Cholinesterase Activity in Skeletal Muscle After Botulinum Toxin

Following section of the cholinergic nerves to various organs, including skeletal muscle, a decreased cholinesterase activity has been found (STRÖMBLAD[1]). It was therefore of interest to study whether blocking of the transmission in cholinergic nerves by botulinum toxin would also affect the cholinesterase activity.

The toxin has no acute effect on cholinesterase activity (BURGEN *et al.*[2]).

Methods. The effect of Botulinum toxin A on the weight and cholinesterase activity of the anterior tibial muscles of 12 months old female rats was studied. The toxin was infiltrated intramuscularly in four series of rats, each consisting of five animals. The doses used in the four series were: 0·005, 0·01, 0·02, and 0·04 μg of toxin into each leg. In addition, in one series of five rats, the sciatic nerve in the thigh was cut aseptically under ether and in another series of five rats both botulinum toxin 0·02 μg was given and the nerve cut. The control series consisted of 11 normal rats.

Fourteen days after the toxin had been given, or the nerve cut, the animal was killed in ether. The right and left anterior tibial muscles were removed, cleaned, washed in saline, weighed and homogenized together in 8 ml of Krebs' bicarbonate solution. The volume was finally adjusted to 10 ml with the buffer. 1·8 ml of the homogenate was brought into the main compartment of Warburg vessels. The substrates, in 0·2 ml in a side arm, were the chlorides of acetylcholine and metacholine in amounts giving final reaction concentrations of 0·06 and 0·2% respectively. The gas phase was 5% CO_2 in nitrogen. Readings were taken, after tipping of the substrates, every 5 min for 30 min. Corrections were made for changes in a thermobarometer and enzyme blank as well as for non-enzymic hydrolysis of the substrates. All estimations were made in duplicate.

Results. When the figures for rats given toxin (Table I) are compared with the corresponding ones in the series of normal rats, it is found that the injection caused a reduction in weight and in cholinesterase activity expressed on a whole muscle basis. If the figures for cholinesterase activity are expressed on a weight basis, there is no decrease. Obviously the atrophy has been of the same order as the decrease in cholinesterase activity in the muscle. Comparisons between the four series given toxin show that the smalles dose had less effect than the other ones. The three bigger doses have in general caused the same effects; there is no indication of a greater effect when the dose was increased. The highest dose given seemed to be also the highest tolerable. The rats given 0·04 μg in each leg were, at the end of the experimental period, in a very bad general condition.

After section of the sciatic nerve, there was an atrophy of the muscles and a decreased cholinesterase activity.

Tab. I. Weight and cholinesterase activity in anterior tibial muscles

The figures are given with ± S. E. Each value in the experimental series was compared with the corresponding one in the series of normals. The significance of a difference is given by $^a = P < 0{\cdot}05$, $^b = P < 0{\cdot}01$, and $^c = P < 0{\cdot}001$. Ach = acetylcholine as substrate. Mch = methacholine as substrate.

Procedure	Weight in mg	μl CO_2/30 min/2 muscles		μl CO_2/30 min/g	
		Ach	Mch	Ach	Mch
Normal muscle ($n = 11$ for figures under weight and Ach. $n = 8$ for figures under Mch)	850 ± 45	339 ± 24	186 ± 24	407 ± 34	222 ± 38
0·005 μg of botulinum toxin A in each leg 14 days previously ($n = 5$)	660 ± 39^b	268 ± 40	132 ± 23	395 ± 36	196 ± 23
0·1 μg of botulinum toxin A in each leg 14 days previously ($n = 5$)	370 ± 21^c	149 ± 23^c	74 ± 9^b	399 ± 51	202 ± 28
0·02 μg of botulinum toxin A in each leg 14 days previously ($n = 5$)	333 ± 29^c	211 ± 35^b	92 ± 16^b	632 ± 79^a	272 ± 33
0·04 μg of botulinum toxin A in each leg 14 days previously ($n = 5$)	480 ± 20^c	188 ± 23^c	87 ± 10^b	399 ± 62	182 ± 27
Section of sciatic nerve 14 days previously ($n = 5$)	514 ± 13^c	103 ± 11^c	48 ± 7^c	200 ± 22^c	92 ± 13^b

Tab. II. Weight and cholinesterase activity in anterior tibial muscle after injection of botulinum toxin, section of the sciatic nerve, or after a combination of these two procedures.

The figures after nerve section and after toxin plus nerve section are compared with those after toxin only. The significance of a difference is given by $^a = P\ 0{\cdot}05$, $^b = P\ 0{\cdot}01$, and $^c = P\ 0{\cdot}001$.

Procedure	Weight in mg	μl CO_2/30 min/2 muscles		μl CO_2/30 min/g	
		Ach	Mch	Ach	Mch
0·02 μg of botulinum toxin A in each leg 14 days previously ($n = 5$)	333 ± 29	211 ± 35	92 ± 16	632 ± 79	272 ± 33
Section of sciatic nerve 14 days previously ($n = 5$)	514 ± 13^c	103 ± 11^a	48 ± 7^a	200 ± 22^c	92 ± 13^c
0·02 μg of botulinum toxin A plus section of sciatic nerve 14 days previously ($n = 5$)	353 ± 8	87 ± 7^b	45 ± 10^a	246 ± 21^b	127 ± 26^b

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[3] R. COUTEAUX, Exp. Cell. Res., Suppl. 5, 294 (1958).

[4] A. J. BULLER, J. C. ECCLES, and ROSAMOND M. ECCLES, J. Physiol. *150*, 417 (1960).

