EFFECT OF ADENOSINETRIPHOSPHORIC ACID AND CYSTEINE ON THE CONTRACTION OF ISOLATED FROG HEART POISONED BY BOTULINUS TOXIN

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In our previous investigations [3] it was found that the depressant action of botulinus toxin on the isolated frog heart is dependent on the direct action of the toxin on heart muscle. In connection with this, the problem of the possible effect of botulinus toxin on the metabolic processes of energy-rich phosphoric combinations in the heart muscle interested us, since there are indications that in a number of cases the fall of the efficiency of the heart is connected with the disruption of the resynthesis of adenosinetriphosphoric acid and phosphocreatine [4, 8, 9]. It has been established, in particular, that the contractions of an isolated heart which has been brought into a state of hypoergy is strengthened considerably by the addition of sodium adenosinetriphosphate (ATP) solution to the perfusion fluid [1, 6].

However, if the hypoergy of the heart was evoked by the use of thiol poisons, which can inactivate enzymes containing sulfhydryl groups, then the ability of the heart to strengthen its contractions in the presence of ATP was spent. The amplitude of the cardiac contractions increased only when substances containing sulfhydryl groups, such as cysteine, were added to the perfusion fluid.

In the present work we set ourselves the problem of establishing whether the introduction of ATP and cysteine from without had any stimulating effect on the contraction of isolated frog heart poisoned with botulinus toxin.

EXPERIMENTAL METHODS

A frog heart was isolated by Straub's method and perfused for 5-15 minutes with Ringer's solution (1 ml). After the initial contractions were recorded, the perfusing fluid was replaced by a solution of botulinus type A toxin (120 observations). 1 ml of the solution contained 0.75-1.5 mg of toxin, corresponding to 15,000-30,000 MLD (1 MLD = 0.00005 mg). After 5-15 minutes, the ventricle was washed with Ringer's solution. If the heart contractions were stronger after this manipulation, botulinus toxin was introduced again (the effect of ATP and cysteine was tested, as a rule, only when the replacement of the toxin solution by Ringer's solution was not accompanied by an increased amplitude of cardiac contractions).

In 3 series of experiments (52 experiments), the action of ATP on a heart poisoned with botulinus toxin was studied. ATP was used at a dilution of $1 \cdot 10^{-5} - 1 \cdot 10^{-4}$ g/ml (1 ml of the fluid perfusing the heart contained correspondingly 0.01-0.1 mg of ATP).

In the 4th series (45 experiments), the action of cysteine and ATP together and of cysteine alone was studied. 1-2 drops of cysteine solution at a dilution of $1 \cdot 10^{-3}$ to $1 \cdot 10^{-4}$ g/ml was used [6].

In addition, in 7 experiments we watched the effect of the toxin on the isolated heart of a frog with botulinus intoxication. For this purpose, 0.15-0.3 mg of diluted toxin was injected into the spinal lymphatic sac of

the frog. The heart was removed after 2-8 days, when paralysis of the extremities developed, while in 2 frogs respiratory movements were absent.

All the substances used in the experiments were dissolved in Ringer's solution. To avoid accumulation of the metabolic products, the fluids perfusing the heart were replaced by fresh portions from time to time.

EXPERIMENTAL RESULTS

Botulinus type A toxin, administered to the isolated heart, caused a quick and considerable fall in the amplitude of the cardiac contractions. In 40 experiments the amplitude was only 25-15% of the original (Fig. 1, a), which agrees with the data of S. G. Serebryanaya and G. L. Shkaver [7]. The toxin had the same action on the isolated heart of a frog with preliminary botulinus intoxication.

In our experiments the following fact called attention to itself: the more the cardiac contractions were weakened by the use of the toxin, the less they were strengthened when the ventricle was perfused subsequently by Ringer's solution. Washing the heart with Ringer's solution after a second administration of toxin usually did not result in a noticeable increase in the amplitude of the cardiac contractions or remained totally ineffective.

In the first series of experiments we tested the action of ATP on the heart, whether the cardiac contractions, weakened by the effect of the toxin, were re-established by the subsequent washing of the ventricle with Ringer's fluid.

