EFFECT OF BOTULINUS TOXIN ON EXCITABILITY OF THE CAROTID SINUS AND THE VASOMOTOR CENTER OF IMMUNIZED AND NON-IMMUNIZED RABBITS

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(Received March 31, 1955. Presented by G. V. Vygodchikov, Member Acad. Med. Sci. USSR)

A considerable number of papers have been published over the past 10 years, dealing with the mechanism of action of various substances on visceral receptors, but the study of the effect of antigens on nervous reception is still only in the stage of collection of basic observations.

It has been shown [1] that the carotid sinus reflexogenic zone is highly sensitive to botulinus toxin. The present paper is devoted to a study of the effect of the toxin on excitability of carotid sinus receptors and on reflex excitability of the vasomotor center.

The objects of our research were: 1) to obtain data on the part played by neuroreflex mechanisms in the pathogenesis of the condition resulting from introduction of botulinus toxin into the carotid sinus, 2) to determine the effect of the toxin on the carotid sinus and on reflex excitability of the vasomotor center, and 3) to ascertain whether the reactions of immunized animals differ from those not immunized.

EXPERIMENTAL METHODS

The large blood vessels of the carotid sinus of rabbits were ligated in the region of the bifurcation of the carotid artery. The excitability of the sinus was assessed from the changes in blood pressure observed in response to stimulation with an induction current before and after introduction of toxin. In some experiments the excitability of the sinus was studied after previous section of the vagus and depressor nerves in the neck (below the sinus, on the same side as that on which toxin was introduced). The reflex excitability of the vasomotor center was derived from the changes in blood pressure following stimulation of the contralateral carotid sinus or of the proximal end of the severed cervical depressor nerve. Blood pressure was measured by means of a mercury manometer, and was registered on a kymograph. The strength of the stimulating current was above the threshold value.

Duration of stimulation was 5 seconds. Excitability was determined before introduction of toxin, and 5-10 minutes after its introduction into the carotid sinus.

EXPERIMENTAL RESULTS

Experiments on Non-immune Animals

The excitability of the carotid sinus was studied in 32 rabbits, and reflex excitability of the vasomotor center in 20 rabbits.

Blood pressure either did not vary or was slightly raised during introduction of toxin into the carotid sinus, and during the following 10 minutes, whereas the excitability of the sinus was considerably depressed, to an extent commensurate with the dose given. Introduction of 0.00025-0.0025 mg of toxin caused slight enhancement or depression of excitability, while 0.005 mg caused a fall in the direct excitability of the sinus and of the reflex excitability of the vasomotor center, as is shown by the diminution in the response of blood pressure to test stimulation.

In the control experiments 0.1 ml of physiological saline was introduced into the isolated sinus, and its excitability was tested as above; it was unaffected by introduction of saline.

The significance of neuroreflex mechanisms was further analyzed by means of experiments in which the excitability of the carotid sinus and the reflex excitability of the vasomotor center after introduction of toxin into the carotid sinus were measured against a background of local procaine anesthesia of the region of the sinus.

The sinus was isolated, and its excitability, and that of the vasomotor center were measured. The region of the sinus was then treated with 2% procaine, and excitability was again tested 10-15 minutes later. Having established the development of anesthesia, we introduced 0.005 mg of toxin, and again tested excitability. The sinus was removed 10 minutes later.

It was seen that anesthetization of the carotid sinus region abolished the usual depressive effect of toxin on excitability of the carotid sinus and the vasomotor center. This did not, however, prevent development of botulism and the death of the rabbits. In view of the short duration of action of procaine, this result might be explained as being a result of diffusion of toxin out of the sinus, after cessation of action of procaine, and so causing generalized disease.

In order to test this supposition, we repeated the experiment with a long-acting anesthetic, containing procaine, anesthesin, benzyl alcohol, and almond oil; the oil was included in order to achieve slow absorption of the anesthetics.

Experiments were done, in which we tested the excitability of the sinus during 7 days after a single application of local anesthetic; this period corresponds with the mean survival time of botulism in the rabbit. One ml of long acting anesthetic was introduced into the region of the sinus (percutaneously or around the exposed sinus). The contralateral sinus was not anesthetized; it served for measuring excitability of the intact sinus, as a control.

The excitability of the anesthetized sinus was determined 1, 2, 3, 5, and 7 days after application of the anesthetic (2 animals for each test); excitability was also tested for some animals before anesthetization.

