Restoration of Function in Botulinum Paralysis by Experimental Nerve Regeneration.* (29146)

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Poisoning with *Clostridium botulinum* toxin produces an enduring neuromuscular block. When the toxin is applied locally to rat skeletal muscle the resulting paralysis lasts for several weeks to months. The neuromuscular block is characterized by lack of transmitter release from the motor nerve without apparent degenerative changes of the nerve terminals. The toxin does not interfere with impulse transmission in either nerve or muscle (1,2). Present evidence indicates that the toxin is irreversibly bound to cholinergic nerve terminals and that this binding interferes with the mechanism by which acetylcholine is released from the nerve(3-5).

Since the longstanding paralysis is apparently the result of fixation of the toxin to existing nerve endings it occurred to us that it might be possible to restore function by inducing the formation of new nerve terminals. Such regeneration is easily obtained following a crush of the nerve supplying the poisoned muscle.

Methods. In a series of experiments *Cl. botulinum* toxin type A (O,1-O,2 μg) was applied locally to the extensor digitorum longus muscle in both hindlegs of 22 adult rats. In 17 the peroneal nerve was crushed on one side by a forcep at a distance of about 5 mm from the muscle, and in another 5 animals the sciatic nerve was crushed high in the hip, at a distance of about 3 cm from the muscle. The crush was made either simultaneously with the poisoning or 2 days later. The other poisoned side served as control. All animals showed marked paralysis of both hindlimbs one day after poisoning. The function of the m.ext.dig. longus was examined daily thereafter by suspending the rats briefly by the tail. In the intact animal such a maneuver evokes the spreading reflex of the hind toes.

Results and discussion. Normal fanning of the toes was clearly absent bilaterally in all animals for several days after poisoning. In the poisoned leg with the distally crushed nerve toe fanning reappeared between 5 and 8 days after the operation. In the group of animals with the high crush the recovery of function was restored after 11-15 days. On the poisoned side with the intact nerve, however, function started to return only after 25-30 days.

Additional evidence for the difference in recovery rate was obtained by recording in a total of 8 animals the isometric twitch tension in the 2 ext. dig. longus muscles during indirect stimulation one to four weeks, successively, after the original unilateral nerve crush. In all rats thus examined it was possible to show increasing twitch tension of the muscle with the previously crushed nerve, while the muscle of the other leg showed nearly complete paralysis. The records obtained from test and control muscles one and three weeks after the nerve crush are shown in the Figure.

The present experiments have shown that motor nerve terminals formed after application of botulinum toxin are not functionally affected by the poison and that they can reestablish neuromuscular transmission. The closer to the muscle the nerve is injured the sooner will the regenerating terminals make effective contact. This approach has made it possible to shorten experimentally the otherwise prolonged paralysis following botulinum poisoning. However, the technique is unlikely to be of clinical value unless a more suitable means is found with which to stimulate regeneration of cholinergic nerve

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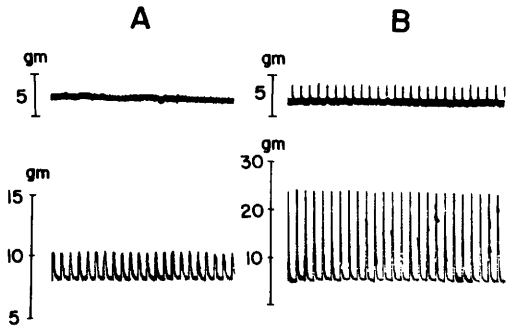

FIG. 1. Isometric twitch tension of m.ext.dig. longus of the rat one (upper traces) and three weeks (lower traces) after poisoning with botulinum toxin. In the right leg (records B) the nerve innervating the muscle was crushed at time of poisoning while the nerve to the muscle of the other leg (records A) was left intact. Electrical stimulation of sciatic nerve with single shocks every second.

terminals.

Summary. Results are presented showing that it is possible to restore neuromuscular transmission in botulinum paralyzed rat skeletal muscle by inducing the formation of new motor nerve terminals. The toxin, bound to the original endings, does not interfere with the regeneration following a crush of the motor nerve, and the new terminals can establish effective neuromuscular transmission after a few days. In muscles treated with the toxin alone the paralysis lasts for several weeks.

1. Bishop, G. H., Bronfenbrenner, J. J., *Am. J. Physiol.*, 1936, v117, 393.

2. Guyton, A. C., MacDonald, M. A., *Arch. Neurol. & Psychiat.*, 1947, v57, 578.

3. Lamanna, C., *Science*, 1959, v130, 763.

4. Thesleff, S., *J. Physiol.*, 1960, v151, 598.

5. Zacks, S. I., Metzger, J. F., Smith, C. W., Blumberg, J. M., *J. Neuropath. and Exp. Neurol.*, 1962, v21, 610.

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The Flexible Nature of the Amino Acid Pool in L Strain Fibroblasts.* (29147)

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Mammalian cells maintained or growing in tissue culture contain intracellular pools of free amino acids. The internal concentration of any amino acid is usually greater than the external concentration; this implies that a permeable barrier exists and that amino acids are actively transported into these cells. The essential amino acids in the pool must come from the external medium; however, non-essential amino acids are derived both from exogenous sources and endogenous synthesis. Regardless of the origin of the pooled amino acid, the internal level is dependent on the external concentration(1).

Previously, we showed that L strain fibroblasts have a greater capacity to accumulate non-essential than essential amino acids when both are present in the medium at approximately the same levels(2). This finding has led us to speculate that non-essential amino acids may interfere with the pooling of essential amino acids, so we proceeded to investigate the quantitative nature of the free amino acid pool during exponential growth when cells are cultured in a medium which omits the non-essential amino acids.

Materials and methods. A replicate series of L strain fibroblast cultures was set up in 100 milk dilution bottles in medium 199 supplemented with 0.5% bacto-peptone (199P). Ten milliliters of a suspension containing 2.5×10^5 L cells/ml was used as inoculum for each bottle. These cultures were incubated at 37°C in a stationary position for 36 hours, at which time the medium bathing the cells was decanted, and the cells

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