Selective Block of Synaptic Transmission in Ciliary Ganglion by Type A Botulinus Toxin in Rabbits. (24388)

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The work of Ambache(1,2) has demonstrated that type A botulinus toxin can be used as a pharmacological agent to block selectively both synaptic transmission in autonomic ganglia and post-ganglionic cholinergic transmission in end-organs (*e.g.* the iris and nictitating membrane of the cat). Because the botulinus toxin was lethal, the experiments were necessarily acute. No animal survived after the fourth or fifth day following toxin administration. It is the purpose of this paper to demonstrate that a prolonged block of synaptic transmission may be induced by local application of type A botulinus toxin to the ciliary ganglion when this is followed in a short time by parenterally administered type A botulinus antitoxin. This specific block of synaptic transmission in the ciliary ganglion persists in the intact animal for at least 6 months. The block so produced affects only the parasympathetic efferent nerves, that is, those which synapse in the ciliary ganglion and innervate the ciliary body and iris. There is no interference of nervous transmission of sympathetic or somatic nervous pathways.

Material and methods. Type A botulinus toxin* was prepared as outlined by Lamanna et al.(3). Dilutions of toxin were carried out so that 1.0 ml of toxin contained 8.0 x 10^4 LD_{50} mouse units. Type A botulinus antitoxin contained 50 antitoxin units/ml.† There were 94 rabbits‡ in all including those used for preliminary studies. 1. *Retrobulbar injection.* After lightly anaesthetizing the rabbit (100 mg pentobarbital intramuscularly), the right eye was proptosed. A 2-inch #25 needle was used to inject 0.5 ml of botulinus toxin into the apex of the orbit in the region of the ciliary ganglion. Fifteen minutes following retrobulbar injection of the toxin, 1.0 ml type A botulinus antitoxin was injected intramuscularly in the interscapular area; then the right eye was dressed with terramycin ointment. This procedure was performed on 45 albino rabbits. 2. *Intravitreal injection.* An additional twenty rabbits were anaesthetized and the right eye proptosed; 0.05 ml of aqueous humor was withdrawn from the anterior chamber and 0.1 ml type A botulinus toxin injected into the vitreous humor through the pars plana region, making an attempt to penetrate the sclera obliquely so as to minimize sub-conjunctival leakage. Twenty-five minutes following toxin injection, 1.0 ml of type A botulinus antitoxin was injected intramuscularly and the right eye dressed. The ciliary ganglion (CG) was isolated using a technic developed by Holland (personal communication); the superior cervical ganglion (SCG) was isolated in routine fashion. The third nerve was isolated intracranially in the decerebrate rabbit. All electric stimulation of the nerves and ganglia was performed with the Lab-Tronics Physio-Stimulator Model N-103-B using 2 silver wire electrodes. Pharmacological preparations used were eserine sulphate 1/4%, pilocarpine hydrochloride 2%, adrenalin 1:1000 and cocaine hydrochloride 4%. Measurements were made of pupillary diameter with a straight millimeter ruler in daylight. In 8 rabbits, retrobulbar injection of boiled botulinus toxin was performed as controls on the left side. The injection was made in the same manner as outlined above.

Results. 1. Retrobulbar toxin, right eye. Despite protection by systemic administration of antitoxin as outlined in the time schedule, 4 animals died of botulinus intoxication within 72-96 hours. Of the remaining 41 animals, 23 had a dilated fixed pupil on the injected side, indicating interruption of the motor innervation to the sphincter muscle of the iris. The remaining 18 rabbits demonstrated either no pupillary dilatation or minimal dilatation. We will confine our remarks to the 23 animals

* Kindly supplied by Dr. Carl Lamanna.

† Fort Dodge Laboratories, Iowa.

‡ Research Supply Co., Philadelphia.

TABLE I. Pupillary Reaction following Electrical Stimulation.

Area stimulated	Pupillary reaction	
	Right eye*	Left eye†
Superior cervical ganglion	Pupil dilates briskly 2/2‡	Pupil dilates briskly 2/2
Third nerve—intracranial portion	No reaction noted 4/4	Pupil constricts slowly 3/5
Ciliary ganglion	Pupil constricts slowly 2/4	Pupil constricts slowly 3/5

* Right retrobulbar inj. of ciliary ganglion with botulinus toxin.
† Normal control.
‡ No. of animals responding as indicated as compared to No. of animals tested.

that developed a dilated fixed pupil.

Within 24-36 hours after injection, these animals exhibited a dilated fixed pupil on the injected side measuring about 9.0 mm as compared to the left eye, the pupil of which measured 5.5 mm. The dilated fixed pupil persisted unaltered for at least 6 months before some of the animals began to show a sluggish pupillary constriction to light. Of the 23 animals, 16 still had dilated fixed pupils 9 months later. In those eyes receiving a retrobulbar injection of boiled toxin (which destroys its activity), there were no pupillary abnormalities whatsoever and these animals were indistinguishable from the non-injected controls. The rabbits were allowed to recover from the experimental procedure over a period of 7 days before further experiments were performed.

