EFFECT OF 4-AMINOPYRIDINE ON DEVELOPMENT OF EXPERIMENTAL BOTULINUM POISONING

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Botulinum toxin (BT) specifically blocks synaptic transmission of excitation in cholinergic synapses by acting on the presynaptic region and disturbing spontaneous quantal and induced release of acetylcholine from nerve terminals [6, 8-10]. Meanwhile secondary functional and biochemical changes develop in various organs and systems, giving rise to a complex disease pattern. Effective methods of pathogenetic treatment of botulism are not yet available.

It was accordingly interesting to study the effect of drugs facilitating transmitter release in cholinergic synapses on the development of botulinum poisoning, and an investigation with this aim is described below.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 20-22 g and albino rats weighing 200-230 g. Botulinum toxin (BT)of type C was injected intramuscularly into the hind limb: into mice in doses of 0.2, 0.14, 0.12, and 0.1 μg, into rats in a dose of 50 μg/kg body weight. In the experiments on mice the effectiveness of experimental treatment of BT poisoning was studied with respect to survival rate and duration of survival of the animals. The effectiveness of treatment was calculated by the use of a four-field table and by Fisher's exact test [2, 4]. Each group contained 20 mice. LD_{50} for BT was titrated on mice. The mean lethal doses were calculated by the probit analysis method of Miller and Tainter [1].

As the substance activating acetylcholine release in synapses, 4-aminopyridine (4-AP) (from Reanal, Hungary) was chosen; it was injected intraperitioneally in doses of 1, 2, and 5 mg/kg once or twice daily. The first injection of 4-AP in this group of experiments was given immediately after the injection of BT (prophylactic injection). In some experiments 4-AP began to be inejcted into mice in the same doses 24 h after injection of BT, against the background of local paralysis of the limb (therapeutic injection).

EXPERIMENTAL RESULTS

After injection of BT into the mice, the appearance of symptoms of the disease and the time of death depended on the injected dose of the toxin. BT, in a dose of 0.2 μg ("rigorous" model of poisoning) caused local paralysis of the limb, which after 1.5 days was replaced by generalized lesions. The paralysis progressed and led to death of the animals 4 days after injection of BT (Fig. 1A). The mean duration of survival was 2.5 ± 0.15 days. As will be clear from Fig. 1B, C, after injection of BT in a doses of 0.14 and 0.12 μg 100% of the animals died after 6-7 days; the duration of their survival was 4.0 ± 0.24 and 5.3 ± 0.12 days respectively. When the mice were poisoned with a dose of 0.1 μg approximately 40% of the animals survived for 10 days after injection of the toxin.

The survival rate, duration of survival, and LD_{50} of BT against the background of prophylactic injection of 4-AP were next studied. We know that 4-AP is a powerful activator of

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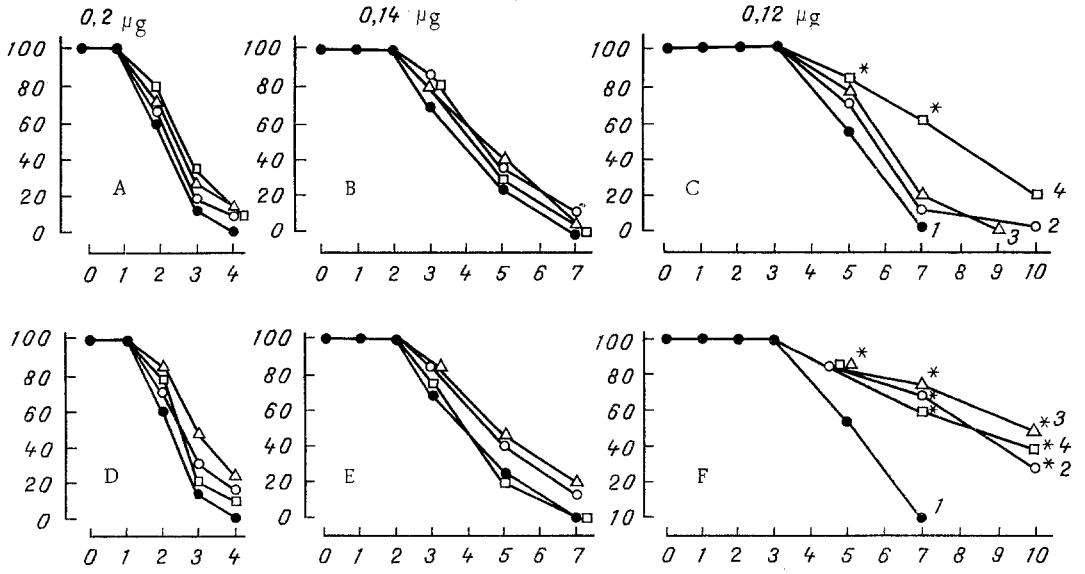

Fig. 1. Effect of prophylatic injection of 4-AP on survival rate of mice with botulinum poisoning. Abscissa, time of investigation (in days); ordinate, percentage of animals which survived. 1) BT, 2-4) BT + 4-AP in doses of 1, 2, and 5 mg/kg respectively. A, B, C) Injection of 4-AP once daily, D, E, F) the same, twice a day; *P < 0.05. Dose of BT shown above graph.

