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Effect of botulinum toxin on extraocular muscle proprioception

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Abstract. Injections of botulinum toxin type A (BoTox) in one extraocular muscle (EOM) induce long lasting paretic lengthening of the muscle permitting realignment to occur in strabismus, while eye movements appear to be unaffected after the transitory period of induced paresis. It has been hypothesized a BoTox-induced change in the spindle discharge of EOMs to explain the effect in EOM length. In decerebrate lambs and goats, first order neurons of eye muscle spindles were identified in a cellular pool located in the medial dorsolateral portion of the semilunar ganglion. The belly of the muscle to which the recorded unit belonged was infiltrated with BoTox. A decrease in afferent discharge of the spindle and in its stretch sensitivity was observed. This effect began 10–15 minutes after the injection. There was no corresponding decrease in muscle tension during the first 45 minutes. This finding suggests that the block of release of acetylcholine at motor endings is earlier and more efficacious in γ- than in α-motoneurons. As a result of the proprioceptive input reduction, an unbalance between the agonist and antagonist muscles should occur favouring the ocular realignment.

Introduction

For several years, some ophthalmologists have been using botulinum toxin (BoTox) for correcting strabismus [15, 27] and blepharospasms [9, 10, 22, 28]. The toxin binds to motor end-plates causing paralysis [5, 12]. It interferes with the Ca^{++} disposal system in the nerve terminals, reducing neurotransmitter release [23]. Repeated injections of BoTox in the EOMs induce dose-dependent weakness producing lengthening of the muscles [8, 27]. However, after a transitory period of eye motility impairment (varying between two weeks and eight months depending upon the concentration of the drug) [26] full recovery is assured by sprouting of new motor end-plates from the axon [6] leaving only a permanent change of ocular alignment. This change results from contracture of the antagonist muscle and lengthening of the injected one. It could be suggested that this steady change in the EOM static tensions, without motility deficit [26], could be explained by a selective damage of the motor units *involved in controlling* eye position *in* the injected muscle and/or by a tonic increase of activity in the postural units of the antagonistic one. Although it has been shown that sprouting in the injected muscles takes longer in fast skeletal fibers than in slow ones [7], recently light and electron microscopy studies showed that only the single-innervated fibres of orbital layer of EOM, possibly involved in eye position control [1], are altered by repeated EOM toxin infiltrations [30]. Besides the BoTox direct effect on the injected muscle and the corresponding contracture of the antagonistic one, another mechanism involving the eye proprioception could be taken into account to justify the permanent change of ocular alignment. In fact it cannot be excluded that BoTox also binds to the motoneuron endings whieh innervate intrafusal muscular fibres, thus reducing proprioceptive afferent input from eye muscle spindles which are abundant in EOMs [16, 29].

Although a stretch reflex has not been demonstrated in the EOMs territory [13], a permanent change in proprioceptive input could lead to a central reorganization of the neurons controlling eye position. Thus we decided to explore such a problem in lambs and goats. These animals are particularly fit for this purpose since it has been demonstrated that their semilunar ganglion contains the first order neurons of eye muscle proprioception [3, 19, 25]. A cellular pool has been identified in the medial dorsolateral portion of the semilunar ganglion from which unitary responses to the stretch of single eye muscle can be recorded [17, 18]. A somatotopic organization has been found with the superior rectus (SR) and oblique (SO) muscles represented dorsal to the inferior rectus (IR) and oblique (IO) muscles, while the medial rectus (MR) and the lateral rectus (LR) have medial and lateral projection respectively [3, 20].

The aim of this study was to verify whether BoTox injected to a given extraocular muscle could influence its afferent proprioceptive input.

Preliminary report has been published [21] as a brief communication.

Techniques and methods

Six lambs and 2 goats (weighing 10–12 Kg) were used. The animals were anesthetized with ketamine hydrochloride (Ketalar, Parke-Davis, 50 mg/kg) and diazepam (Valium, Roche, 2 mg/kg) i.m., intubated and put on a

Transvertex stereotaxic apparatus. After craniotomy, the cerebral cortex and the thalamus were removed by suction. The left semilunar ganglion was identified, isolated and protected by warm mineral oil. The distal insertion of EOMs was cut and eyeball was removed. Single EOMs were isolated and protected by warm mineral oil. Tungsten microelectrodes (tip diameter 1–4 μ; resistance 800–1000 KΩ) were used to localize, in the medial dorsolateral portion of the semilunar ganglion, the units which innervate eye muscle spindles. The microelectrode was connected through conventional preamplifiers to a beam of a Tektronix 5440 oscilloscope. The discharge rate of the units was recorded on tape and measured by means of a window discriminator and a timed counter (Frederick Haer and Co). A Sanborn FTA 100 transducer (maximum working range: 100 g; minimum reliable force 10 mg) for measuring the EOM tension and a length transducer (Sanborn 7 DCDT/500) were employed for measuring EOM displacement during stretch. Muscle length was adjusted by a micromanipulator.