a. *Results of electric stimulation.* Table I summarizes the pupillary response on the injected side and control side. Stimulation of the SCG produced good pupillary dilatation. Third nerve stimulation (preganglionic parasympathetic efferents) yielded no pupillary response on the injected side whereas direct CG stimulation (post-ganglionic parasympathetic efferents) produced a slow pupillary constriction on the injected side in half the rabbits. It would appear then that the site of block of the nerve impulse is at the synapse. There was constriction of the pupil on the control side in 3 of the 5 animals with both pre- and post-ganglionic nerve stimulation.

b. *Results of pharmacological stimulation.* Table II summarizes the pupillary response on the injected and control side. Although topical eserine caused some pupillary constriction on the injected side, it was not as marked as the constriction on the control side. Pilocarpine further constricted the pupil on the injected side. There was a normal reaction to adrenalin and cocaine on both sides. These pupillary reactions to topical drugs give further support to the previous evidence that while the sympathetic nervous pathway to the iris appears intact, there is a block of the parasympathetic efferent pathway. The localization of this block is better indicated by electrical stimulation experiments.

2. *Intravitreal injection, right eye.* There were no deaths in this group and 16 of the 20 rabbits had a dilated fixed pupil within 24 hours. Following injection, there was an inflammatory reaction in the vitreous with formation of a retrolental membrane. These eyes became blind.

a. *Electrical stimulation.* The pupil dilated briskly on electrical stimulation of the SCG indicating that the sympathetic pathways were intact. The pupil failed to constrict on the injected side to CG stimulation, indicating that the site of block of the nerve impulse is at the post-ganglionic cholinergic nerve ending of the iris and ciliary body. The control eye responded as before.

Discussion. 1. Retrobulbar injection of botulinus toxin. By following a retrobulbar injection of type A botulinus toxin at an appropriate time interval with parenterally ad-

TABLE II. Pupillary Reaction following Pharmacological Stimulation.

	Avg pupillary size in mm*			
	Right eye†		Left eye‡	
	Before	After	Before	After
Adrenalin + cocaine	9.0	10.0	5.5	11.0
Eserine	9.0	4.5	5.5	1.5
Pilocarpine	9.0	3.0	5.5	3.0

* This data is purely qualitative in nature and is not meant to truly quantitate the effect of each particular drug used.
† Right retrobulbar inj. of ciliary ganglion with botulinus toxin.
‡ Normal control.

ministered type A botulinus antitoxin, there develops a dilated fixed pupil on that side in the majority of surviving animals. Since parasympathetic efferent fibers are the only ones to synapse in the ciliary ganglion, and electrical and pharmacological data presented in this paper indicate an interruption of these pathways at the synaptic level, one may conclude that the toxin has been fixed at the sites of action in the ciliary ganglion by the time antitoxin is given systemically. The antitoxin then serves to prevent the further acton of the toxin at other sites such as neuromuscular junctions or other ganglionic synapses. Such an animal has a highly localized nerve defect, *i.e.* synaptic block of the parasympathetic efferent nerves to the eye. Other than in those animals which died, there were no gross signs or symptoms of impairment of other functions such as ocular motility, blinking reflex, corneal sensation or general neuromuscular dysfunction.

This nerve block persists for at least 6 months and then appears to be reversible in only a small number of animals. Thus ample time is available for experimentation during this period.

2. *Intravitreal injection of botulinus toxin.* By utilizing the same technic but using the intravitreal route for introduction of the toxin, a dilated fixed pupil is again produced, presumably due to a block of the nerve impulse at the post-ganglionic (cholinergic) nerve endings in the iris and ciliary body. The toxin diffuses forward from the vitreous and is selectively affective at this site. Since the pupil does dilate further to SCG stimulation (and topical cocaine-adrenalin) it is evident that the post-ganglionic adrenergic nerve endings to the iris are still intact. This data supports Ambache's contention that the toxin selectively blocks post-ganglionic cholinergic nerve transmission while leaving post-ganglionic adrenergic nerve transmission unaffected.

Summary. 1. It has been demonstrated that retrobulbar injection of type A botulinus toxin in rabbits followed in 15 minutes by systemic administration of type A botulinus antitoxin produces a dilated fixed pupil in the eye on the injected side. 2. The dilated fixed pupil results from a block of the parasympathetic efferent nerves synapsing in the ciliary ganglion. This block is produced by the botulinus toxin. 3. Such a block persists in the intact animal at least 6 months without interference to nerve transmission of sympathetic or somatic pathways elsewhere in the animal.

1. Ambache, N., *J. Physiol.*, 1949, v108, 127.
2. ———, *ibid.*, 1951, v113, 1.
3. Lamanna, C., Jensen, J. I., Bross, D. J., *Am. J. Hyg.*, 1955, v62, 21.

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Compatibility System Controlling Heterokaryon Formation in *Streptomyces coelicolor.** (24389)

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Most of the mutant characters which have been studied genetically in *Streptomyces coelicolor* have been morphological variations and growth-factor requirements. In the streptomycetes, heterokaryons and recombinants which were formed by interaction of 2 growth-factor dependent mutants have been found to grow readily without added supplements if the genes involved were nonallelic(1,2). This increased growth has been attributed to reciprocal sustenance of different nuclei in a common cytoplasm in the case of heterokaryosis, and in the case of recombination to reformation of the full complement of normal genes within a nucleus. Conversely, if the genes involved are allelic, no growth of the growth-factor de-

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