Reports

Pharmacologic weakening of extraocular muscles.

ALAN B. SCOTT, ARTHUR ROSENBAUM, AND CARTER C. COLLINS.

Currently, the correction of nonaccommodative strabismus involves surgical manipulation to loosen or tighten extraocular muscles to balance the forces and align the eyes. The present study was designed to evaluate the effects of various neurotoxic agents when injected locally into specific extraocular muscles in an attempt to seek pharmacologic alternatives for the correction of strabismus. Conrad Behrens seems to be the originator of this notion. He injected alcohol into human extraocular muscles, but the effects were inadequate.[1] A project to evaluate drugs placed by electromyograph (EMG) guided injection was proposed by Jampolsky[2] in 1963, but experiments were not done for lack of an animal model. Anesthetic agents such as barbiturates and halothane abolish EMG activity. However, one of us (A. S.) observed that ketamine would give surgical levels of anesthesia in both the monkey and the human with preservation of an active EMG signal recorded by an electrode at the tip of the injection needle to guide drug placement.

All experiments were performed on adult rhesus monkeys weighing between 2 and 5.5 kilograms. Each animal was anesthetized with an intramuscular injection of ketamine in a dose of 5 mg. per kilogram of body weight. A hypodermic injection needle containing 75 μ insulated stainless steel electrode exposed at the tip was inserted transconjunctivally into either the medial or lateral rectus muscle of the animal until a robust EMG signal was obtained. Solutions of the various drugs described below were then injected through this needle into the belly of the selected muscle. The needle was then withdrawn and the animal was allowed to recover from the anesthesia. The animals were photographed preoperatively and at various stages postoperatively to assess eye position and were examined periodically by a veterinarian.

The following drugs were injected: (1) di-isopropyl-fluoro-phosphate (DFP); (2) a-bungarotoxin isolated from the venom of *Bungarus multicinctus* (cobra neurotoxin); (3) botulinum neurotoxin, Type A; and (4) alcohol.

Di-isopropyl-fluoro-phosphate (DFP). One horizontal rectus muscle in each of seven monkeys was injected with DFP in an aqueous solution. The dosage ranged from 0.25 mg. to 1 mg. in volumes varying between 0.1 and 0.5 c.c. Cholinesterase levels were determined before injection and at one hour after injection. The injection of DFP resulted in the pharmacologic production of a horizontal strabismus that lasted from two hours to thirty-six hours. Four monkeys were severely ill, and two monkeys died of acute DFP intoxication produced by injections of 0.5 mg. and 1 mg., with cholinesterase levels which dropped from 4,162 milliunits per milliliter before injection to 168 milliunits per milliliter after injection. Because of the transient pharmacologic response coupled with the severe systemic toxicity, experimentation with this drug was discontinued.

A-bungarus neurotoxin. Individual horizontal rectus muscles were injected with A-bungarus toxin in four monkeys. The dose ranged between 10 and 20 μg in volumes between 0.1 and 0.3 c.c. All animals injected experienced ptosis occurring three to six hours after injection and clearing two to three days after injection. The expected horizontal strabismus which occurred approximately six hours after injection lasted four days in each case. No systemic or ocular toxicity was noted with the exception of a mild conjunctival inflammation lasting for a day after the injection. The limited duration of effect is useful to know about, but was insufficient to our purpose.

Alcohol. Absolute alcohol (0.3 c.c.) was injected into the lateral rectus muscle of one monkey. One day postoperatively marked ptosis, edema of the lids, and chemosis was seen. These effects cleared after four days. At no time was esotropia seen.

Botulinum Type A neurotoxin. To assure consistency of drug potency, toxin was assayed by mouse intraperitoneal injections carried out to determine the L.D./50 for each batch of toxin being utilized. In each instance, the L.D./50 was about 1×10^{-4} μg for Swiss Webster mice weighing approximately 20 grams.

Thirteen horizontal rectus muscles of eight rhesus monkeys were injected with this drug. The amount injected has varied between 1×10^{-5} μg and 1.6×10^{-3} μg in volumes between 5 μl and 500 μl. We have been able to produce both transient weakness of individual horizontal muscles varying between two weeks and eight months, and permanent changes of ocular alignment depending upon the concentration of drug injected. Fig. 1 demonstrates the range of doses and volume of neurotoxin injected and the observed duration of effect as judged by ocular alignment. Fig. 2 demonstrates the change in ocular alignment in the monkey receiving the largest single dose of botu-