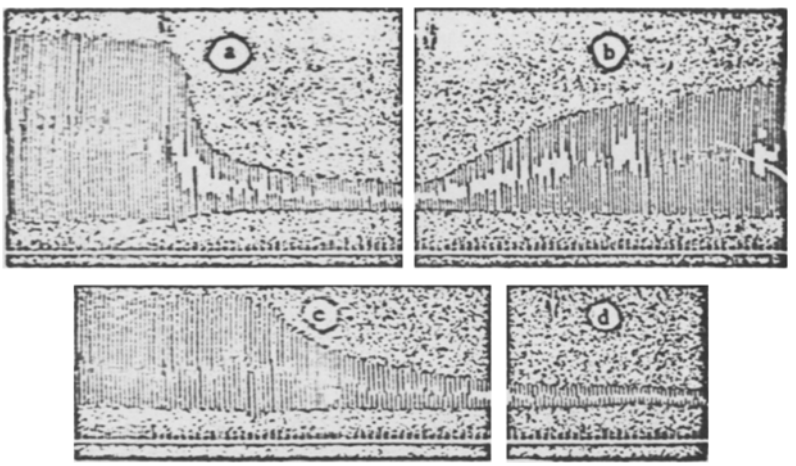

Fig. 1. Effect of ATP on the contractions of isolated frog heart poisoned with botulinus toxin.
a) Administration of botulinus toxin ($1 \cdot 10^{-3}$); b) administration of ATP ($1 \cdot 10^{-4}$)
c) administration of toxin after washing the ventricle with Ringer's solution;
d) second administration of ATP.
Curves (top to bottom): record of heart contractions; time marker (3 seconds);
▼ – moment of administration of the test substances into the heart.

Under these conditions, stimulation of cardiac activity typical of ATP was observed. The amplitude of the cardiac contractions increased 400% and more, not infrequently exceeding the level registered before the administration of botulinus toxin into the heart.

In the 2nd series of experiments we studied whether the stimulant action of ATP would persist if it was administered against a background of continuing perfusion of the heart with botulinus toxin. After persistent depression of the heart action due to the action of toxin, the heart was not washed with Ringer's solution and 0.1 ml of ATP solution diluted to $1 \cdot 10^{-3}$ to $1 \cdot 10^{-4}$ was added to the cannula. The volume of the perfusing fluid practically did not change under these conditions, while the ATP concentration remained the same as in the first series of experiments, i.e., $1 \cdot 10^{-4}$ to $1 \cdot 10^{-5}$ g/ml. The amplitude of the cardiac contractions increased noticeably

(by 200-400% and more) in the presence of ATP, in spite of the continuing perfusion of the ventricle with botulinus toxin (Fig. 1, b).

In the 3rd series of experiments we investigated whether the depressant action of botulinus toxin on the heart persists if it is introduced into the ventricular cavity simultaneously with ATP. Experiments showed that ATP administered with the toxin initially promoted the strengthening of the cardiac contractions and at first glance forestalled the inhibitory action of the toxin on the heart. However, after 2-4 minutes the amplitude of the contractions fell, and repeated administration of ATP remained without effect.

Essentially, in the first two series of experiments the strengthening of the action of the heart poisoned by botulinus toxin was observed only during the initial use of ATP also. If the heart was washed with Ringer's solution after being acted upon by ATP and then again perfused with toxin (Fig. 1, c), the stimulant action of ATP on the heart was not evoked by repeated administration of ATP (Fig. 1, d) or was very weakly evidenced.

Thus, ATP administered together with toxin or against a background of toxin activity temporarily blocked, but did not eliminate, the inhibitory effect of botulinus toxin on the heart. Consequently, ATP cannot be regarded as an antidote for botulinus toxin.

