CHANGES IN THE AMMONIA AND GLUTAMINE CONTENT OF THE BRAIN IN RATS AFTER ADMINISTRATION OF SODIUM BROMIDE AND CAFFEINE AND IN CARBON TETRACHLORIDE POISONING

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(Received February 28, 1959. Presented by Active Member AMN SSSR V. N. Chernigovskii)

In previous research [1, 2] carried out jointly with N. V. Veselkin, we found an increase in the content of ammonia and glutamine in the brain of animals during disturbances of the liver activity caused by surgical interference and by poisoning of the animal with CCl_4.

In the present investigation we studied the effect of sodium bromide and caffeine on the content of ammonia and glutamine in the brain of white rats during CCl_4 poisoning.

EXPERIMENTAL METHOD

We know from the work of the Pavlov school that, depending on the state of the nervous system and the type of higher nervous activity of an animal or man, the dose of sodium bromide necessary to produce a beneficial effect varies considerably. In our investigation we therefore used both high and low doses of sodium bromide: either a dose of 20-50 mg/100 g body weight subcutaneously 2-3 times a day for 2-5 days (large doses) or 1-5 mg/100 g body weight once a day for 24-30 days (small doses); Caffeine (sodium benzoate) was injected subcutaneously in a dose of 3-5 mg/100 g body weight once or twice a day for 2-5 days.

CCl_4 was injected intraperitoneally as a single dose of 0.1-0,2 mg/100 g body weight in 1 ml of mineral oil. The animals were sacrificed by immersion in liquid oxygen. The frozen brain was extracted and the content of ammonia and glutamine determined. Ammonia was estimated by vacuum distillation of a trichloroacetic centrifugate of the brain (in a Parnas apparatus); the amide nitrogen content of the glutamine was investigated in a separate sample of the same centrifugate by the increase in ammonia after acid hydrolysis for 11 minutes on a boiling water bath.

EXPERIMENTAL RESULTS

No increase in ammonia and glutamine was found in rats after administration of caffeine, for our results were the same as those which we obtained in normal rats in a state of increased physiological activity (Fig. 1, a). In these conditions the rats appeared healthy, with the exception that they were more restless and timid.

After the prolonged administration of small doses of sodium bromide to the rats, as might have been expected, we found no increase in the content of ammonia and glutamine in the brain (Fig. 1, b). Outwardly, the rats appeared perfectly healthy, very alert and mobile. After administration of large doses, however, the general condition and behavior of the rats was greatly modified. During the first few days of the injections the rats slept soundly at unusual times of day, and signs of bromide poisoning became apparent and developed in intensity. Under these circumstances the animals appeared very sluggish, their fur became ruffled and their skin pale or cyanotic. They often did not react even when grasped with forceps during freezing.

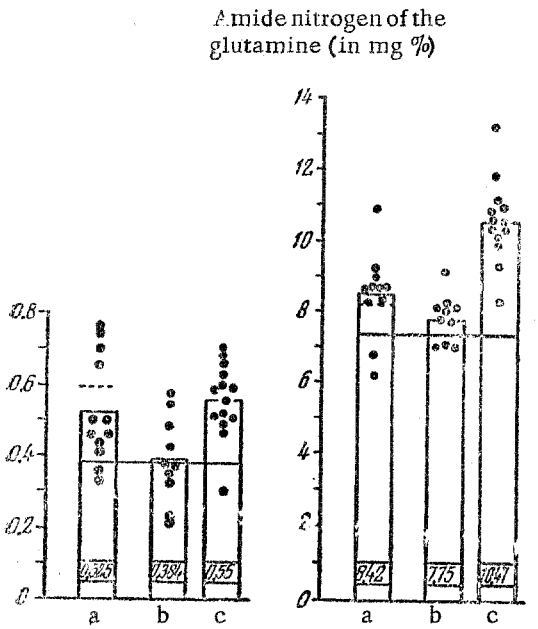

Fig. 1. Content of ammonia and glutamine in the brain of rats. a) After injection of caffeine; b) of sodium bromide (small doses); c) sodium bromide (large doses). Continuous line — a normal animal in a state of physiological rest. Broken line — normal animal in a state of physiological activity. Along the ordinate axis — NH_3 as nitrogen (in mg %).

