The Effect of Neuromuscular Blocking Agents on Acetylcholine Release[1] (35970)

S. D. GERGIS, K. L. DRETCHEN, M. D. SOKOLL, AND J. P. LONG

Departments of Anesthesiology and Pharmacology, School of Medicine, University of Iowa, Iowa City, Iowa 52240

Neuromuscular transmission seems to be a more complex phenomenon than had been originally suggested by Bernard in 1851, who showed that curare acts on the myoneural junction (1). Brown, in 1936 and 1937, suggested that these agents had a postjunctional site of action (2). This was further emphasized by Eccles in 1941 (3). Standaert and Riker, in 1967 (4), suggested a prominent presynaptic site of action. The purpose of this work was to test the hypothesis that these drugs did in fact act presynaptically. If these agents did have a presynaptic blocking action, then they should reduce the amount of the acetylcholine released upon nerve stimulation.

Methods. Frog sciatic nerve gastronemius muscle preparation. Frogs, weighing between 20–25 g, of the species *Rana pipiens,* were stunned and decapitated. The skin was removed to expose the gastrocnemius muscles. The sciatic nerve was dissected free, ligated, and cut. The gastronemius muscle was freed from the surrounding tissue and removed along with the sciatic nerve. The origin of the muscle was connected to a stationary metal rod, while its tendon was attached to a transducer. The muscle was then placed in a 15 ml bath containing eserinized frog Ringer's solution (NaCl, 111.23; KCl, 1.88; NaH_2PO_4, 0.04; glucose, 11.10; $NaHCO_3$, 4.76; $CaCl_2$, 0.82; physostigmine, 0.31 mmoles/liter) and bubbled with 95% O_2–5% CO_2. Interrupted supramaximal tetanic stimulation was applied to the nerve for 0.2 sec every 10 sec. The stimulation parameters were a frequency of 250 Hz, 1 msec duration, and 5 V. The contractions of the muscle were measured by a Statham strain gauge transducer and recorded on an Offner Dynograph recorder.

After 0.5 hr of stimulation, samples from the bath were tested for acetylcholine content on the superfused guinea pig ileum and compared with acetylcholine standards. The muscles were then dried in an oven for 1 hr at 175° and weighed. The acetylcholine content was expressed as micrograms of acetylcholine per gram of dry muscle.

Guinea pig ileum preparation. Guinea pigs, weighing between 200–350 g, were stunned by a blow to the back of the head; and a 20–30 mm segment of the ileum was removed. One end of the ileum was fastened to a stationary rod, while the other end was connected by string to a Statham strain gauge transducer. The force of contraction was measured on an Offner Type RS Dynograph. All preparations were subjected to an initial tension at 1 g. The pieces of ileum were superfused (5) with eserinized Tyrode's solution (NaCl, 136.87; KCl, 2.68; $CaCl_2 \cdot 2H_2O$, 1.36; $MgCl_2$, 1.67; NaH_2PO_4, 0.36; $NaHCO_3$, 11.90; dextrose, 10.09; physostigmine, 0.31 mmoles/liter) at 37°, bubbled with 95% O_2–5% CO_2. The flow rate was 7 ml/min and it was controlled by a Holter pump. The dynograph was calibrated so that 1 cm of displacement was equal to 1 g of force of contraction.

The frogs were divided into four groups. Each group received two concentrations of a myoneural blocking agent. The agents employed in the study were *d*-tubocurarine, gallamine, succinylcholine, and decamethonium.

[1] This investigation was supported by U.S. Public Health Service Grants Nos. NS-1396 and NS-4431 and U.S. Public Health Service Training Grant 5 T01 GM00141-11 and by the Department of Anesthesia Trust Fund.

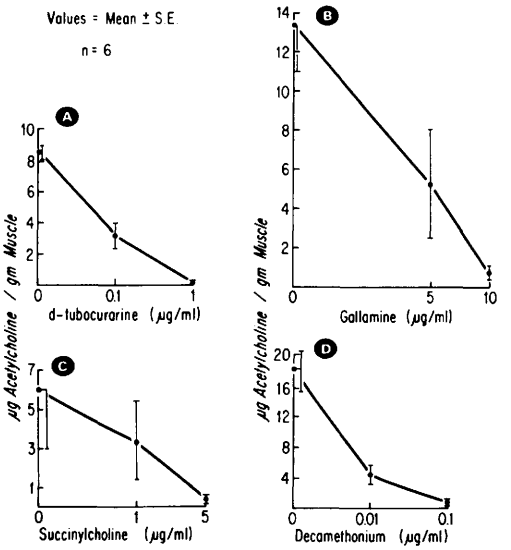

FIG. 1. Effect of various neuromuscular blocking agents on acetylcholine release from the frog sciatic nerve–gastrocnemius muscle preparation: (A) *d*-tubocurarine; (B) gallamine; (C) succinylcholine; and (D) decamethonium.

