *3*) another rat which was not administered NH_4Cl and in which the area of the PR site had been removed in the C1 region of the left hemisphere. Calibration: 500 μV (cortex) and 100 μV (RF) on the ordinate and 20 msec on the abscissa.

Stimulation of both paws in NH_4Cl-injected curarized rats led to a slight decrease of the primary response in the cortex and to increases in both the amplitude of EP and in the area under their curve in the RF (Fig. 2, *1* and *2* - dashed lines). When the C1 zone was removed additionally in the left cortex, high-amplitude, though somewhat modified, EP were recorded from the RF in response to stimulation of the right as well as the left leg, despite the absence of a primary response (Fig. 2). Removal of this zone resulted, in most instances, in the generation of EP in response to stimulation of the paw whose representation in the cortex remained intact (Fig. 2) (see also [3]). This indicates that in the NH_4Cl-poisoned rats the impulse traffic descending from the cortex did not exert its specialized influence on the formation of EP in the RF. In the amygdala, by contrast, EP remained dependent on cortical integrity after the toxic dose of NH_4Cl.

The reduction in EP amplitudes on the ECoG could be due either to its desynchronization because of an afferent input from muscles and/or RF or to impaired postsynaptic inhibition [4, 11]. The activating system of the RF is believed to make only an insignificant contribution to EEG desynchronization in ammonium intoxication. We, however, observed a substantially elevated activity level in the RF. A similar pattern of shifts in the cortex and RF has been witnessed in hypoxia, hypercapnia, and in terminal states [1,2].

The RF may be activated as a result of altered acid-base balance (ABB) and the excitation of chemoreceptors. We noted two phases of deviation in ABB after NH_4Cl injection: an initial increase in pCO_2, that lowered to 7.1 the pH of the blood without altering its buffer capacity, was followed 15 min later by a fall in pCO_2 without any alteration in pH because of the concurrent twofold decrease in the buffer capacity of the blood ($HCO^-_3 + CO_2$). The effect of blood plasma acidification is usually attributed to the entry of ammonium, in the form of NH_3 ($NH^+_4 \rightleftharpoons NH_3 + H^+$; pH of the reaction = 9.2), into erythrocytes and other cells along a concentration gradient, i.e., there is no membrane barrier to ammonium in the gaseous form. The entry of NH_3 into cells leads to alkalinization of the intracellular medium and acidification of the extracellular medium. On the other hand, in ammonium intoxication the proportion of the curare-dependent flow to the RF also increases, as is indicated by the reduced RF activation when more curare is administered and by the protective effect of the latter. A part of the afferent flow (or of the efferent inhibitory flow) may be glutamate-dependent [9]. Possibly, excitation of both the curare-dependent and NMDA receptors results in the activation of a Ca^{2+}-dependent NO-producing enzyme with subsequent activation of soluble guanylate cyclase [8].

The present results warrant the conclusion that ammonium alters the state of different functional brain systems in a differential manner and increases the afferent input and the excitation of the RF in different ways; as a result, vital functions, in particular respiration, may be deranged and death may ensue.

REFERENCES

1. E. L. Golubeva, *Formation of the Central Mechanisms Regulating Respiration* [in Russian], Moscow (1971).
2. A. M. Gurvich, *Electrical Activity of the Dying and Reviving Brain* [in Russian], Leningrad (1966).
3. I. A. Zhuravin, B.F. Tolkunov, and A.K. Dobrylko, *Neirofiziologiya*, **15**, № 1, 42-49 (1983).
4. A. J. L. Cooper and F. Plum, *Physiol. Rev.*, **67**, № 2, 440-481 (1987).
5. E. Fifkova and J. Marsala, *Electrophysiological Methods in Biology* (1960), pp. 653-731.
6. J.-F. Giguere and R. F. Butterworth, *Neurochem. Res.*, **9**, № 3, 1309-1321 (1984).
7. E. Holm, K. F. Hamann, *et al.*, in: *Recent Progress in Ammonia Metabolism* (M. Imler and I. Szam, eds.) (1974), pp. 92-110.
8. G. J. Lovenstein and S. H. Snydes, *Cell*, **70**, 705-707 (1992).
9. G. Marcaida, V. Felipo, *et al.*, *FEBS Lett.*, **296**, № 1, 67-68 (1992).
10. M. D. Minana, V. Felipo, A. Quel, *et al.*, *Neurochem. Res.*, **14**, № 12, 1241-1243 (1989).
11. W. Raabe and S. Lin, *Exp. Neurol.*, **87**, № 3, 519-532 (1985).
12. C. Torda, *J. Pharmacol. Exp. Ther.*, **107**, 197-203 (1953).