MECHANISM OF DEVELOPMENT OF PARALYSIS IN CATS POISONED WITH TYPE A BOTULINUS TOXIN

V. V. Mikhailov

From the Chair of Pathological Physiology (Director: Corr. Member Acad. Med. Sci. Prof. A. D. Ado), Second I. V. Stalin Moscow Medical Institute.

(Received December 10, 1954. Presented by Member of the Acad. Med. Sci. USSR V. N. Chernigovsky)

Published work on the effect of poisoning cats with Type A Botulinus toxin is scanty and contradictory. Legroux and Levaditi [9] consider that cats cannot be poisoned by this material, even when 10,000 MLD of the toxin are injected subcutaneously. However, Ambache [2] found that retrobulbar injection, or introduction into the anterior chamber of the eye of cats, led, after 24-48 hours, to paralysis of the oculomotor nerves. Brooks [3] found in acute experiments on cats that introduction of 10^8 LD_{50} into the blood stream produced neuromuscular block.

Paralysis of motor innervation by Type A Botulinus toxin is regarded as being the result of its action on the function of the terminal nerve structures [3]. It is not thought that there is any significant interference with transmission in nerves or with the activity of nerve centers [6]. Nevertheless, histological studies reveal considerable changes in the structure of the large cells of the anterior horns of the spinal cord of animals poisoned with Botulinus toxin [6].

In view of the contradictory opinions on the mechanism of Type A Botulinus toxin poisoning in cats, we have initiated a study of the dynamics of the process, following intravenous and intramuscular introduction of the toxin, and of the mechanism of paralysis of motor innervation.

EXPERIMENTAL METHODS

Type A Botulinus toxin (1 mouse MLD = 0.00005 mg) was administered to cats intravenously or intramuscularly in doses of from 800 to 22,000 MLD (0.04-1.1 mg) per kg body weight. The inhibitory effect of stimulation of the vagus on the heart was then studied under conditions of acute experimentation, as well as the effect of stimulating the sciatic nerve on contraction of the flexor digitorum longus. In the experiments involving registration of contractions of the heart muscle artificial respiration was applied, the thorax was opened, and the heart was connected by a thread to the registering lever of a direct myograph. The vagus nerves were exposed in the neck, and were stimulated by an induction current storage battery (emf 2.5 volts). The strength of the stimulating current was slightly above the threshold value.

Registration of contractions of the flexor digitorum longus was effected by means of a thread connecting the tendon with a stylus; in some experiments the lever was connected with the third phalanx of the middle digit.

The sciatic nerve was exposed in the thigh, cut, and the peripheral portion was stimulated, using currents slightly greater than threshold strength.

Altogether, 59 experiments were performed.

EXPERIMENTAL RESULTS

Symptoms of poisoning were not observed with intravenous doses of 800 to 20000 mouse MLD per kg body weight. Levels of from 6000 to 20000 mouse MLD per kg body weight were regularly followed by severe intoxication, within 2-6 days, and by death of the animals (after various times, from 15 to 4 days, depending on the dose). Various trophic disorders were observed (ulcerative stomatitis, ulceration of the extremities). The cats became very inactive after intravenous injection of the toxin, but paralysis of the skeletal muscles was not observed at any time, including the agonal period.

In our next experiments we gave intramuscular injections of the toxin, at the same dosage levels as before, into the muscles of the lower third of the calf. We found that even quite small doses (800-1600 mouse MLD) caused paralysis of the injected limb, but the animals all survived. Similar effects were obtained with larger doses of toxin (over 10,000 mouse MLD per kg body wt.), but the animals died of botulism on the 4th to 6th day after the injection. In some experiments injection into the neck muscles was similarly followed by their paralysis, 2-3 days later.

It should be noted that paralysis developing as a result of injection of toxin into a muscle affects only the injected limb – the innervation of other organs was not affected.

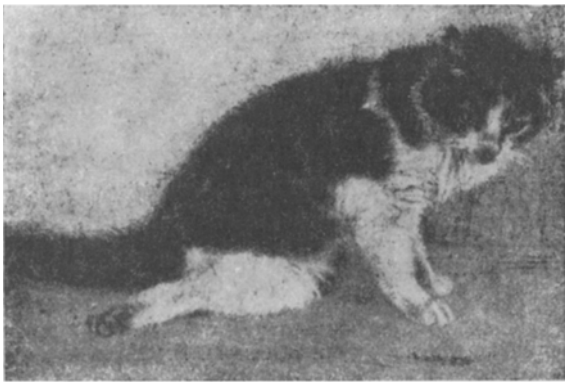

Fig. 1. Paralysis of the deafferented leg of a cat 6 days after intramuscular injection of Type A Botulinus toxin (10,000 mouse MLD per kg body wt.)

