The Effect of Intravitreal Botulinum Toxin on Rabbit Visual Evoked Potential

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ABSTRACT
Intravitreal injections of botulinum A toxin in two doses, 1.25 and 25 units, were performed in two rabbit eyes. The fellow eyes were injected with an equal volume of saline to serve as controls. The visual evoked potential was unchanged at one and two weeks post-injection when compared to pre-injection recordings in both botulinum and saline injected eyes.

Botulinum toxin has been injected under electromyographic control into human extraocular muscles for the treatment of some forms of strabismus since 1978. Interest in the effect of intravitreal botulinum has been generated by reports of five scleral perforations during attempted botulinum injection. Previous investigation of intraocular injection of botulinum showed no effect on the electroretinogram, gross fundus, or light microscopic appearance of rabbit eyes. The purpose of this study is to investigate further possible adverse effects of intravitreal botulinum toxin by assessing ganglion cell function using the visual evoked potential (VEP).

MATERIALS AND METHODS
Stainless steel supradural recording electrodes were implanted over the visual cortex of two New Zealand white rabbits under ketamine/acepromazine anesthesia. Reference and ground wire electrodes were implanted in the skin behind the right ear and at the nape of the neck, respectively. Each rabbit weighed approximately 1.8 kg.

An identical volume (0.1cc) was used for all intravitreal injections. In each eye a small amount of vitreous was aspirated after passing a 25 gauge needle through the pars plicata inferiorly. The intravitreal injection was then given with a 30 gauge needle passed through the pars plicata superiorly. Doses employed and details regarding the injections are shown in Table. Doses selected are the minimum and maximum amounts injected in adult human strabismus patients.

The monococular VEP stimulus consisted of a green

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<table>
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<tr>
<th>TABLE</th>
<th>DOSAGE OF INTRAVITREAL BOTULINUM</th>
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<tr>
<td>Rabbit</td>
<td>Right eye (Saline)</td>
</tr>
<tr>
<td>A</td>
<td>0.1 cc</td>
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<tr>
<td>B</td>
<td>0.1 cc</td>
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* Initial dose refluxed out of the eye into the subconjunctival space and was repeated 6 days later.
light emitting diode glued to a polymethylmethacrylate contact lens. VEP recordings were obtained under ketamine/acepromazine anesthesia prior to, and serially for at least 16 days after the intravitreal injections. Each recording was based on 500 averaged responses from stimuli at one second intervals.

RESULTS
There was no apparent difference in one and two week post-injection VEP recordings compared to those obtained pre-injection for eyes receiving saline, 1.25 units of botulinum, or 25 units of botulinum (Figure). An unexpected finding was the prolonged pupillary dilation first noted on the day after the initial injection in the left eye of rabbit B, in which most of the 25 units of botulinum refluxed from the injection site to form a subconjunctival bleb.

DISCUSSION
The normal electroretinogram (ERG) after intravitreal botulinum injection found by Wienkers et al. suggests that botulinum toxin does not significantly affect the outer and middle retinal layers. However, the flash ERG does not reflect ganglion cell function, and a selective effect on this layer would go undetected. For example, intraocular tetrodotoxin does not significantly alter the ERG yet completely abolishes ganglion cell activity and the VEP. The failure of botulinum toxin to suppress the VEP in the current study suggests a lack of effect on ganglion cell function as well as the outer and middle retinal layers.

The mechanism of action of botulinum involves a presynaptic alteration (perhaps by changing its calcium sensitivity) of the exocytic mechanism responsible for acetylcholine release. Most cholinergic synapses in the rabbit retina appear to be in the inner plexiform layer. The ganglion cells which show sensitivity to acetylcholine do so selectively, based on their receptive field characteristics. Lack of change in the VEP does not rule out suppression of a small subset of ganglion cells by botulinum.

Three cases of pupillary dilation have been reported in humans after botulinum injection into extraocular muscles. The specific mechanism of this complication is unclear and deserves further investigation.

REFERENCES