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AMMONIA INTOXICATION AND ITS EFFECTS ON BRAIN AND BLOOD AMMONIA LEVELS*

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Abstract—Since the evidence for a relationship between convulsive disorders and brain and blood ammonia content was not conclusive, the changes in brain ammonia levels were compared with those occurring in the blood after acute ammonia intoxication, produced in rats by intraperitoneal injection of ammonium acetate in LD_{50} dose. Blood and brain ammonia estimations were performed on the survivors at 5 min intervals from 5 to 60 min after the injection. An unusual time-course of ammonia content with two peaks at 5 and 20 min after the injection of the toxic agent was found in the blood, whereas in the brain an increase in ammonia was observed until 25 min after intoxication. The level of ammonia then remained approximately unchanged until 50 min, after which a slow decrease was observed. Hyperexcitability and convulsions occurred in the rats in the first 20–25 min, while lethargy and coma were observed later. These results together with those obtained by other authors point to the possible existence of a biphasic action of ammonia on the central nervous system. The mechanism of action is discussed.

INTRODUCTION

IN PREVIOUS WORK[2, 3] we studied the blood ammonia levels in rats given ammonium acetate in LD_{50} doses and protected by a mixture of L-ornithine and L-aspartic acid, i.e. amino acids concerned in the biosynthesis of urea. At different times after the injection of the ammonium salt, the animals of the various groups showed different blood ammonia levels, e.g. 60 min after injection these levels were very much lower than at 15 min. However, in unprotected rats the fall of blood ammonia level did not parallel the alleviation of toxic symptomatology: coma, dispnea and other similar symptoms were still present 60 min after injection and even later, though at this time the ammonia content in the blood was about 60 per cent lower than at 15 min after intoxication. In other words, neurological symptoms showed no clear relationship with blood ammonia levels.

Since the evidence for a relationship between convulsive disorders and brain and blood ammonia content was not conclusive,[4–10] it appeared interesting to observe the time-course of ammonia concentration in brain and blood in ammonia poisoning, before, during and after the onset of convulsions, coma and related symptoms. There-

* A brief communication[1] of some results reported here was given at the 418th Meeting of the Biochemical Society (Cambridge, 12–13 July, 1962).

fore a convulsive and comatose symptomatology was provoked in rats by intraperitoneal injections of LD_{50} doses of ammonium acetate.

EXPERIMENTAL

About 250 male rats of the Long–Evans strain, weighing from 250 to 300 g, were used in the experiments. The animals were fasted overnight and injected intraperitoneally with 0·66 M phosphate buffer (pH = 7·4), and 1 hr later with ammonium acetate in the dose of 8·2 ± 0·8 m-moles/kg body weight (LD_{50}),[11] dissolved in the same buffer. The amount of solution administered was 1·0 ml/100 g body weight. In different groups of surviving rats heart blood was drawn at 5 min intervals until 60 min after the intoxication. Sodium oxalate was used as an anticoagulant.

Similarly treated rats were killed at the same intervals of time by immersion of the heads in liquid nitrogen. The brains were then removed, weighed, homogenized in phosphate buffer and diluted to a 15% concentration. Ammonia estimations were performed by the microdiffusion technique according to Seligson and Hirahara.[12] The ammonia contents of the brains and blood of rats receiving two injections of phosphate buffer served as controls (values referred to the 0 time in Table 1 and Fig. 1).

RESULTS

The means of the values of brain and blood ammonia levels at different times after intraperitoneal injection of an LD_{50} dose of ammonium acetate are recorded in Table 1.

TABLE 1. BRAIN AND BLOOD AMMONIA LEVELS IN RATS AFTER INTOXICATION WITH AMMONIUM ACETATE IN LD_{50} DOSES

(Each value is the mean of estimations performed on 7–10 rats. The figures of blood ammonia at 5, 10 and 20 min were obtained with 20–23 animals each.)

Time after intoxication	Blood ammonia (μmole/ml)		Brain ammonia (μmole/g wet weight)	
	Mean values ± S.D.†	Range	Mean values ± S.D.†	Range
0*	0·12 ± 0·02	0·09–0·17	4·65 ± 0.25	3·66–5·46
5	4·49 ± 0·68	3·45–5·69	6·68 ± 0·44	6·08–6·98
10	3·67 ± 0·59	2·66–5·38	8·29 ± 0·75	7·52–9·70
15	4·28 ± 1·26	2·57–5·68	9·55 ± 0·90	8·96–11·24
20	4·85 ± 0·78	3·49–5·99	10·21 ± 0·60	9·70–10·82
25	3·90 ± 0·67	3·21–5·08	10·35 ± 0·85	8·80–11·24
30	3·44 ± 0·84	2·60–4·73	10·75 ± 0·95	9·50–11·36
35	—	—	10·88 ± 0·80	10·34–11·56
40	—	—	10·76 ± 0·85	9·80–12·20
45	2·45 ± 0·75	0·97–4·52	10·73 ± 0·90	9·54–12·20
50	—	—	10·75 ± 0·80	8·68–12·26
60	1·57 ± 0·47	0·86–2·11	10·04 ± 0·95	7·20–9·80
120	0·23 ± 0·08	0·13–0·31	6·02 ± 0·52	4·98–7·01