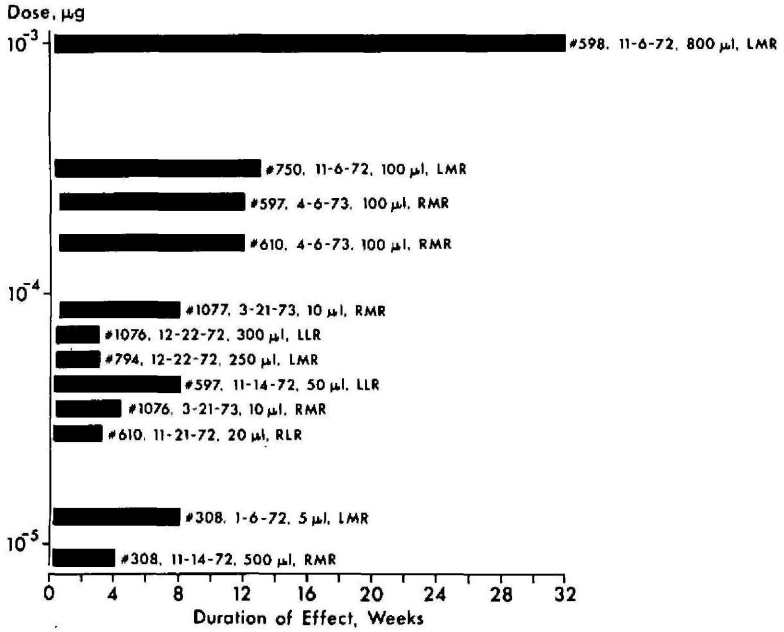

Fig. 1. The duration of induced strabismus for each injection is represented by the horizontal bar. The animal number, date of injection, volume injected, and muscle injected follows each bar. One injection of old, ineffective toxin is not shown.

linum neurotoxin. A left ptosis cleared six weeks following injection. The exotropia reduced slightly beginning at two months, but has been stable at about 30 prism diopters since three months after injection. This exotropia has persisted until the present time, eight months after injection. Horizontal rotation amplitudes are normal. Three and one-half months following injection, the EMG recorded from the injected muscle was of full normal amplitude (Fig. 3). Saccadic velocity determinations using electrooculography showed the velocity for medial and lateral saccades was equal. Sutures placed under anesthesia from the globe to a strain gauge holding the eye in 40° abduction measured the pulse of force produced by the left medial rectus muscle during saccades to right gaze as the monkey, after awakening and with the head held by skull bolts, looked between two targets 30° apart with the unrestrained right eye. The force pulses produced for such saccades by the (injected) medial rectus muscle acting as agonist were equal to the force pulses produced for return saccades in the opposite direction when the eye was held nasally 40° and the (uninjected) lateral rectus muscle was acting as agonist.

In all cases, the monkeys awakened promptly from light ketamine anesthesia and proceeded to thrive in their cages without evidence of systemic side effects. There has been no recognized systemic toxicity in these monkeys and their dietary habits and activity remain essentially unchanged from the pre-injection status. A mild conjunctival

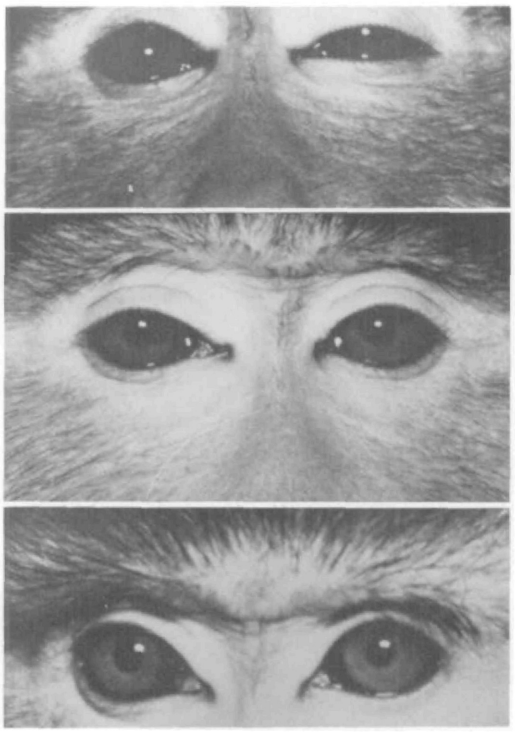

Fig. 2. Exotropia following injection of left medial rectus muscle. Top: before injection (anesthetized); middle: five months after injection; and bottom: eight months after injection (animal No. 598).

