EFFECT OF GLUTAMIC ACID ON CHOLINERGIC EXCITATION IN CENTRAL AND PERIPHERAL SYNAPSES

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One of the ways ammonia is detoxified in the animal organism is by combining with glutamic acid, with the formation of glutamine. This is why preliminary introduction of glutamic acid into the organism of rabbits, rats, and dogs prevents the excitation of the central nervous system caused by ammonium salts [3, 4, 14].

It is well known that in any excitation of the central nervous system the concentration of ammonia, which is liberated from glutamine and adenylic acid, increases in the brain tissue. For this reason various authors have tested the effect of glutamic acid on convulsions produced by administration not only of ammonium salts but also of other substances. In experiments on mice, rats, cats, rabbits, and monkeys, however, it was ineffective in preventing convulsions occurring under the influence of pentylenetetrazol (Corazol), and electroconvulsions [11]. An insignificant anticonvulsive effect was found when glutamic acid was tested in experiments on mice and rats subjected to the action of elevated oxygen tension [5]. Nevertheless, the clinical literature of the last fifteen years contains a large number of works devoted to the administration of glutamic acid in small epileptic seizures [1, 2, 13, etc.]. An antiepileptic effect, however, is seen only when glutamic acid is used in combination with anticonvulsive substances (phenobarbital, bromides, etc). Observations have been described in which agents with an anticonvulsive action failed to give a positive result in the treatment of patients with small epileptic seizures; on the other hand, the combination of these agents with glutamic acid proved effective. Many authors explain this therapeutic effect of glutamic acid by its capacity, established previously, to combine with ammonia.

In view of the hypothesis that epileptic seizures are cholinergic in nature [15, etc.], we undertook a study of the influence of glutamic acid on the effects of neostigmine methylsulfate, which promotes cholinergic excitation by depressing the enzymatic hydrolysis of acetylcholine.

METHODS AND RESULTS

In the first series of experiments we studied the effect of glutamic acid on neostigmine methylsulfate intoxication in white mice. The experiments were carried out on 100 adult male animals weighing 16 – 22 g. The substances under investigation were injected subcutaneously.

Neostigmine methylsulfate in a dose of 0.2 γ per g weight of mouse produced obvious muscular fasciculation in all the animals. When neostigmine methylsulfate was injected in a dose of 0.3 γ per g weight, convulsions occurred, terminating in death in 62.5% of the cases. When this difference had been established, the effect of preliminary injection of glutamic acid on the muscular fasciculation caused by neostigmine methylsulfate was ascertained: 10 mice were injected with glutamic acid (in a dose of 300 γ per g weight) 10 minutes before injection of neostigmine methylsulfate (in a dose of 0.2 γ per g weight). In this dose, glutamic acid by itself caused some limitation of activity in the mice. Ten control animals received no glutamic acid. Glutamic acid had essentially no effect on the muscular fasciculations: It delayed the time of appearance only insignificantly, slightly reduced their intensity, and had no effect on the duration.

In subsequent experiments, 40 mice were injected with glutamic acid (300 γ per g weight) 10 minutes before injection of neostigmine methylsulfate (0.3 γ per g weight); 40 control animals were injected with neostigmine methylsulfate only. For comparison, the effect of glutamic acid on pentylenetetrazol-induced convulsions in white mice was tested (according to published data, convulsions of this type are not in the main of cholinergic origin [15]). This series of experiments was carried out on 80 adult male animals weighing 16 – 22 g; 40 mice were injected with glutamic acid in the same dose as in the experiments with neostigmine methylsulfate, 10 minutes before injection of

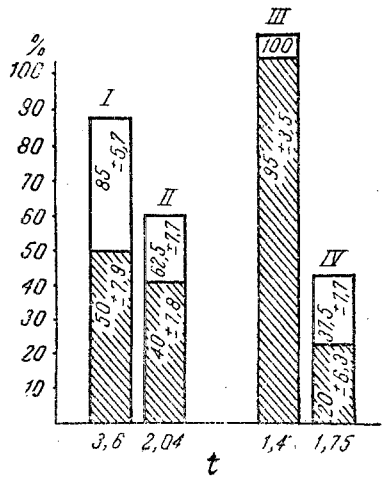

Fig. 1. Effect of glutamic acid on the course and outcome of intoxication with neostigmine methylsulfate and pentylenetetrazol. I, II) Experiments with neostigmine methylsulfate; III, IV) experiments with pentylenetetrazol.

pentylenetetrazol (80 γ per g weight). Control mice received only pentylenetetrazol (80 γ per g weight).

