PARTICIPATION OF THE NEURO-REFLEX MECHANISMS IN THE PATHOGENESIS OF DISEASE PRODUCED BY INTRODUCTION OF BOTULIN TOXIN IN THE CAROTID SINUS

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The object of the work was to clarify the possibility of reproducing a specific pathological process in conditions of temporary influence by toxin on the neuro-receptor apparatus.

EXPERIMENTAL METHODS

The carotid sinus of a rabbit isolated from the vessels was chosen as the test object. The absence of lesion of the nerve fibers of the sinus upon its isolation was ascertained by the blood pressure reaction upon stimulation of the sinus by an induction current. In the isolated sinus from 0.00025 to 0.005 mg of the toxin in a volume of 0.05-0.1 ml was introduced. Within 10 minutes the sinus with the toxin contained in it was removed. The operated surface was treated with streptocide and sutured. The animal was kept under observation for 10-12 days. Toxin of series A IEM-2 was used in the experiments. The assay of the toxin in our laboratory showed that 0.00025 mg dose of toxin produced death of mice within 24-48 hours. In certain cases the MLD for the mice equalled 0.00015 mg.

EXPERIMENTAL RESULTS

Upon introduction in the sinus of 0.00025 mg to 0.002 mg toxin in only a few animals (3 out of 11) was disease apparent. The introduction of 0.0025-0.005 mg of the toxin produced in 36 animals out of 56 lethal disease with a manifestation of rales, increased frequency of respiration and occasional lowering of the tonus of the skeletal muscles, chiefly of the neck and forelimbs. On intensification of these signs, death of the animals occurred in most cases within 3-4 days.

Upon dissection, hemorrhagic lesions of the upper sections of both lungs were established. The tissue of the lungs in external appearance was similar to the tissue of the liver or spleen. Upon plunging into water, the lungs sank. In some rabbits the stomach and small intestine were distended.

As is known, the reflex zone of the carotid sinus is characterized by the presence of a large number both of nerve fibers and very fine blood vessels with a thick network interwining the region of bifurcation of the common carotid artery. The abundance of fine blood vessels in this region and the complexity of the blood supply to the carotid ball, permit one to assume the possibility of escape from the sinus of a small amount of toxin. Determination of the escape dose of the toxin was carried out in the mice by assay of the residual toxin in the sinus at the moment of its removal. The experiments were conducted as follows. The sinus containing the toxin, was removed from the organism, placed in a porcelain dish and pulverized. Then 5 ml physiological solution was added. From this primary solution, various attenuations of the toxin were prepared, calculated so that

in 0.5 ml was contained 0.00025 mg, 0.0005 mg and 0.00075 mg of the toxin. In the other group of experiments for the assay attenuations containing in 0.5 ml solution 0.000125 mg, 0.00015 mg, 0.0002 mg, 0.00023 mg, 0.00027 mg and 0.00037 mg of the toxin were prepared. In the control experiments, the mice were given the same doses of the original solution. Estimation of the results was made by comparing the lethal doses of the toxin and the times of death of the mice after introduction of different concentrations of the control solution of the toxin and the toxin taken from the sinus.

20 experiments were carried out which may be broken down into three groups according to the results.

In the experiments of the first group, a significant difference in the lethal dosages of the toxin taken from the sinus, in comparison with the control solution was established. This indicated escape of the toxin from the sinus. All the rabbits in this group in which the sinus was extracted died after injecting the toxin into the sinus.

In the experiments of the second group, death of the mice after introducing the toxin taken from the sinus occurred earlier than with introduction of the same dose of the control solution of the toxin. In this group, 9 rabbits died and 2 remained alive.

In the experiments of the third group, death of the mice occurred simultaneously both upon introduction of the control solution of the toxin and the toxin taken from the sinus. In this group, 2 rabbits died and 4 survived.