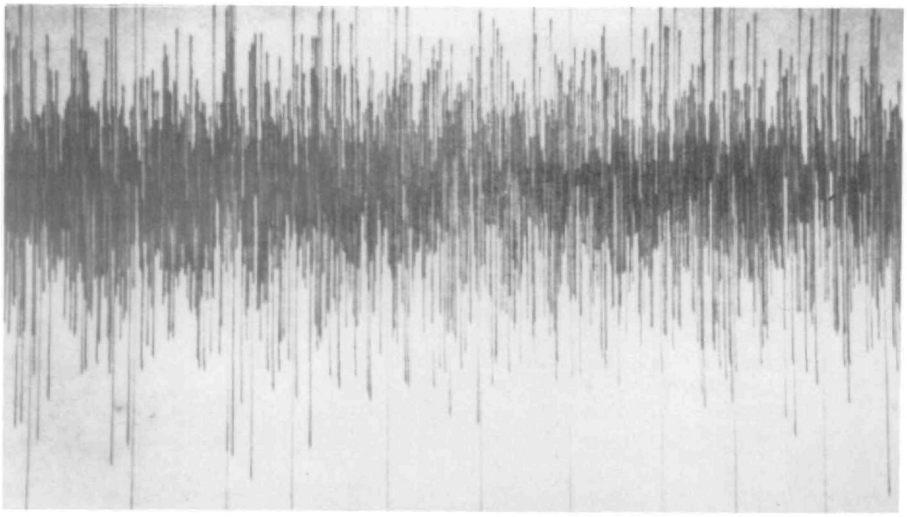

Fig. 3. EMG of left medial rectus, same animal as in Fig. 2 (animal No. 598).

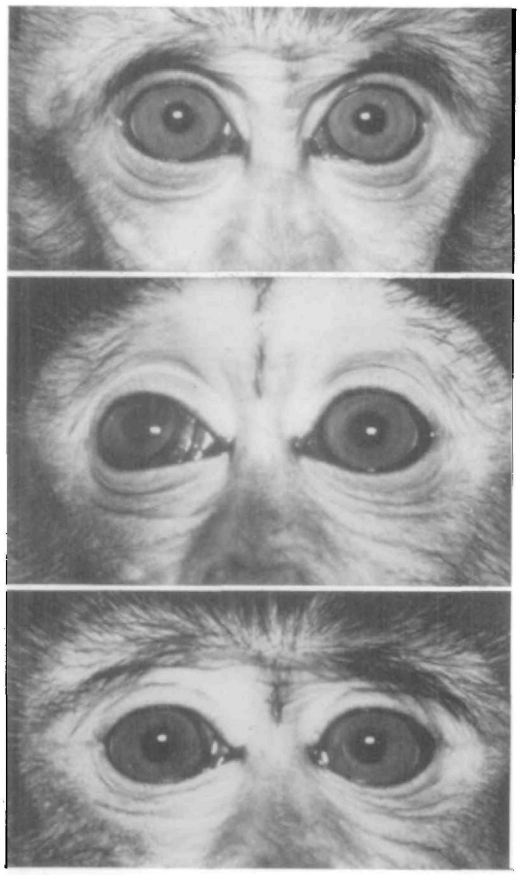

Fig. 4. Exotropia following injection of right medial rectus muscle (animal No. 1076). Top: before injection; middle: four weeks after injection; and bottom: 7.5 weeks after injection.

redness followed injection for a day or so. The only side effect of the injection has been transient involvement of adjacent muscles to the one injected, presumably due to diffusion of the toxin into these structures. This effect was reduced or eliminated by the use of small volumes of injected material (10 to 100 μl). Also, simultaneous injection of 5 to 10 units of antitoxin into the antagonist and adjacent rectus muscles of animals seemed helpful in preventing paralysis of them. When ptosis did occur from involvement of the levator palpebral muscle, this always cleared completely. In no case was pupil size altered. An interesting aspect is the delayed onset of effect. In smaller doses it may not show for days, and then increase for three or four days to its height about one week after injection. This was the sequence in the animal shown in Fig. 4.

Two monkeys died during these experiments, both of severe enteritis and hepatitis unrelated to drug injections. One of these deaths occurred during an extensive Shigella epidemic in the primate colony and the other was found on postmortem examination to have a typical enteric infection as the cause of death. Both of these animals had small doses of toxin and were healthy for many weeks after injection. Histologic examinations of the treated muscle showed it to be indistiguishable from the untreated muscle of the fellow eye.

The injection of botulinum Type A neurotoxin into the horizontal rectus muscles of the rhesus monkey produced prolonged paresis of the injected muscle without serious local side effects, and without any systemic effect. The duration of effect has extended from two weeks to a permanent effect (eight months) depending on the dose injected. Permanent alignment changes

after temporary muscle paralysis are a common clinical occurrence. We have duplicated this clinical sequence pharmacologically in the monkey. The EMG recording technique described insures precise localization of the muscle desired, without the need for conjunctival incision or direct exposure of the muscle. Diffusion of the drug into adjacent muscles has been reduced by the use of small volumes of drug injected. The effect seems able to be reduced or nullified also by the simultaneous injection of a small amount of antitoxin into adjacent areas, a modification of the approach of Kupfer.[3] Effective paresis has been produced in the monkey by injection of 1.0×10^{-5} up to 1.6×10^{-3} μg of botulinum Type A neurotoxin. Relative to the (oral) L.D./50 dose in man, approximately 1 μg,[4] this represented from 1/100,000 to 1/1,000 of the L.D./50.