Once a gasserian unit was isolated, the motor nerve of the corresponding muscle was stimulated to characterize the nature of the unit. Then the muscle was lengthened from -2 to $+8$ mm above the resting length ($Lr = 0$), which corresponds to the length of the muscle when the eye is in its "primary position". It was difficult to accurately judge the "primary length" of the detached extraocular muscles. However, from previous measurements of the intact eye, it was determined that the average stiffness which could be attributed to purely passive factors could be no greater than 100 mg/0.1 mm. If the passive stiffness of a particular muscle exceeded 100 mg/0.1 mm at its estimated primary length, the primary length was redefined to bring the passive stiffness into conformity with this upper limit. Unit discharge rate and muscle tension were repeatedly measured during static periods and averaged. After that, the muscle was fixed at length $Lr + 4$ and subsequently infiltrated with 100 units ($4 \times 10^{-2}\,\mu g$) of Oculinum° in 0.1 ml [27], kindly provided by Dr. AB Scott. This dosage is about 20 times the initial dose used in strabismus [15, 27]. Such a large amount of toxin administration was chosen in order to shorten the observation time as we were not able to record from the unit for more than 1 hour. Tension and afferent discharge were measured every 10 minutes at different lengths for a maximum of 45 minutes following injection. Controls consisted of an EOM infiltration with the same volume of saline solution as that utilized for Oculinum°. Statistical signifieance was calculated by analysis of variance and Tukey's tests.

At the end of the experiments the animals were sacrificed under deep anaesthesia.

Results

In agreement with our previous investigations, proprioceptive units were found in the medial dorso-lateral portion of the semilunar ganglion which responded exclusively to single eye muscle stretch. Eight units from muscle spindles (2 from LR, 2 SO, 1 SR, 1 MR, 1 IR, 1 IO) were identified by their low threshold to muscle stretch and by their response to tetanic stimulation of the motor nerve [4]. The discharge frequency of the proprioceptive units and tension of the muscle to which the unit belonged were measured at different static muscle lengths from Lr − 2 to Lr + 8. The tension showed small spontaneous oscillations in the range of 20–50 mg. Larger oscillations with slow and fast components indicated central unbalance; when they were present the experiment was interrupted.

After fixing the muscle at Lr + 4, BoTox or saline was injected into the eye muscle belly. In both cases the injection caused a temporary increase in tension of about 250–500 mg and also in frequency discharge of 20% (Figs. 1 and 2). These variations disappeared within a few minutes (2–5 min) and were attributed to the mechanical disturbance caused by the fluid volume injected into the muscle. Muscle tension and unitary discharge were measured every 10 min at different lengths (Lr − 2, Lr = 0, Lr + 2, +4, +6,