The quicker death of the mice given the toxin taken from the sinus, as compared with that of the mice given the control solution of the toxin may be explained by the presence in this solution of a mixture of blood and some products of tissue decomposition. For the purpose of verifying this hypothesis, experiments were conducted differing from the former in that as control was used not only the original solution of the toxin, but toxin introduced into the sinus previously removed from the organism. The introduction of the toxin in the sinus was carried out in a dish in the same amount as in the experiment. Then the sinus was pulverized in physiological solution in the same way as in the experiment. From these primary solutions (experiment and control), uniform attenuations of the toxin were prepared and uniform volumes introduced into the mice at the same attenuations. The same volume of the original solution of the toxin was introduced. In some experiments, the investigated solution was subjected to centrifugalization. The results of these experiments were analogous to the findings in the previous experiments.

The times of death of the mice in which the experimental and control solutions of the toxin were introduced were uniform. Consequently, this method of determining the escape of toxin from the sinus can reflect only a significant loss of toxin, taking place in a small number of cases, related obviously to the inadequate, insufficiently accurate preparation of the sinus. It was difficult by the method of mouse assay, to establish the loss in small doses of the toxin, which, it seems, take place in most experiments.

However, for analysis of the findings and for elucidation of the sensitivity of the animals to botulin toxin on its application to the region of the carotid sinus and other receptor zones, the toxin was introduced in the vein of the ear, in the region of the carotid sinus, in the subcerebral cistern, in the internal carotid artery, under the skin and in the intestines of the animals.

Non-uniform sensitivity of the animals upon introduction fo botulin toxin in different zones was observed. Death of rabbits weighing 2.5-3 kg could be induced by application of 0.0003-0.0005 mg of the toxin in the region of the sinus and by introduction of 0.001-0.0015 mg in the vein of the ear, 0.002-0.0025 mg in the subcerebral cistern, under the skin, in the internal carotid artery, and 0.5 mg in the cavity of the small intestine.

The results of the experiments show that death of the animals upon application of botulin toxin to the region of the carotid sinus can in some cases be caused by an amount of the toxin smaller than that introduced in other parts of the organism. With this, the symptoms of disease and the character of the affection of the internal organs whatever the method of introduction of the toxin were similar. In some experiments, the animals died without the usual external signs of disease, but nevertheless hemorrhagic lesions of the lungs were seen.

After establishing high sensitivity of the sino-carotid zone to botulin toxin upon direct administration in the region of the sinus, we conducted further experiments to clear up the question of toxin escape from the sinus.

A nonlethal dose of toxin (0.00015 mg) was introduced in isolated sinuses of 6 rabbits and then 10 minutes later the sinus was removed and in this region, 0.000075 mg, 0.00015 mg and 0.000225 mg of the toxin in a volume of 0.1 ml were administered. In the control experiments in the isolated sinuses of 6 rabbits, 0.1 ml of

physiological solution was introduced and upon removal of the sinus the same amounts of the toxin were administered in this region as in the experiment rabbits.

In the control experiments, death of two animals was induced by administration in the region of the sinus of 0.000?25 mg of the toxin, while in the experimental group, death of two animals occurred upon introduction in the sinus of 0.00015 mg of the toxin with subsequent administration in the region of the sinus of an additional 0.00015 mg. The discrepancy in the lethal doses in the experimental and control groups of the rabbits suggests that escape of extremely small doses of toxin from the sinus (less than 1 MLD) may lead to death of the rabbit.

On analysis of the findings, it was found that the rabbits were more sensitive to botulin toxin than the mice. Thus, the MLD of this toxin for the mice on conversion to 1 g weight was equal to 0.015-0.016 γ, for the rabbit 0.0006-0.0004 γ, and with administration of the toxin in the sinus of the rabbits, the MLD was equal to 0.00012γ.

In a further series of experiments we induced with the help of novocain, anaesthesia of the sinus before introducing the toxin into it. It was noted than even slow introduction of novocain (1 ml a minute) produced a rise in blood pressure, increased frequency of respiration and a motor reaction. The anaesthesia occurred after introduction of 10-12 ml of a 2% novocain solution. After the onset of anaesthesia, the sinus was isolated and 0.005 mg of the toxin was introduced. Novocain anaesthesia did not prevent death of the animals. Thus, all 7 experimental rabbits died and 5 out of 6 control rabbits died. In other experiments 0.0025 mg of the toxin was introduced in the sinus. Of 4 experimental rabbits, 3 died and in the control group also 3 rabbits out of 4 died. Upon introduction of 0.00125 mg of the toxin, 3 out of 7 experimental rabbits died and of the 4 controls, 1 died.