* Control value.
† Standard deviation.

The time-course of ammonia concentration in blood and brain together with a Gauss-type curve constructed on the basis of mortality during the time are shown in Fig. 1.

A sharp increase of blood ammonia is evident in the first 5 min following the injection of the toxic agent, a slight decrease was then observed and a further increase

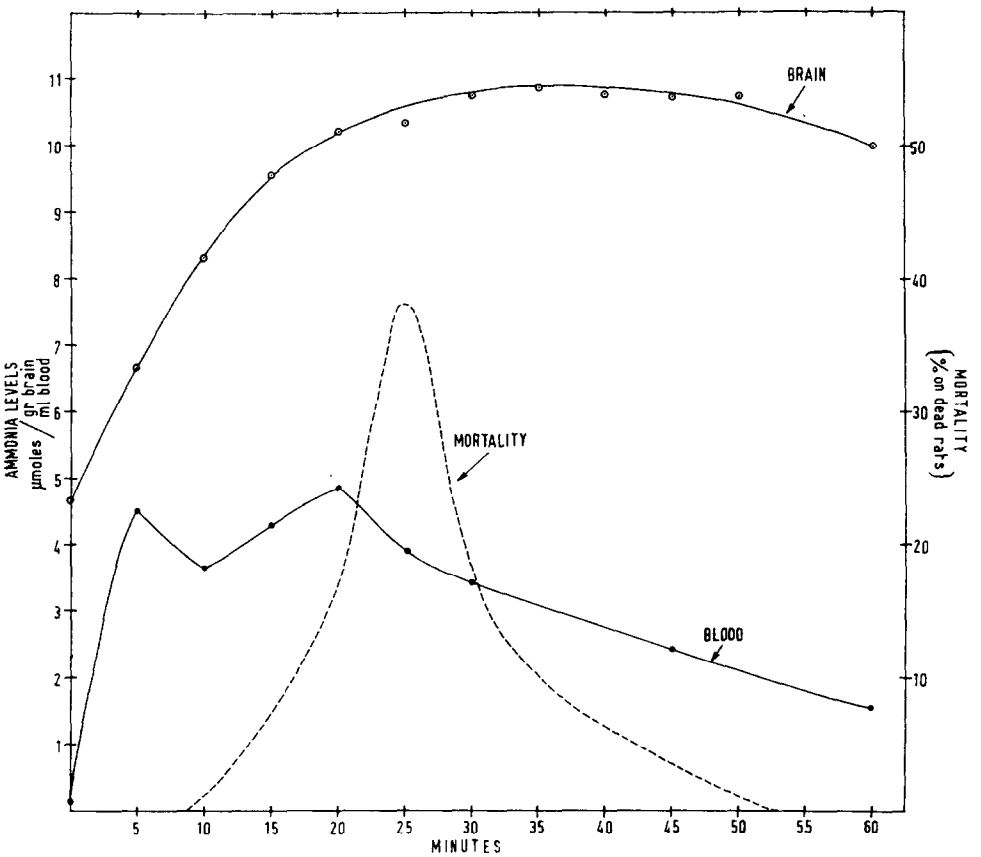

FIG. 1. Incidence of mortality and time-course of ammonia concentration in brain and blood in rats intraperitoneally injected with ammonium acetate in LD_{50} dose (the percentage of mortality at different times was calculated on the number of the dead rats).

occurred with a peak at the twentieth minute; a gradual fall of ammonia was then observed. This unusual and previously never observed behaviour was confirmed by statistical analysis. In fact, the P of the differences between ammonia values at 5 and 10 min and at 10 and 20 min after the intoxication were less than 0·001. Furthermore, the decrease of blood ammonia from the twentieth to the twenty-fifth minute was also significant (P was less than 0·05).

In the first 20–25 min a clear increase of ammonia was observed in the brain, until it reached a value of about 10·5 μmole/g wet weight. This level remained approximately unchanged until 50 min afterwards, when a certain decrease occurred. As shown in Table 1 the value at the 120th min was 6·02 μmole/g wet weight.