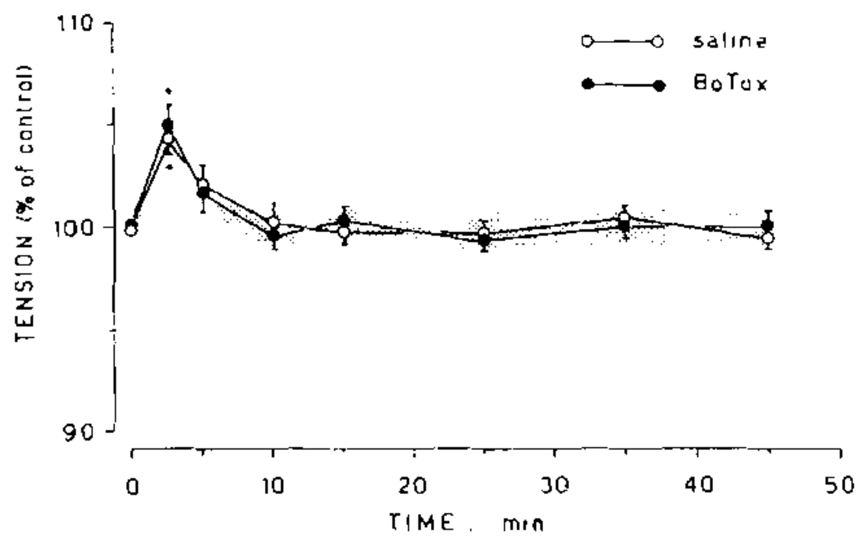

Fig. 1. Time course of change in tension of EOMs caused by injection of 100 U of BoTox and saline in 0.1 ml. Average obtained from 8 muscles (2 LR, 1 IR, 1 SR, 1 MR, 1 SO, 2 IO). The muscle was fixed at length Lr + 4 mm. Tension variation is expressed as percent of control. The amplitude of shadow area is related to the SEM of normal values. Asterisks indicate significant differences from control ($P < 0.01$). Temporary tension increase disappeared within 5–7 minutes after injection.

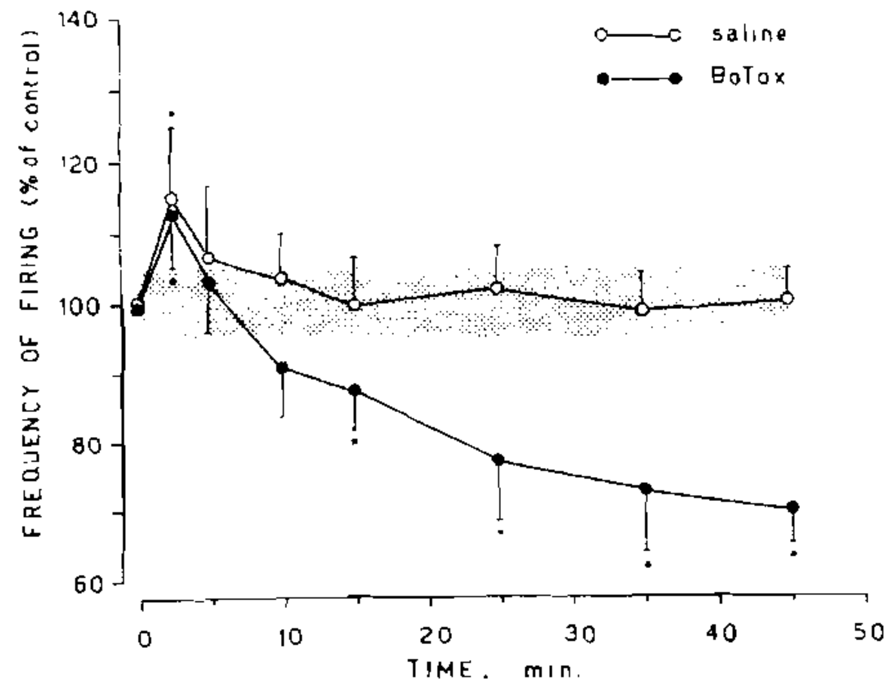

Fig. 2. Time course of change in frequency of firing of EOMs afferents caused by injection of 100 U of BoTox and saline in 0.1 ml. Average obtained from 8 muscles (2 LR, 1 IR, 1 SR, 1 MR, 1 SO, 2 IO). The muscles were fixed at length Lr + 4. Frequency variation is expressed as percent of control. The amplitude of shadow area is related to the SEM of normal values. Asterisks indicate significant differences from control ($p < 0.01$). Note temporary frequency increase for both saline and BoTox curves in the first 5–7 minutes and the significative decrement of firing after 10 minutes post BoTox injection ($P < 0.01$).

+8) for 45 minutes. No significant changes in tension and frequency occurred after saline injection (Figs. 1, 2, 3 and 4). On the contrary, the administration of 100 units of Oculinum° induced, starting 10 minutes after the injection, a gradual decrease in discharge frequency in all the eight units, with progressive reduction of up to 35% of the initial value at 45 minutes (Fig. 2). Furthermore, a decrease in spindle stretch sensitivity was found by comparing unit discharge frequency at different lengths before and after injection (Fig. 4). In all cases tension did not show any effect (Figs. 1 and 3).