The findings may be explained by the fact that novocain, by excluding vasoconstriction innervation, produces dilation of the blood vessels, which contributes to the escape of a larger amount of the toxin from the sinus, than in the absence of novocain administration. Penetration of even small amounts of botulin toxin from the sinus into the surrounding area leads to lethal disease of the rabbits.

In the subsequent experiments denervation of the carotid sinus was carried out by denudation of the common carotid artery in the region of its bifurcation. The lethal dose of toxin (0.005 mg) introduced in the denervated sinus, produced neither disease nor death of the animals (4 rabbits), while the control rabbits died (2 rabbits). It should be noted that such a method of denervation of the sinus is accompanied by traumatization of the very fine blood vessels of the region of bifurcation of the carotid artery; this complicates evaluation of the results, from the point of view of the participation of these vessels as possible channels of transport of the toxin from the sinus, in the genesis of the disease of the animal.

In this connection, we shought to exclude the nervous formations of the sino-carotid zone by means of a long-acting anesthetizing preparation, the composition of which included novocain, benzocaine, benzoline spirit and peach oil (the presence of oil is required for the slow absorption of the anesthetics included in the formula). One day previous to the experiment, 1 ml of 2% novocain solution was introduced in the rabbit in the unilateral or bilateral cervical region, then 1 ml of the long-acting preparation. On the following day, a lethal dose of the toxin was introduced in the isolated sinus. Upon removal of the sinus (10 minutes later) 0.5 ml of the anesthetic preparation was also administered in this region. In 10 rabbits out of 11 no signs of disease were noted. In the control experiments, 5 out of 8 rabbits died. Upon introduction into the sinus of twice the amount of toxin (0.01 mg) death was not prevented by the long-acting preparation.

The pathological effect of the toxin, when introduced in the carotid sinus, can be brought about in different ways. It is possible that disease of the animals was a result of the penetration of the toxin into the lungs through the nerves lying directly next to the bifurcation of the carotid artery and anatomically connected with it by numerous nerve fibers and vessels. Also possible is another path of inclusion of the nerve formations of the region of the sinus in the development of a pathological process – the neuro-reflex path.

These questions were clarified by resection of the various nerve formations adjacent to the region of the carotid sinus. Introduction of the toxin in the carotid sinus was carried out in some experiments after unilateral transverse section of all the cervical nerve trunks below the sinus, and in others above the sinus. Of 6 rabbits, on administration of the toxin in the sinus after transverse cut of the nerves below the sinus, 5 died. Of 4 rabbits in which the toxin was introduced in the sinus after transverse cut of the nerves above the sinus, only 1 died. In the control with 4 rabbits, three died.

SUMMARY

1. The receptor zone of the carotid sinus possesses exceptionally high sensitivity to botulin toxin in connection with which escape from the sinus of extremely small doses of toxin induces death of the animal.

2. Upon introduction of the toxin in carotid sinus, absence of its escape through the very fine vessels can not be guaranteed. In this connection, disease induced by temporary presence of the toxin in the sinus is the result, not only of the ten minute stimulation of the latter, but of the further prolonged stimulation of the nearest nerve formations by that minimum amount of toxin which penetrates into them from the sinus.

3. Hemorrhagic affections of the lungs, basic to the syndrome of botulin intoxication, appear whatever the method used for introducing the toxin in the organism, including introduction of botulin toxin in the sinus. It may be postulated that the pathogenesis of the affection of the lungs with botulism is associated with the influence of botulin toxin on the vascular-reflexogenic zone of the carotid sinus. Botulin toxin, belonging to the group of vascular-dystrophic poisons, by producing lesion of the vasomotor reflexogenic zones, thereby disturbs the neuro-reflex regulation of the tonus of the vessels, in particular in the lesser blood circulation, which leads to signs of congestion in the lungs.