ON THE MECHANISM OF THE ACTION OF BOTULINUS TYPE A TOXIN ON FROG HEART

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(Received December, 10, 1954. Submitted by Active Member of the Academy of Medical Sciences of the USSR V. N. Chernigovskii)

There are indications in the literature that botulinus toxin attacks the peripheral apparatus of the vagus nerves [6].

In the earlier work of one of us [1], the condition of the parasympathetic innervation of the heart of warm-blooded animals was studied at various periods of botulinus intoxication. It was found that botulinus type A toxin injures the central portions of the vagal innervation of the heart, for the most part. The interference with the parasympathetic innervation was evidenced by the disappearance of the inotropic effect of the vagus nerves on the heart at the beginning of the intoxication, and then of the chronotropic effect also.

Some clinicians [4] allow the possibility that botulinus toxin attacks not only the neural apparatus of the heart, but also the heart muscles themselves independently.

With regard to the attack on the heart of cold-blooded animals by botulinus toxin, the literature only contains information on the action of botulinus toxin on isolated frog heart [2, 3, 5].

Thus, S. G. Serebryanaya and G. L. Shkavera noted that botulinus toxin leads to a noticeable weakening, sometimes to a decreased frequency, of the contractions of isolated frog heart; atropinization of the heart averts the action of the toxin. The authors came to the conclusion that botulinus toxin has a vagotropic action, i.e., stimulates the parasympathetic nerve endings and as a result causes a weakening of heart action.

In this work we tried to discover whether the vagal influence on frog heart is disrupted at various periods of botulinus intoxication and to establish whether the effect of botulinus toxin on isolated frog heart is really connected with stimulation of the parasympathetic nerve endings or whether the toxin is able to act on the heart muscle independently.

EXPERIMENTAL METHOD

In the first series of experiments (27 experiments), the heart of frogs, both poisoned and not poisoned by means of botulinus toxin, isolated by Straub's method with the vago-sympathetic nerve trunks preserved intact, were used.

Since frogs are usually only slightly sensitive to this toxin, we used large doses – from 3000 to 6000 (mouse) MLD (1 MLD was 0.00005 mg) – to produce a marked intoxication (primarily development of paralyses). The toxin was introduced into the spinal lymph sac; after this, the animals were kept for 3-14 days at room temperature. Then the heart was removed and the vago-sympathetic trunk was stimulated with current from an induction coil (with an electromotive force of 2.5 V in the primary circuit).

In the second series of experiments (23 experiments), the effect of the toxin on isolated frog heart which had been atropinized beforehand was tested, and on the isolated ventricle as well.

A cannula was inserted in the ventricle, which was separated from the auricles. Platinum electrodes which were connected to the secondary winding of an induction coil were brought in contact with the ventricle muscle, and rhythmic stimuli, above threshold in strength, were administered with a frequency of 30 or 20 per minute.

Isolated frog heart is very stable to botulinus toxin. G. L. Shkavera and S. G. Serebryanaya used concentrations from 1:2000 to 1:400 [5]. We diluted the botulinus type A toxin in Ringer's solution and used it in concentrations of from $1 \cdot 10^{-3}$-$1.5 \cdot 10^{-3}$. The heart was perfused with the solution, containing 1-1.5 mg of toxin, for 5-30 minutes.

In order to eliminate the accumulation of metabolites, the toxin in the cannula was replaced with fresh portions from time to time.

EXPERIMENTAL RESULTS

At first we investigated the inhibitory effect of the vagus nerve on the heart 3-14 days after administering botulinus toxin to the frogs. The investigation showed that stimulation of the vago-sympathetic trunk produced the usual inhibitory effect on the heart until the fourth to fifth day after administration of the toxin. On the

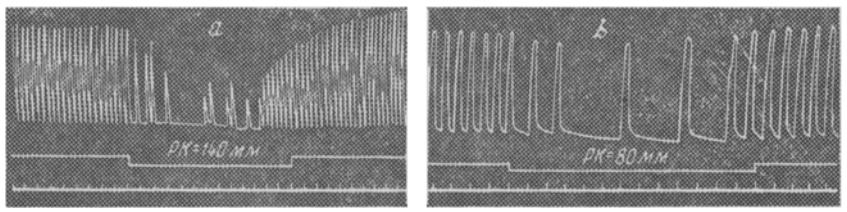

Fig. 1. Stimulation of the vago-sympathetic trunk of the isolated frog heart on the 5th (a) and on the 8th (b) day after poisoning with botulinus toxin. The time mark for curve (a) 2 seconds; for curve (b), 3 seconds; (d) is the distance between the induction coils.