The results obtained from analyses of the brain showed that the content of ammonia and glutamine in the brain of these rats was greatly increased, by as much as 45% in the case of ammonia and 43% for glutamine (Fig. 1, c).

From 1 to 2 hours after the injection of CCl_4, in spite of the very poor general condition of the rats, the content of ammonia and glutamine in their brain was within normal limits (Fig. 2, A). These results were in agreement with those in the literature [9], showing that obvious histological changes begin to appear in the liver 4 hours after exposure to the action of CCl_4.

Twenty four hours after a single injection of CCl_4 central necrosis developed in the liver, affecting from a quarter to a half of each lobule [10], and as a results of this the ammonia content of the liver was increased [2]. Evidently this was also the cause of the increase in the content of ammonia and glutamine in the brain tissue also, as we found in our previous work. According to the results of our present investigation, after 24 hours the content of ammonia in the brain tissue had increased on the average by 87% and that of glutamine by 83% (Fig. 2, B).

These findings confirm that the presence of extensive damage to the liver tissue may result ,in fact, in the appearance of excessive amounts of ammonia in the brain.

If CCl_4 was injected 2-5 days after administration of caffeine, the general condition of the rats both in the hours immediately after injection and after 24 hours was rather better than in control animals (given CCl_4 only), but as a rule their characteristic hypermobility was absent. If the results obtained were compared with the normal values for a resting state, the increase in the ammonia content was +61%. That an increase in the ammonia content of the brain did in fact result from the harmful effect of CCl_4 on the liver was supported by a simultaneous absolute increase in the glutamine content of +67% compared to the resting state or of +49% compared to a state of physiological activity (see Fig. 2, a).

If rats were injected with CCl_4 after receiving small doses of sodium bromide for a long time or large doses for a short time, the increase in the content of ammonia and glutamine in the brain was very high, namely +74% and +80% respectively with the small doses and +66% and +77% with the large doses (Fig. 2, b and c). The general behavior of the rats during the 36 hours after injection of CCl_4, especially when large doses of sodium bromide had been given, was very seriously affected, and they appeared much worse than those animals which received CCl_4 alone in parallel experiments. If all three groups of rats are compared under these circumstances (receiving CCl_4 alone or CCl_4 after caffeine or sodium bromide), the impression is created that caffeine supported the general condition of the rats but sodium bromide caused it to deteriorate considerably. If, however, attention is directed towards the rate of survival of the rats, it is apparent that among the rats which received CCl_4, after caffeine, cases occurred in which the animals died in spite of their outwardly better condition, whereas after receiving even large doses of sodium bromide and CCl_4, in spite of their serious condition, the animals did not die. This may be due to the lowering, by the action of sodium bromide, of the sensitivity of the central nervous system to the toxic agents (CCl_4 and ammonia). A similar phenomenon has been observed by other authors [4, 5]. In a state of prolonged drug-induced sleep, extremely high contents of ammonia and glutamine were found in the brain of the animals as the result of the disturbance of the urea-forming function of the liver under these conditions, although the animals did not die [5]. In our experiments, therefore, the administration of caffeine, and especially of sodium bromide, during poisoning with CCl_4 had no appreciable effect on the content of ammonia and glutamine in the brain. At the same time we consider of interest the fact that the content of ammonia and glutamine in the brain was increased when the rats were poisoned with large doses of sodium bromide. The