As far as the Gauss-type curve is concerned the highest incidence of mortality was around 25 min after the intoxication. It is noteworthy that this time does not completely agree with the highest level of the ammonia in blood—hyperexcitability and convulsions occurred only in the first 20 min. In the following period confusion, tremor, lethargy and coma were observed; this behaviour did parallel the high content of ammonia in the brain rather than that in the blood.

DISCUSSION

As far as the blood curve is concerned (see Fig. 1), a rapid intraperitoneal absorption of injected ammonia in the first 5 min occurs. This absorption occurs so quickly that at first the enzymic systems usually involved in the blockage of ammonia are ineffective. Though these systems eventually become effective, after 10 min they are no longer capable of counteracting the further rise of ammonia concentration, which increases until the twentieth minute. Later the absorption of ammonia is complete and its detoxication in organs and tissues occurs.

While our researches were in progress we came upon the findings obtained by Navazio and co-workers[13] in similar experiments. A rise in brain ammonia content was only evident in Navazio's experiments when the blood ammonia concentration increased to more than twenty times its basal value, that is 10 min after the injection of ammonium acetate. This fact was explained by assuming that brain ammonia is regulated by the blood–brain barrier; when high blood ammonia levels are reached, this regulatory mechanism is altered, and a sudden rise in brain ammonia is observed. However, our results show an immediate brain ammonia increase; moreover, in the first 5 min the ammonia curve of the brain has a slope which is nearly the same as that of the blood. Therefore, our findings would suggest that there is no critical blood ammonia concentration for its diffusion through the blood–brain barrier. Our results agree with those of Torda,[4] who observed a dramatic increase of brain ammonia even at 2 min after the intraperitoneal injection of ammonium chloride, and such an increase was also observed by Du Ruisseau and co-workers[14] 5 min after intraperitoneal administration of $LD_{99\cdot99}$ doses of ammonium acetate.

The fall of brain ammonia was observed by Navazio[13] 25 min after the intoxication, just as in the blood. Since in our experiments the brain ammonia values remained very high at a much later time, while blood ammonia was diminishing, it should be affirmed that brain ammonia levels are not maintained by blood ammonia; in addition Benitez and co-workers[8] found that in rats fluoroacetate poisoned ammonium ion, once released in the brain, does not escape rapidly into the blood stream nor is it quickly converted to glutamine. Therefore, it appears likely that the observed high ammonia levels in the brain might be related either to the failure of detoxifying mechanisms in the central nervous system (see Bessman[15]) or to the increased cerebral activity.

In agreement with the latter hypothesis some of the work of other authors may be mentioned. Torda[4] found that in rats injected with pentamethylentetrazole and

physostigmine salicylate (non-ammoniogenic convulsive drugs) an increase of brain ammonia was observed only after the onset of convulsions and not in the preconvulsive phase. Richter and Dawson[6] showed that an increase of cerebral activity induced by anoxia, electroshock and picrotoxin produced high brain ammonia levels. The finding of Benitez[8] that an increase of ammonia content in the brain occurred after the convulsions induced by pentamethylentetrazole, also supports the hypothesis of a release of ammonia related to an augmented cerebral activity; in addition Budaniova[16] described a considerable increase in the ammonia content of the brain in the course of experimental epilepsy. From these and many other researches and from the data of our experiments, it appears possible to conclude that an increase of cerebral ammonia occurs after the excitation of the central nervous system, whatever kind of stimulus is used.[17–20]

On the other hand it was demonstrated that a decrease of cerebral acetylcholine occurs after the intraperitoneal administration of many drugs that exhibit a depressive and anticonvulsant action on the central nervous system.[12] According to Torda[4] ammonia appears capable of inducing a decrease of cerebral acetylcholine, and such a decrease seems to be responsible for the depressive action of ammonia on the central nervous system.

In conclusion, from our experiments and in the light of those described by other authors, it is possible to draw some inferences. (1) Even though the mechanism of action of ammonia in the central nervous system is still poorly understood, it appears likely that ammonia is responsible for a biphasic action. At first it does induce a definite excitability (as shown by a greater sensibility to many kinds of stimulus) followed by convulsions; next a depressive action followed by lethargy and coma is more evident. (2) Since many agents are able to provoke hyperexcitability and convulsions, and others to provoke a depressive action on the central nervous system terminating in lethargy and coma, the existence of two different kinds of biochemical mechanisms separately influenced by the two types of drugs appears likely; the ammonia would be able to affect both these mechanisms.

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