Discussion

The present experiments demonstrate that infiltration of an EOM with

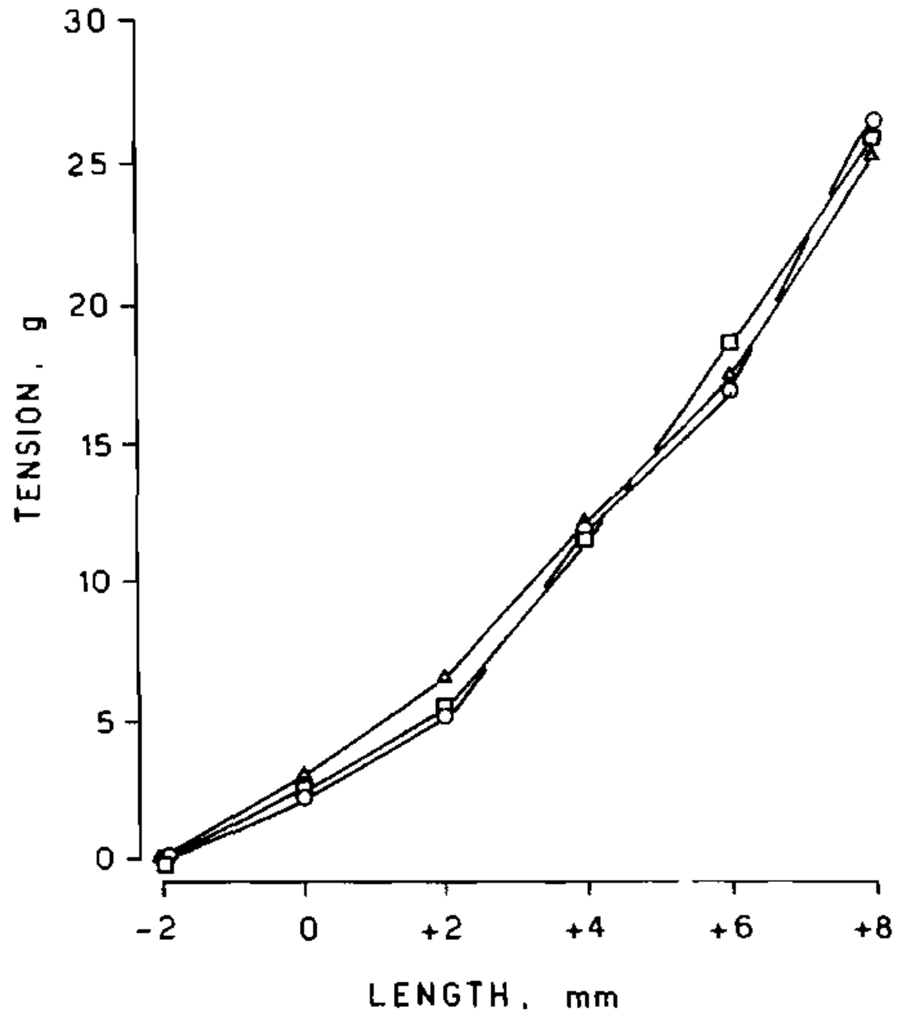

Fig. 3. Relation between tension and length of Lateral Rectus (LR). Triangles indicate the curve in normal conditions, circles 45 minutes after saline and squares 45 minutes after BoTox injection.

BoTox (100 U) reduces afferent discharge frequency from its neuromuscular spindles starting from 1–15 minutes after the infiltration of BoTox into a single eye muscle. Also, unit stretch sensitivity was reduced by the toxin injection. Since EOM mechanical characteristics remained constant as shown by length/tension curves (Fig. 3), the effect on the spindle unit discharge could be attributed to an effect of the toxin on the spindles. Since BoTox has been reported to interact at the motor endings [5, 12], the decrease in stretch spindle sensitivity is probably due to the toxic effect on