TABLE 1. Effect of 4-AP on Duration of Survival of Mice (in days) with Experimental Botulinum Poisoning

Experimental conditions	Dose of BT, μg		
	0,2	0,14	0,12
Prophylactic injection of 4-AP			
BT	2,50±0,15	4,00±0,24	5,3±0,12
Injection of 4-AP once daily			
BT + 4-AP(1 mg/kg)	2,65±0,18	4,60±0,3	6,1±0,25**
BT + 4-AP(2 mg/kg)	3,03±0,21*	4,68±0,27	6,3±0,23**
BT + 4-AP(5 mg/kg)	2,88±0,2	4,30±0,27	7,7±0,48***
Injection of 4-AP twice daily			
BT + 4-AP(1 mg/kg)	2,98±0,2	4,80±0,33*	8,6±0,46***
BT + 4-AP(2 mg/kg)	3,38±0,27*	5,20±0,36*	9,9±0,72***
BT + 4-AP(5 mg/kg)	2,85±0,21	4,23±0,3	9,1±0,87***
Therapeutic injection of 4-AP twice daily			
BT	2,45±0,15	3,85±0,27	4,98±0,24
BT + 4-AP(1 mg/kg)	2,73±0,21	4,33±0,33	5,55±0,45
BT + 4-AP(2 mg/kg)	3,25±0,27*	5,28±0,45**	5,86±0,44
BT + 4-AP(5 mg/kg)	2,68±0,18	4,60±0,32	5,87±0,43

Legend. *P < 0.05; **P < 0.01; ***P < 0.001.

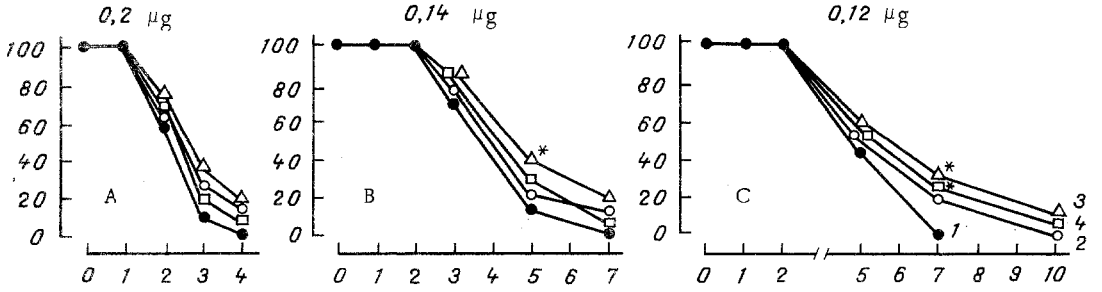

Fig. 2. Effect of therepeutic injection of 4-AP on survival rate of mice with botulinum poisoning (4-AP was injected twice daily). Legend as to Fig. 1.

transmitter release in various cholinergic synapses and can even reverse a neuromuscular block induced by BT [3, 7]. Injection of 4-AP increased the motor activity of the mice after 3-5 min without causing the appearance of seizures; the effect lasted 1.5-2 h, i.e., synaptic conduction was improved for this time. As Fig. 1, A-C shows, a single injection of 4-AP had a protective effect against botulinum poisoning, the intensity of which depended to a certain degree on the severity of the poisoning and the dose of the compound injected. 4-AP was most effective when injected in doses of 2 and 5 mg/kg.

Considering the short duration of action of 4-AP, its effectiveness was studied when it was injected twice daily. It will be clear from Fig. 1, D-F that administration of 4-AP in this way had a more marked therapeutic effect, especially on the "mild" model of botulinum poisoning (injection of 0.12 μg BT). When given twice a day, 4-AP was most effective in a dose of 2 mg/kg. When 4-AP was given in this way, two or three of 20 mice under observation, treated with 0.12 μg of BT, survived for 20 days (time of observation). Injection of 4-AP was accompanied by a regular increase in the mean duration of survival of the animals. The maximal effect was observed when 4-AP was injected twice a day in a dose of 2 mg/kg (Table 1).

The mean lethal doses of the toxin were increased by prophylactic administration of 4-AP from 4.7 ± 0.79 μg/kg in untreated animals to 6.7 ± 0.59 μg/kg when 4-AP was given in a dose of 1 mg/kg ($P < 0.05$), to 7 ± 0.54 μg/kg when 4-AP was given in a dose of 2 mg/kg ($P < 0.02$), and to 6.8 ± 0.65 μg/kg when 4-AP was injected in a dose of 5 mg/kg ($P < 0.05$). Therapeutic injection of the compound also gave an effect which, however, was much weaker than that of the prophylactic injection of 4-AP (Fig. 2; Table 1).

When LD_{50} of the toxin was determined after "therapeutic" injection of 4-AP an increase in the mean lethal doses of the toxin was observed only in the case of injection of 4-AP in a dose of 2 mg/kg.

The therapeutic effect of 4-AP also was observed on albino rats poisoned with lethal doses of BT. The rats developed total paralyses of the skeletal musculature, inability to move, and signs of respiratory failure 3 days after injection of the toxin. When 4-AP was injected in a dose of 5 mg/kg against this background, the animals were able to lift their heads after 5-6 min, and even to move about in circles. This effect lasted 1-1.5 h. Repeated injection of 4-AP again led to an improvement of the animals' general condition. If, however, 4-AP was injected 3 or 4 times at intervals of 2 h, after temporary improvement the animals died.

It can be concluded from analysis of these data that 4-AP, which facilitates synaptic transmission in synapses of different types, had a marked protective action against the development of experimental botulinum poisoning. In view of the results of these experiments, and also of clinical data [5] of the effectiveness of 4-AP during treatment of botulism, it can be concluded that this drug may be regarded as an additional remedy for the pathogenetic treatment of botulism.

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