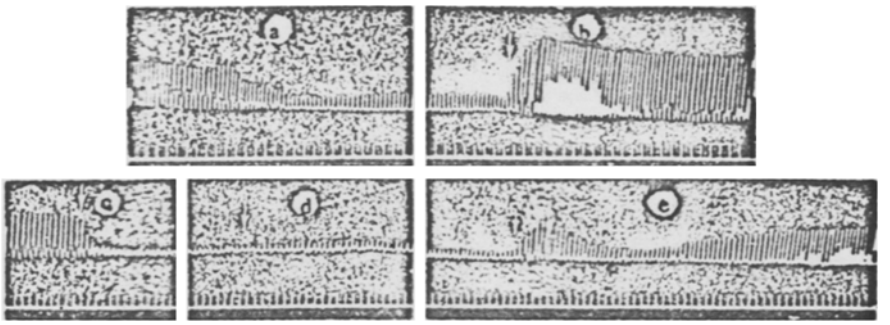

Fig. 2. Action of ATP in combination with cysteine on the contraction of isolated frog heart poisoned by botulinus toxin.
a) Repeated administration of ATP ($1 \cdot 10^{-4}$) together with botulinus toxin ($1 \cdot 10^{-3}$); b) addition of cysteine ($1 \cdot 10^{-3}$); c) administration of toxin after washing the ventricle with Ringer's solution; d) administration of cysteine; e) addition of ATP solution. Symbols are the same as in Fig. 1.

In the 21 experiments of the 4th series we tested the effect of cysteine on the contractions of the heart poisoned with toxin, when repeated application of ATP did not show any stimulant action (Fig. 2, a). Immediately after the addition of cystine, the amplitude of the cardiac contractions grew considerably (Fig. 2, b) and was kept at a high level for 3-4 minutes; in addition, we observed that, in the presence of cysteine alone, a heart poisoned with botulinus toxin began to contract more energetically. However, in 5 cases out of 19, such an effect was absent. However, if ATP solution was added to the cannula containing the mixture of cysteine and toxin, the amplitude of the cardiac contractions increased and reached the original level. In Fig. 2, d it is apparent that cysteine had no noticeable action against a background of toxin perfusion of the heart. It was enough to add ATP solution to the cannula for the cardiac contraction to increase considerably (Fig. 2, e), although ATP used before this in the absence of cysteine did not evoke the usual stimulation of the cardiac action (see Fig. 2, a).

It follows from the experiments of the 4th series that if the heart poisoned by toxin stops reacting to the separate administration of ATP and cysteine, their concurrent administration evokes anew a strengthening of the cardiac contractions. Thus, cysteine and ATP mutually maintain the ability to stimulate the work of the heart poisoned by botulinus toxin.

All the experiments described above indicate indirectly that botulinus toxin acts on the energy metabolism of the heart muscle, disrupting the processes of resynthesizing adenosinetriphosphoric acid. The fact that ATP added to the perfusion fluid of the heart poisoned by botulinus toxin facilitates the strengthening of the cardiac

contractions favors such a hypothesis. Repeated ATP administration is not accompanied by such action. Apparently, the initial administration of ATP stimulates the decomposition of the reserves of energy substances still stored in the heart muscle. Repeated administration of ATP does not have the same effect because the energy reserves of the heart affected by toxin are depleted, and resynthesis of them does not occur. We observed a similar picture of absence of the effect due to repeated administration of ATP when the resynthesis of energy-rich phosphorus compounds was deliberately disrupted by the elimination of the respiratory and glycolytic processes in the heart tissue [2].

On the other hand, on the basis of experiments with cysteine, the hypothesis is not excluded that botulinus toxin inhibits the activity of adenosinetriphosphatase by blocking its sulfhydryl groups and disrupts the utilization of ATP when in prolonged contact with heart tissue. It is possible that the strengthening of the cardiac contractions under the influence of cysteine, the bearer of sulfhydryl groups, depends on this when the ATP expands its ability to stimulate cardiac activity.

However, the question still remains unexplained which specific metabolic reactions are disrupted, which enzyme systems blocked by botulinus toxin. Nevertheless, the fact of the independent action of botulinus toxin on the cardiac muscle and its part in the energy metabolism of the heart is sufficiently interesting and requires further study.

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