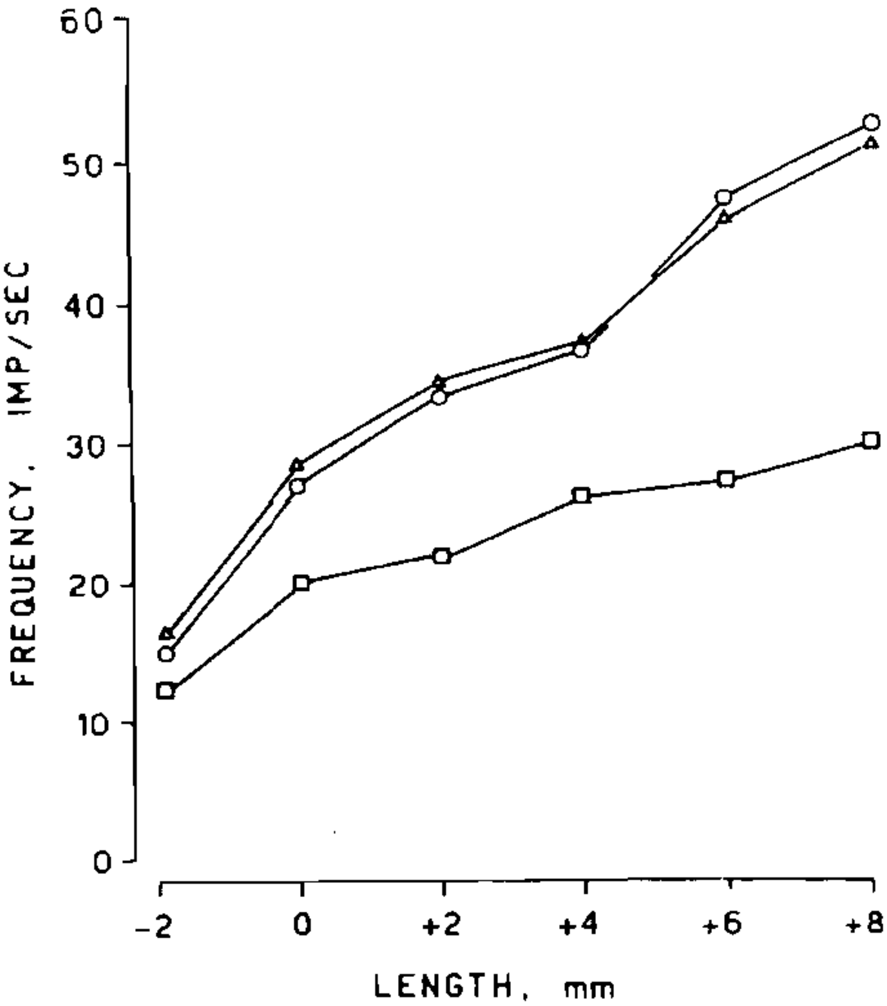

Fig. 4. Relation between frequency discharge of a single unit of LR and muscle length. Symbols relate to the same condition as in Fig. 3. Note the reduction of stretch sensitivity of the unit.

the motor endings connected to the intrafusal muscular fibres of spindles. Such a reduction in proprioceptive input without simultaneous tension changes also suggests that the γ-motor neurons are affected earlier and more powerfully than the α-motor endings innervating extrafusal fibres. Paresis, in fact, is reported to occur many hours later [26, 27]. Since intrafusal fibres show a richer grape innervation than the extrafusal ones (the EOM G fibres presenting these endings do not contribute to muscle tension)[11], one can suggest that the grape endings are specifically affected by the toxin. However, recently γ-plate endings also have been shown to differ from the α-plate endings in the amount of synaptic boutons [14]. As a consequence of that, the first influence of BoTox in EOMs would result in a reduction of spindle discharge without significant tension changes. Whether and how this effect could induce or facilitate ocular realignment, such as that occuring after repeated injections in EOMs of low doses of BoTox in strabismus, can not now be assessed. Since myotatic reflex is elicited by spindle activity, a decrease in EOM proprioceptive input should lead to an immediate direct reduction in EOM tone and eye position change. However, no stretch reflexes in the EOMs were found and no significant tension reduction paralleling the frequency decrease of the proprioceptive afferents was observed in the present experiment. On the other hand, a local loop controlling EOM tension has been reported in decerebrate animals [24]. Studies are in progress in our laboratory to solve this discrepancy by selectively blocking EOM proprioception. In addition to a possible local proprioceptive control of EOM tension, another mechanism would be taken into consideration when trying to explain the long lasting ocular deviation observed after BoTox injection. It has been suggested that ocular proprioception interacts with oculomotor centers to give a continuous orbital eye position signal [2]. In this case, a reduction in the spindle aetivity of one muscle should cause an illusory eye deviation toward the injected EOM because of the proprioceptive unbalance between the agonist and antagonist muscles. Consequently, a tension reduction in the same muscle would occur. Therefore, the mechanisms by which BoTox exerts a long lasting effect on ocular alignment in strabismus may be related to changes not only in the tonic motor fibres [30] but also in the spindle afferent discharge.

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