Two preparations were obtained from each animal, one was used to test a single dose of the chosen relaxant, while the other served as control.

Results. The addition of the nondepolarizing agents, *d*-tubocurarine and gallamine, and the depolarizer succinylcholine and decamethonium, to the frog neuromuscular preparation produced a dose-related decrease in the acetylcholine release as measured on the guinea pig ileum (Fig. 1). When the muscle was washed with normal Ringer's solution, the acetylcholine content returned to normal.

d-Tubocurarine also caused a dose-related decrease in muscle twitch height concomitant with an inhibition of acetylcholine release (Fig. 2a). The other agents did not show such effect (Fig. 2b, c, d). Decamethonium and high concentrations of succinylcholine produced a contracture of the muscle (Fig. 2d). This is the normal response seen with frog muscle. A lengthening of the muscle was observed upon the addition of 1 μg/ml of the depolarizing agent succinylcholine, but not decamethonium (Fig. 2c). The reason for this phenomenon is unknown.

Discussion. Depolarizing and nondepolarizing neuromuscular blocking agents reduced the amount of acetylcholine released in response to supramaximal tetanic stimulation. This would suggest that both classes of agents act on the presynaptic nerve terminal, blocking the release of the neurohormone. The exact site of this action is not yet known.

d-Tubocurarine blockade of muscle twitch, a phenomenon not shared by the other agents, would suggest that the main site of action of curare could be presynaptic and this agrees with the work of Galindo (6), who claims a presynaptic nature of action of

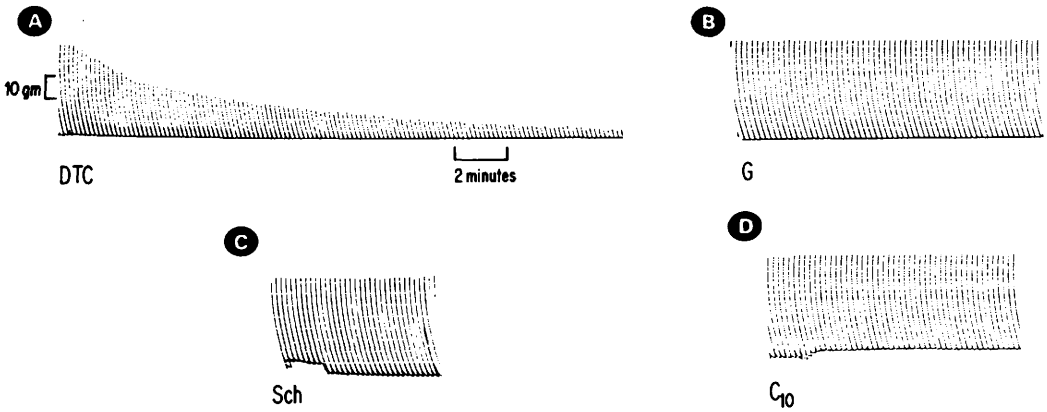

FIG. 2. Effect of various neuromuscular blocking agents on the muscle twitch of the frog sciatic nerve–gastrocnemius muscle preparation (μg/ml): (A) *d*-tubocurarine, 0.1; (B) gallamine, 10; (C) succinylcholine, 1; and (D) decamethonium, 0.1.

the drug in clinical use. This observation also suggests that the other agents act both pre- and postsynaptically.

It is interesting that with close to 90% depression of acetylcholine released by the nerve stimulation there was no effect on the muscle twitch height with most of the agents. This suggested that on nerve stimulation there is acetylcholine released in abundance to what is actually needed to affect the motor end plate. This might be needed to overcome the action of cholinesterase at the synaptic cleft.

The authors are indebted to Mr. L. R. Davies for his excellent technical assistance.

1. Bernard, C. C., Roy. Soc. Biol., Paris **2**, 195 (1952).

2a. Brown, G. L., Dale, H. H., and Feldberg, W. J., J. Physiol. (London) **87**, 394 (1936). (b) Brown, G. L., J. Physiol. (London) **89**, 220 (1937).

3. Eccles, J. C., Katz, B., and Kuffer, S. W., J. Neurophysiol. **4**, 362 (1941).

4. Standaert, F. G., and Riker, W. F., Ann. N.Y. Acad. Sci. **144**, 517 (1967).

5. Gaddum, J. H., Brit. J. Pharmacol. **8**, 321 (1953).

6. Galindo, A., J. Neurophysiol. **34**, 289 (1971).

Received June 14, 1971. P.S.E.B.M., 1971, Vol. 138.