In experiments with neostigmine methylsulfate, preliminary administration of glutamic acid reduced the number of animals in which convulsions appeared (from 85% to 50%), and the number of animals that died (from 62.5% to 40%). The same dose of glutamic acid, tested in experiments with pentylenetetrazol, did not have a clear-cut anticonvulsive effect (100% of the animals showed convulsions in the control, and 95% in the experimental group), but reduced the number of deaths to some extent (from 37.5% to 20%). Thus the inhibiting effect of glutamic acid was more clearly apparent in convulsions caused by neostigmine methylsulfate, which are evidently cholinergic in nature.

Figure 1 shows the results of experiments with neostigmine methylsulfate and pentylenetetrazol. The number of mice in which convulsions developed (see Fig. 1, I and III), and the number of animals that died (see Fig. 1, II and IV) are expressed in percentages with the error calculated according to the formula:

$$m = \pm \sqrt{\frac{v(100 - v)}{n}},$$

where m is the error of the index; v is the index (percent of animals in which convulsions occurred, or percent of animals that died); and n is the number of animals.

The percent of animals in which convulsions appeared, and the percent of animals that died, in the control experiments, are indicated in Fig. 1 in the upper, unshaded part of the column; and the percent of mice in which convulsions occurred, and the percent of animals that died, after preliminary injection of glutamic acid, are indicated in the shaded portion of the column. Under the columns are shown the reliability criteria t, which are obtained by dividing the differences between the indices in percent by the root-mean-squares of the errors. Whereas the positive effect of glutamic acid on the course and outcome of neostigmine methylsulfate intoxication was statistically reliable (t = 3.6 and 2.04), the corresponding effect on the course and outcome of pentylenetetrazol intoxication was not reliable (t = 1.4 and 1.75); for the number of animals that we used in the experiment, the value of t for reliability should be no less than 2.02.

In a second series of experiments we studied the effect of glutamic acid, as well as that of glutamine and asparagine, on the contractile activity of the isolated rectus abdominis muscle of the frog.

We described this contractile activity in 1948 [6]. It was first found in the rectus abdominis muscle and the gastrocnemius muscle of the frog, and later also in the sartorius muscle [10]. Its occurrence is explained by enhancement of the effect of acetylcholine: In the isolated muscle acetylcholine is liberated spontaneously in amounts insufficient to cause contractions; but under the influence of neostigmine methylsulfate, which depresses cholinesterase and apparently also facilitates the response to acetylcholine, contractile

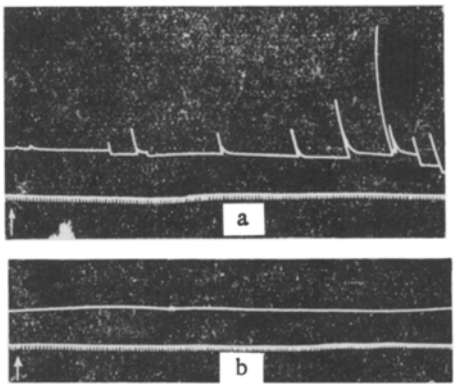

Fig. 2. Contractions of frog rectus abdominis muscle (a) under the influence of neostigmine methylsulfate (1: 250,000). The segment of the myogram corresponds to 30 minutes (during this time the kymograph was stopped repeatedly, for a total of 19 minutes). The arrows indicate the addition of neostigmine methylsulfate Time marker (5 seconds). Myogram of the same muscle (b) after the neostigmine methylsulfate had been repeatedly washed out and replaced with Ringer's solution containing glutamic acid (1: 1000), with subsequent addition of neostigmine methylsulfate (1: 250,000). Segment of myogram corresponds to 30 minutes (during this time the kymograph was repeatedly stopped, for a total of 20 minutes). Designations and time marker are the same as in Fig. 2a.