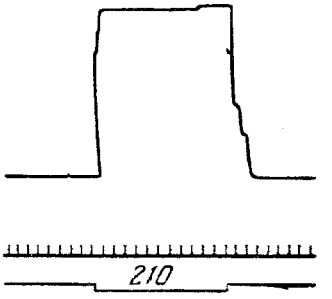

Fig. 2. Contraction of the flexor digitorum longus in response to stimulation of the sciatic nerve after intramuscular injection into the same limb of Type A Botulinus toxin (12,000 mouse MLD per kg body wt.). The posterior spinal roots had been cut on the same side 10 days before the experiment.

Thus, stimulation of the vagus nerves gave the usual inhibitory effect on contraction of the heart muscle, and stimulation of the sciatic nerve of the contralateral limb caused contraction of its muscles.

It could be supposed that the development of paralysis depended on the action of the toxin on the receptor apparatus of the muscles, stimulation of which by the toxin led, by a reflex action, to paralysis. In order to verify this supposition we repeated the experiments with cats in which the posterior spinal roots had been cut on one side, from L_3 to S_4, 10 to 14 days before injection of toxin into leg muscles of the same side. In this way we achieved total abolition of sensory perception in one of the hind limbs. The operated animals had an ataxic gait, characteristic of abolition of extero-and intero-ceptor innervation, which is evidence that the anterior roots were not damaged during the operation. Toxin was injected into the deafferentated limb of the operated cats, in the same dosages as before, and paralysis followed within 48-72 hours, as for cats with intact posterior root innervation (Fig. 1).

It follows that botulinus toxin acts on the muscles, irrespective of exclusion of afferent innervation. The effect of the toxin does not appear to depend on its action on sensory nerve terminations. A similar observation was made by Davis, Morgan, Wright, and Wright [5] in their researches on localized tetanus caused by intramuscular introduction of tetanus toxin.

Further experiments were done in order to determine whether botulinus toxin acts in the first place at the peripheral motor nerve terminations, or whether it attacks spinal motor centers. With this object, the sciatic nerve of limbs paralyzed by previous injection of toxin was stimulated; these acute experiments were done without anesthetic by the same methods as described for the foregoing experiments. It is evident from Fig. 2 that stimulation of the sciatic nerve of a paralyzed limb gives a strong contraction of the flexor digitorum longus.

It follows from our experimental data that botulinus toxin acts chiefly on motor neuron centers in the spinal cord, and not at the myoneural junction, as was supposed by Guyton and MacDonald [7] and others. In this respect our present results agree with our earlier ones on rabbits [1], in which we studied the mechanism whereby the parasympathetic innervation of the heart is affected by botulinus toxin. We intend in our subsequent researches to make a more detailed study of the effects of botulinus poisoning on the functions of the spinal cord.

LITERATURE CITED

[1] V. V. Mikhailov, Byull. Eksptl. Biol. i Med. 41, 1 (1955).

[2] N. Ambache, J. Physiol., 1951, Vol. 113, No. 1, pp. 1-17.

[3] V. B. Brooks, Science, 117, 3039, 334-335 (1953).

[4] A. S. V. Burgen, F. Dickens and L. J. Zatman, J. Physiol., 109, 1-2, 10-24 (1949).

[5] J. R. Davies, R. S. Morgan, E. A. Wright, and G. Payling Wright, Arch. intern. Physiol., 62, 248-263 (1954).

[6] J. R. Davis, R. S. Morgan, E. A. Wright, et. al., J. Physiol. 120, 4, pp 618-623 (1953).

[7] A. C. Guyton and M. A. MacDonald, Arch. Neurol. psychiat., Chicago, 57, 578-592 (1947).

[8] W. Kempner and B. Pollack, Dtsch. med. Wschr., Jg. 23, No. 32, p. 505 (1897).

[9] R. Legroux and J. Levaditi, cit. by A. R. Prevot and E. K. Brygoo, Inst. Pasteur, 85, 544-575 (1953).