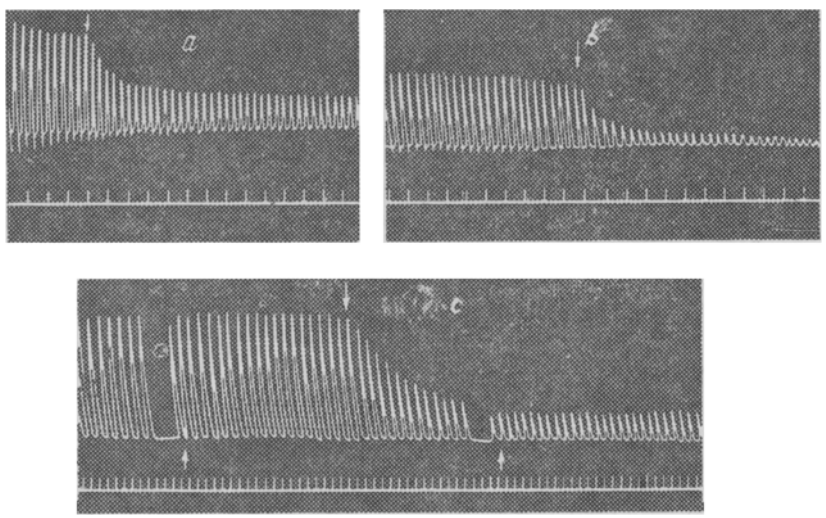

Fig. 2. Action of botulinus type A toxin on isolated frog heart (a) unatropinized, b) atropinized and on isolated heart ventricle (c).
The time marker for curves a and b is 3 seconds; marker for stimulation of the ventricle in curve c is every 2 seconds; (↓) – moment of toxin administration.

eight to thirteenth day we observed noticeable disruptions of the inhibitory action of the vagus nerve: during stimulation of this nerve, the negative inotropic action on the heart decreased (Fig. 1). In addition, the irritability of the vago-sympathetic trunk decreased. Thus, if a noticeable vagal effect on the heart was observed in the control animals when the distance between the induction coils was 120-140 mm, then in the poisoned animals it was observed at 60-100 mm. No disruptions whatsoever of the vagal action on the heart were observed in the control experiments on frogs which had not been poisoned and which were kept under the same conditions as the experimental frogs.

Thus, experiments on frogs poisoned with botulinus type A toxin showed that by the 8-14th day of intoxication, a noticeable disruption of the parasympathetic innervation of the heart developed.

Next we were posed with the question whether the parasympathetic innervation of the isolated heart of a frog which had not been poisoned is attacked during perfusion of the heart with massive doses of botulinus toxin (up to 1.5 mg per 1 ml).

Before and after introduction of the toxin into the heart, we checked the effect of stimulation of the vago-sympathetic trunk. We found that perfusion of the heart with botulinus toxin, accompanied by sharp hypodynamics, does not cause a noticeable disruption of the inhibitory effect of the vagus nerve on the heart. Negative ino- and chronotropic action was evidenced to the same extent as before introduction of the toxin into the heart. Consequently, notwithstanding the presence of signs that the isolated heart was poisoned with botulinus toxin, the latter, apparently, was not able to cause disruption of the vagus nerve endings, even though the intramural nervous system of the heart was washed with a solution containing a considerable concentration of toxin.

As regards disruption of vagal influences on the heart of a frog which had previously been poisoned with botulinus toxin, these disruptions, apparently, are connected primarily with attack on the central sections of the prasympathetic innervation of the heart. This conclusion agrees with the results of experiments on warm-blooded animals [1].

The next series of experiments was set up for the purpose of checking whether the inhibitory action of botulinus toxin on isolated frog heart is actually connected with stimulation of the vagus nerve endings. For this purpose, the heart was perfused with Ringer's solution containing atropine sulfate at a concentration of $1 \cdot 10^{-5}$-$1 \cdot 10^{-4}$ before the administration of toxin. 10-20 minutes later, the action of botulinus toxin in the concentrations given above was tested when it was introduced into the ventricle immediately after the removal of the atropine solution from the cannula or after preliminary washing of the heart with Ringer's solution.

The experiments which were carried out showed that elimination of the parasympathetic nerve endings by atropinization did not change the usual picture of the depressant action of botulinus toxin on isolated frog heart (Fig. 2).

In order to prove that atropine eliminated the parasympathetic nerve endings under the conditions of our experiments, we set up 4 control experiments in which the vago-sympathetic trunk of the atropinized heart was stimulated. Consequently, the action of botulinus toxin on isolated frog heart was evident independently on the elimination of preservation of the functioning of the parasympathetic nerve endings. Apparently, botulinus toxin in large doses attacks the heart muscle of frogs independently.

Experiments in which the toxin was introduced into an isolated ventricle which was contracting in response to independent stimulation of the muscle by an induced current, above threshold in strength, also indicated the direct effect of botulinus toxin on heart muscle.

As Fig. 2c shows, the contractions of an isolated ventricle weakened sharply during perfusion with botulinus toxin. It should be observed that the irritability of the ventricular muscle did not change after attack by the toxin: the stimulus threshold remained constant.

Control experiments with preliminary atropinization of the ventricle yielded the same results.

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