ANTAGONISM OF BOTULINUM TOXIN BY THEOPHYLLINE

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SUMMARY Theophylline increased the time required for botulinum toxin to cause neuromuscular blockade in an isolated phrenic nerve-diaphragm preparation. Theophylline also offered some protection against botulinum toxin in mice, in vivo.

The etiological factor in botulism is botulinum toxin, which is secreted by Clostridium botulinum. For some time it has been known that botulinum toxin causes neuromuscular blockade by inhibiting nerve impulse-induced release of acetylcholine (1). However, the biochemical mechanism of action of the toxin remains obscure. We wish to report that botulinum toxin is antagonized by theophylline on isolated phrenic nerve-diaphragm preparations and in living animals.

MATERIALS AND METHODS

Purified type A botulinum toxin was obtained from the late Dr. Daniel Boroff. Our preparation contained 4 x 10^3 mouse intraperitoneal LD_{50} per μg of protein. Phrenic nerve-diaphragm preparations were isolated from Swiss Webster male mice weighing 25-30 g. The isolated neuromuscular preparation was suspended at 37° in pH 7.4 Ringer-bicarbonate buffer containing 10 mM glucose and equilibrated with 5% CO_2, 95% O_2. The nerve was stimulated with supramaximal rectangular pulses of 2 msec duration every sec, and the amplitude of muscle twitch was measured isometrically with a force transducer led to an oscilloscope.

RESULTS

As shown in Table 1, under the conditions described, botulinum toxin at 3 ng/ml caused neuromuscular blockade after 80-110 min in three separate

Table 1. Effect of theophylline on latency of botulinum toxin-induced neuromuscular paralysis

Treatment	Paralysis time (min)
Control (3)	> 240
Theophylline (1)	> 240
Botulinum toxin (3)	97 ± 15
Botulinum toxin + theophylline (3)	207 ± 23

Paralysis time is the interval between addition of toxin and complete inhibition of muscle response to a single nerve pulse. The number in parenthesis is the number of experiments. The values are means ± standard deviations. When both were present, botulinum toxin was added 15 min after theophylline. In all cases the paralyzed preparations exhibited muscle contraction when the muscle was stimulated directly, demonstrating that the paralysis was not due to an effect on the muscle itself.

experiments. In the presence of 2 mM theophylline, the botulinum toxin-induced blockade occurred after 180-220 min. The only observed effect of theophylline by itself was a slightly increased muscle twitch amplitude at each nerve stimulation. This did not account for the antagonistic effect of theophylline because theophylline added at the onset of botulinum toxin-induced paralysis did not restore neuromuscular transmission. Furthermore, theophylline did not affect the time required for paralysis induced by β-bungarotoxin, another presynaptically acting neurotoxin.

The results of Table 2 show that theophylline offers some protection for mice injected with botulinum toxin. All mice injected with botulinum toxin and 2.5 mg of theophylline were alive 24 hr after injection while half of the mice injected with botulinum toxin alone were dead. The protection with theophylline was only temporary since by 48 hr after injection more than half of the mice treated with toxin and theophylline were dead. The amount of theophylline used in these experiments was high as mice died after receiving intraperitoneal injections of 8.5 mg of theophylline alone.

Table 2. Effect of theophylline on *in vivo* toxicity of botulinum toxin

Injection	Fraction dead	
	24 hr	48 hr
Botulinum toxin	4/8	7/8
Botulinum toxin + 0.8 mg theophylline	2/8	4/8
Botulinum toxin + 2.5 mg theophylline	0/8	5/8

Mice were injected intraperitoneally with 0.9% NaCl containing 1.5 ng of botulinum toxin and, in the indicated cases, theophylline. Eight mice were injected for each condition.

DISCUSSION

Our results have considerable mechanistic and therapeutic implications. The ability of theophylline to antagonize botulinum toxin might mean that botulinum toxin, like diphtheria toxin (2) and cholera toxin (3), acts by a NAD-dependent process. Theophylline, by virtue of its purine structure, could compete with NAD for its binding site. Theophylline inhibits cholera toxin (4), apparently by this means. Theophylline, which is a known inhibitor of cyclic nucleotide phosphodiesterase, could also act antagonistically by inhibiting cyclic nucleotide degradation thereby altering cyclic nucleotide levels. Of course these two mechanisms are not mutually exclusive.

Alternatively the effect of theophylline may involve neither NAD nor cyclic nucleotides. It should not be difficult to distinguish among these possibilities.

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