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The atrophy was less, but the decrease in cholinesterase more pronounced, than after a big dose of botulinum toxin (Table II). In contrast to botulinum toxin, denervation caused a decreased enzyme activity also when this was expressed on a weight basis (Table I). In the series of rats both denervated and given toxin, the atrophy was greater than after only denervation, while the enzyme activity was comparable to that after denervation (Table II).

Discussion. The results here reported show that two weeks after the injection of botulinum toxin into the anterior tibial muscle there is a decreased cholinesterase activity. The decrease is, however, less than that obtained after denervation of the muscle. The difference can not be explained by loss of enzyme held in the degenerating nerve terminals, since these are known to contain extremely small amounts of enzyme compared with the end-plate (COUTEAUX[3]). On denervation of the muscle in addition to toxin, the decrease was similar to that after only denervation. The toxin therefore does not seem to have any effect in itself on the enzyme, which can explain why botulinum toxin does not have the same effect as section of the nerves. Thus there seems to be some influence of the nerve on the muscle not blocked by botulinum toxin. In this connexion, it is of interest to compare the suggestion put forward that the nerve has some influence on the muscle apart from that caused by release of acetylcholine (BULLER, ECCLES and ECCLES[4]). It may also be recalled that long-continued blocking of the effect of the cholinergic nerves on salivary glands by an atropine-like agent does not cause changes in the cholinesterase activity of the gland (STRÖMBLAD[5]).

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Zusammenfassung

Bei Zufuhr von Botulinum-A-Toxin in den Muskel Tibialis anterior der Ratte war die Cholinesteraseaktivität nach zwei Wochen herabgesetzt, jedoch weniger ausgesprochen als nach zweiwöchiger Denervierung des Muskels.

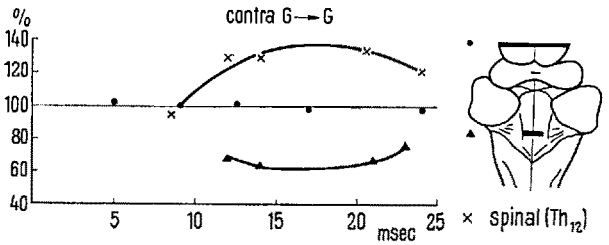
Crossed Reflex Actions Evoked by High Threshold Muscle Afferents

In spinal cats impulses in group II (12–4 μ) and III (4–1 μ) muscle afferents evoke in ipsilateral motoneurones the actions of the general flexion reflex, i.e. widespread excitation to flexor and inhibition to extensor motoneurones. It has, however, been observed that under some conditions these afferents give inhibitory effects to flexors and sometimes also excitatory to extensors[1]. The present investigation is concerned with contralateral effects from these afferents. Unanaesthetized decerebrate cats with both hindlimbs denervated were used. The effect of single conditioning volleys evoked at different stimulus strengths was examined on contralateral monosynaptic test reflexes.

In acute spinal preparations, no group I effects were observed but it has been confirmed that there are usually pronounced effects by volleys in high threshold muscle afferents[2]. Extensors as well as flexors received either inhibition or excitation but in each experiment the same effect was usually exerted on motor nuclei of synergic muscles (extensors or flexors). These effects occurred in combinations which included all four possible variations: (1) generalized excitation or (2) inhibition or (3) excitation to flexors and inhibition to extensors or (4) finally the opposite with inhibition to flexors and excitation to extensors. The last variation was the most frequent one, and would be expected, according to Sherrington's scheme of double reciprocal innervation. In one case a reversal from inhibition to excitation occurred in flexor motor nuclei during the course of the experiment. As group II and III volleys can evoke different crossed effects in different animals under apparently identical conditions, there is, both to contralateral flexor and extensor motoneurones, one inhibitory and one excitatory pathway. Presumably the balance between these channels is labile and easily influenced, whereas on the ipsilateral side the excitatory path to flexor and the inhibitory path to extensor motor nuclei are very predominant in the spinal state.

In the decerebrate cat, the ipsilateral synaptic actions by group II and III muscle afferents may be completely suppressed[3]. This is due to a tonic inhibitory control from the brain stem of interneurones mediating there effects. It has now been found that a similar control is exerted on the interneurones mediating crossed group II and III effects, as is evidenced by the finding that in the decerebrate state stimulation of these fibers never evoked effects in contralateral motoneurones.

The release of supraspinal control of ipsilateral reflex arcs has been investigated after various lesions in the lower reticular formation[4]. After a medial lesion in lower pons, there is a release of the tonic control of the inhibitory path to ipsilateral extensor motoneurones and concomitantly an opening of an inhibitory path to ipsilateral flexor motoneurones. Release of the excitatory pathway to ipsilateral flexors occurs only after a more caudal lesion in the brain stem. Similar investigations have now been made on the supraspinal control of crossed actions. A low pontine lesion gave release of crossed inhibition to extensors. In the Figure a group II + III volley in the nerve to gastrocnemius-soleus had no effect on contralateral extensor motoneurones (gastrocnemius-soleus) in the decerebrate state (•). After a pontine



The effect of a conditioning volley in the nerve to gastrocnemius-soleus on the monosynaptic reflex from the contralateral gastrocnemius-soleus nerve recorded in the ventral root. 100% on the ordinate represents the unconditioned amplitude of the test reflex. Conditioned amplitude, expressed as percentage of control amplitude, is plotted as a function of time interval between the conditioning and testing stimuli. The three series of measurements were obtained after the lesions indicated in the schematic drawing, all at a conditioning stimulus strength supramaximal for group III.

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