activity develops which is accordingly of a cholinergic nature [6]. The data obtained by us have been reproduced by N. A. Kozlova and M. Ya. Mikhel'son [9] and by G. A. Panosyan [10], who have adopted our interpretation of the neostigmine methylsulfate effect.

The isolated frog rectus abdominis muscle was subjected to the action of the substances being tested, 1-2 hours after isolation. Neostigmine methylsulfate was tested in concentrations of 1: 200,000–300,000. The contractions that appeared were recorded on a kymograph for 30-40 minutes, after which solutions of glutamic acid, glutamine, or asparagine were added. (The glutamic acid solutions were neutralized with sodium bicarbonate; solutions of the other amino acids gave a neutral reaction). In another version of the experiment, after recording of the neostigmine methylsulfate contractions the neostigmine methylsulfate was rinsed out and subsequently replaced by Ringer's solution containing glutamic acid,or asparagine, and after 30 minutes neostigmine methylsulfate was again added in the concentration tested earlier.

According to the data of these experiments, glutamic acid (in concentrations of 1: 10,000 and above) suppresses the neostigmine methylsulfate contractions of muscle (Fig. 2a and b). Glutamine and asparagine at higher concentrations (1: 500-1000) inhibit neostigmine methylsulfate contractions, but at lower concentrations (1 : 10,000 -20,000) they enhanced the neostigmine methylsulfate contractions in 4 of the 7 tests. Thus glutamic acid also showed a capacity to disturb cholinergic excitation in experiments on the isolated muscle. Although this effect was also demonstrated under the influence of glutamine and asparagine, glutamic acid produces it in solutions at least 5-10 times more dilute (since the molecular weights of glutamic acid, glutamine, and asparagine are almost exactly equal, the molar ratios are the same).

The experimental material obtained indicates that glutamic acid has the capacity to affect one of the steps in cholinergic excitation. This is displayed more distinctly in the neuromuscular junctions (suppression of contractile activity of muscles occurring under the influence of neostigmine methylsulfate); a less marked effect appears in the central synapses (anticonvulsive action). Presumably such an effect of glutamic acid can be explained by its action on the synthesis or on the binding of acetylcholine. The former idea is supported by the data of D. Nachmansohn and his collaborators [12], who have established that sufficiently high concentrations of glutamic acid are able to inhibit acetylcholine synthesis (in contrast to lower concentrations, which favor this synthesis). The second interpretation conflicts with results obtained on brain tissue sections [16]. According to the experiments of Tower and Elliott [16], in epilepsy, hypoxia, and poisoning with the convulsive poison methionine sulfoximine, the ability of brain tissue to bind acetylcholine is disturbed; glutamine and asparagine eliminate this disturbance, but glutamic acid does not have such an effect. It should be said, however, that the data of Tower and Elliott, obtained with sections, can not be unconditionally applied to processes occurring in the isolated muscle or in the intact organism. For this reason, we do not believe that the second interpretation can be rejected. It is possible that glutamic acid has an effect on the Krebs cycle, which is responsible not only for acetylcholine synthesis, but also for binding of acetylcholine. Finally, glutamic acid might play a role in our experiments as a substance that binds ammonia. Although the fact that ammonia is formed in convulsions cannot be denied, its importance in their production is apparently slight.

SUMMARY

It has been established experimentally that glutamic acid depresses the contractile activity of frog rectus abdominis muscle under the influence of neostigmine methylsulfate, and reduces the percentage of white mice suffering convulsions or death after neostigmine methylsulfate poisoning. The author suggests that these effects of glutamic acid may be explained by its action upon the synthesis or binding of acetylcholine.

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