Clostridium botulinum produces a specific neurotoxin, which, because it appears spontaneously in the culture medium from which it can be isolated, is classified as an exotoxin. To date, six antigenically distinguishable toxins have been identified, and we have done our work exclusively with Type A toxin. These toxins are proteins with a molecular weight of approximately 900,000. Present evidence indicates that botulinum toxin acts presynaptically as an extremely powerful blocker of cholinergic transmission. Evidently toxin directly affects the mechanism by which quanta of acetylcholine are liberated from the nerve endings. This effect is not a consequence of interference with impulse conduction in the motor nerve or of inhibition of synthesis or storage of acetylcholine. Neither the nerve nor the muscles suffer impairment of electrical excitability or conductivity in the presence of complete neuromuscular blocks produced by botulinum toxin, and the changes in nerve or muscle are considered secondary atrophic consequences from loss of cholinergic transmission.[4, 5]

The clinical relevance of the use of botulinum neurotoxin or other drug injections into extraocular muscles awaits appropriate human trials. Such a pharmacologic approach may be used to replace or augment existing methods of surgical correction of strabismus. The weakening of overactive extraocular muscles in comitant squint and the weakening of the antagonist muscles in paralytic squint, both as treatment and in smaller doses to prevent secondary contractures, seem possible. Extension of this approach to reducing lid retraction in endocrine exophthalmos, reducing blepharospasm, and influencing skeletal muscle groups seems entirely feasible.

A. E. Maumenee, M.D., suggested the use of botulinum toxin to us; Edward J. Schantz, Ph.D., provided the botulinum toxin and gave valuable advice in many ways; and Bert L. Tate helped in recording and electrode design.

From the Smith-Kettlewell Institute of Visual Sciences, 2232 Webster St., San Francisco, Calif. 94115. Supported in part by United State Public Health Service Grants No. 5 PO1 EY00299, No. 1 PO1 EY01186, and a grant from the Smith-Kettlewell Eye Research Foundation. Manuscript submitted for publication Aug. 13, 1973; manuscript accepted for publication Sept. 25, 1973.

Key words: strabismus, paralysis, eye muscles, botulinum toxin.

REFERENCES

1. Knapp, P.: Personal communication.
2. Jampolsky, A.: Personal communication.
3. Kupfer, C.: Selective block of synaptic transmission in ciliary ganglion by Type A botulinus toxin in rabbits, Proc. Soc. Exp. Biol. Med. **98**: 474, 1958.
4. Lamanna, C., and Carr, C. J.: The botulinal, tetanal, and enterostaphylococcal toxins: a review, J. Clin. Pharmacol. Therap. **8**: 286, 1967.
5. Drachman, D. B., and Houk, J.: Effect of botulinum toxin on speed of skeletal muscle contraction, Am. J. Physiol. **216**: 1453, 1969.
6. Simpson, L. L.: Neuropoisons. New York, 1971, Plenum Press, pp. 303-323.

In vivo measurement of optic-disk oxygen tension.

J. TERRY ERNEST.

Davies and Brink[1] first demonstrated the feasibility of using a platinum microelectrode to electrolyze dissolved oxygen in animal tissue. The stability of the system is poor because of drift due to change in electrode performance. Thus, slow changes are difficult to analyze but transient qualitative measurements may be obtained with moderate reliability. The present study was undertaken to develop a valid method for measuring the oxygen tension of the optic disk in vivo using a platinum microelectrode.

Fifty-five adult cats, both male and female of mixed breeds, ranging in weight from 2.0 to 3.5 kilograms, were used. The animals were anesthetized with sodium pentobarbital, 30 mg. per kilogram, administered intraperitoneally. General anesthesia was maintained by supplements of sodium pentobarbital administered intravenously. Tracheostomies were performed and the animals were given artificial respiration (Respirator Model 671, Harvard Apparatus Co.) and then paralyzed and maintained in this state with a mixture of tubocurarine chloride, 0.4 mg. per milliliter, and gallamine trithiodide, 2 mg. per milliliter, administered intravenously in 0.5 ml. increments as needed.

The tidal P_{CO_2} was continuously monitored with an infrared absorption carbon dioxide analyzer (Medical Gas Analyzer Model LB-1, Spinco