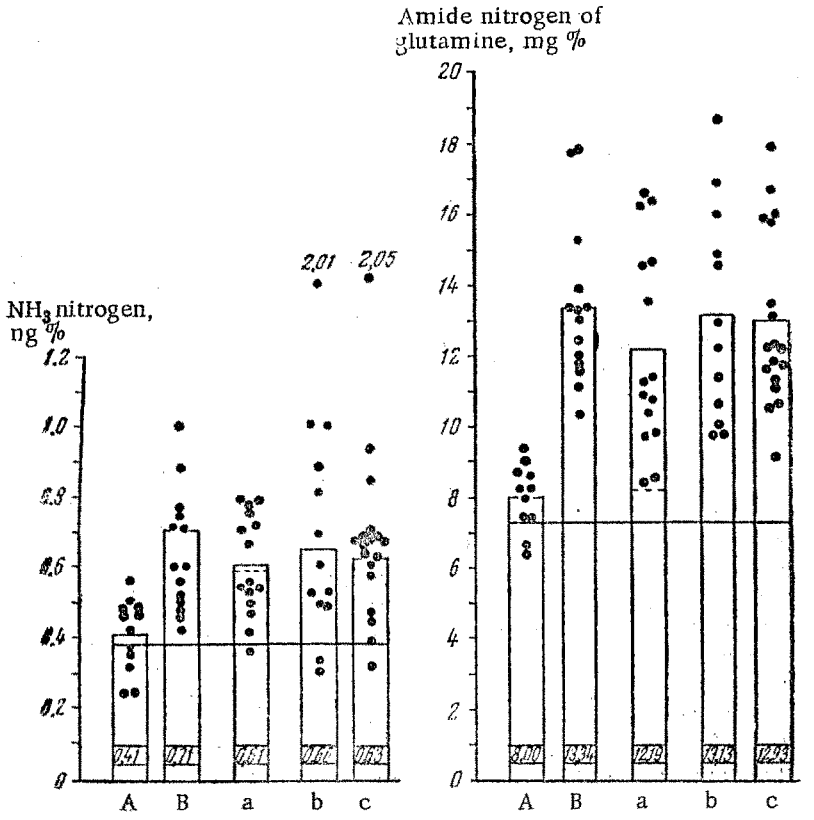

Fig. 2. Content of ammonia and glutamine in the brain of rats. A) 1-2 hours after injection of CCl_4 and B) 24 hours after injection of CCl_4; a) after injection of caffeine + CCl_4; b) of sodium bromide + CCl_4 (small doses); is c) of sodium bromide + CCl_4 (large doses). In columns D and c the means values of the NH_3 nitrogen were calculated without taking into consideration the results of two experiments in which a very high ammonia content was obtained (2.01 and 2.05 mg%).

manifestations of bromism are well known clinically — in persons receiving an overdose of bromide the speech is disturbed and impairment of consciousness, delirium and cachexia, etc., develop. This condition is reminiscent of the state of coma arising in hepatic failure, when low values are established for the oxygen consumption of the brain and for the absorption of ammonia by the brain from the blood (the blood of these patients often contains increased concentrations of ammonia [6, 7, 8]). A fall in the oxygen demand in these cases precedes the appearance of neurological signs. A lowering of the oxygen demand of the brain and liver tissue is also found in prolonged drug-induced sleep, when, as has previously been mentioned, the urea-forming function of the liver is disturbed and, in connection with this, the content of ammonia and glutamine in the brain is increased [3, 5].

The lowered oxygen consumption of the brain in coma is explained by some authors [6, 7, 8] by blocking of the formation of intermediate metabolites of the three-carbon acid oxidation cycle by ammonia, which also disturbs the normal course of the oxidative processes in the brain and leads to the development of a number of neurological disorders.

The sharp increase which we found in the content of glutamine in the brain during disturbance of liver function and after administration of large doses of sodium bromide may be indirect evidence in favor of this point of view, and may also help towards an understanding of the mechanism of the toxic action of large doses of bromides.

SUMMARY

The ammonia and glutamine content in the brain of rats is not increased by the action of caffeine and low doses of sodium bromide. Conversely, the animals' behavior and the content of ammonia and glutamine in the brain rises under the effect of large doses of sodium bromide.

Increase of the ammonia and glutamine content in the brain occurring in carbon tetrachloride poisoning does not drop noticeably under the effect of caffeine or sodium bromide. Investigations of rats' brain at different intervals after CCl_4 administration (in 1-2 hours and in 24 hours) show that the increase of the ammonia and glutamine content in the brain occurs only when marked injury of the liver tissue takes place, i.e., in 24 hours, notwithstanding the fact that the general condition of rats is particularly grave in 1-2 hours. This confirms the supposition that disturbances of the central nervous system occuring in diseases of the liver may be also due to the action of ammonia.

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* Original Russian pagination. See C. B. Translation.