As appears from the Table, the excitability of the anesthetized sinus is considerably depressed (absence of blood pressure response to stimulation with an induction current) one day after anesthetization. The excitability of the anesthetized sinus remains at a low level for 7 days of observation, whereas it is normal or in some cases slightly lowered, for the contralateral, control sinus.

The experiments on excitability of the carotid sinus after introduction of toxin, with preliminary anesthetization with a long-acting anesthetic, consisted of the following.

On the day before the experiment 1 ml of 2% procaine, followed by 1-1.5 ml of long-acting anesthetic, were introduced into the region of one or both carotid sinuses. The sinus was exposed on the following day, and its excitability was determined, after which it was isolated, and toxin was introduced into it. Excitability was again determined, after 5-10 minutes, and the sinus was removed 10 minutes after introduction of toxin.

It was observed that introduction of toxin into the sinus treated with long-acting anesthetic did not cause changes in excitability, and the animals did not develop botulism. Only one rabbit, of 11 used in this group of experiments, died, as compared with 5 of 8 control rabbits.

Experiments on Immunized Rabbits

In these experiments we took 10 rabbits which had been given a single immunizing dose of 1 ml of precipitated botulinus toxoid, followed 8-9 months later by a second 1 ml dose of toxoid. Non-immunized rabbits were used as controls in some of the experiments on immunized animals.

No morbid symptoms followed introduction of a lethal dose (0.005 mg) of toxin into the carotid sinus of immunized rabbits, whereas this dose regularly causes development of botulism, with a fatal outcome, in control rabbits.

Introduction of toxin into the sinus of immunized rabbits led to intensification of the depressor effect of stimulation of the proximal end of the severed depressor nerve, indicating enhanced excitability of the vasomotor center; enhanced excitability of the carotid sinus was also observed. Non-immunized rabbits showed depression of excitability of the carotid sinus and the vasomotor center.

TABLE

Excitability of the Carotid Sinus After a Single Application of Long-Acting Anesthetic Mixture to the Carotid Sinus Region

No. of rabbit	When tested	Test sinus (right) blood pressure, mm			Control sinus (left) blood pressure, mm		
		before stimulation	after stimulation	% change	before stimulation	after stimulation	% change
326	before anesthetization	110	74	— 32	110	96	— 12
	1 day later	76	84	0	70	60	— 14
1	before anesthetization	116	90	— 22	126	90	— 36
	1 day later	90	90	0	80	70	— 12
250	2 days later	106	106	0	114	90	— 21
	7 days later	90	90	0	90	76	— 15
434	2 days later	96	90	— 6	100	80	— 20
	7 days later	86	68	— 20	84	64	— 23
606	3 days later	116	114	0	104	92	— 12
	5 days later	90	84	— 6	80	60	— 20
277	3 days later	96	90	— 6	96	80	— 16
	5 days later	110	80	— 30	106	96	— 10

Our experiments show that introduction of a lethal dose of botulinus toxin into an isolated carotid sinus of non-immune rabbits causes their illness and death within 3-4 days, whereas the same dose of toxin causes no visible symptoms of disease in immunized animals.

Introduction of the toxin into the carotid sinus of both control and immunized rabbits causes changes in the direct excitability of the carotid sinus and of the reflex excitability of the vasomotor center.

These changes vary for immunized and non-immunized rabbits. Carotid sinus and vasomotor center reflex excitabilities are enhanced in the former, and depressed in the latter.

The changes in the functional state of the carotid sinus and the vasomotor center after introduction of botulinus toxin, and the protection from toxic effects given by local anesthetization of the carotid sinus region with a long-acting anesthetic mixture are evidence of the participation of neuro-reflex mechanisms in the pathogenesis of the fatal disease caused by introduction of toxin into the carotid sinus.

Death of non-immunized rabbits following presence of toxin in the carotid sinus for 10 minutes is not, of course due to its effect on blood pressure. It may be supposed that the cause of the fatal outcome of the condition is the primary change in excitability of carotid sinus receptors and in the reflex excitability of the vasomotor center, complicated by subsequent prolonged action on adjacent nerves of toxin which had diffused out of the sinus.

LITERATURE CITED

[1] M. M. Gromakovskaya, Byull. Eksptl. Biol. i Med., 1953, No. 4, 56-70.