Anesthesiology
V 36, No 6, June 1972

d-Tubocurarine Effects on Nerve-terminal and Neuromuscular Conduction

M. D. Sokoll, M.D.,* K. L. Dretchen, M.S.,† S. D. Gergis, M.D.,‡
J. P. Long, Ph.D.§

Using the frog sartorius muscle preparation, *d*-tubocurarine in concentrations between 10^{-12} and 10^{-11} g/l was found to increase miniature endplate potential (MEPP) frequency with no effect on MEPP amplitude. The agent at 10^{-10} g/l was able to increase the twitch height of the submaximally stimulated muscle preparation. Between 2.5×10^{-9} and 5×10^{-7} g/l, it decreased MEPP frequency and twitch height of indirectly-stimulated muscle preparations to 10 per cent of control. At the latter doses, MEPP amplitude and sensitivity to iontophoretically-applied acetylcholine were reduced by only about 40 per cent. At this degree of depression of endplate sensitivity, the ability of postjunctional membrane to generate an action potential is unaltered. It is believed that the primary blocking effect of *d*-tubocurarine is presynaptic. (Key words: *d*-Tubocurarine; Nerve terminal; Presynaptic; Neuromuscular blockade.)

SINCE THE EARLY WORK of Bernard,[1] *d*-tubocurarine has been assumed to have solely postsynaptic effects on neuromuscular transmission. The studies by Fatt and Katz[2] and Auerbach and Betz[3] using microelectrode techniques also suggest a primary postsynaptic locus of action. However, the work of Riker and Standaert[4,5] has suggested that this drug has definite nerve-terminal activity. Galindo[6] has offered evidence that the primary blocking effect of *d*-tubocurarine is prejunctional. Recently, Gergis *et al.*,[7] Beani *et al.*,[8] and Hubbard *et al.*[9] showed that, while causing neuromuscular blockade, it produced a concomitant decrease in release of acetylcholine from the nerve terminal. The purpose of this work was to examine the effects of a wide range of concentrations of *d*-tubocurarine on spontaneous acetylcholine release, twitch height, and endplate sensitivity to acetylcholine in order to gain more information regarding the exact site of action of *d*-tubocurarine.

Methods

Microelectrode Studies

All experiments were carried out *in vitro* with the isolated frog (*Rana pipiens*) sartorius muscle. The preparation was mounted in a lucite bath of 3-ml volume on a paraffin-lined plexiglass plate, in the center of which was a planoconvex lens. The muscle was constantly perfused (flow rate 7 ml/min) with frog Ringer's solution (NaCl 116, KCl 1.9, $CaCl_2$ 1.0, NaH_2PO_4 1.0, Na_2HPO_4 1.0, mM/l). Intracellular potentials were measured with glass capillary microelectrodes of 8 to 15 MΩ resistance filled with 3 M KCl in a conventional manner similar to that employed by del Castillo and Katz[10] and Akerman and Sokoll.[11] Potentials were monitored on a Tektronix 564B storage oscilloscope and recorded with an Elema-Schonander Mingograf 81 (a polygraph having a flat frequency response to 500 Hz). The time constant of the recording circuit was approximately 40 μsec. Endplate regions were located by finding miniature endplate potentials (MEPP's) with a rapid rising phase. The MEPP's were recorded for continuous one-minute intervals and analyzed for frequency and amplitude before and after application of *d*-tubocurarine (Sigma Chemical Co.).

* Associate Professor of Anesthesia.
† Predoctoral Fellow in Pharmacology.
‡ Assistant Professor of Anesthesia.
§ Professor of Pharmacology.
Received from the Departments of Anesthesiology and Pharmacology, School of Medicine, University of Iowa, Iowa City, Iowa 52240. Accepted for publication December 1, 1971. Supported in part by USPHS grants NS4431 and NS1396 and USPHS training grant 5 T0 1 GM-000141-15 and the Department of Anesthesia Trust Fund.

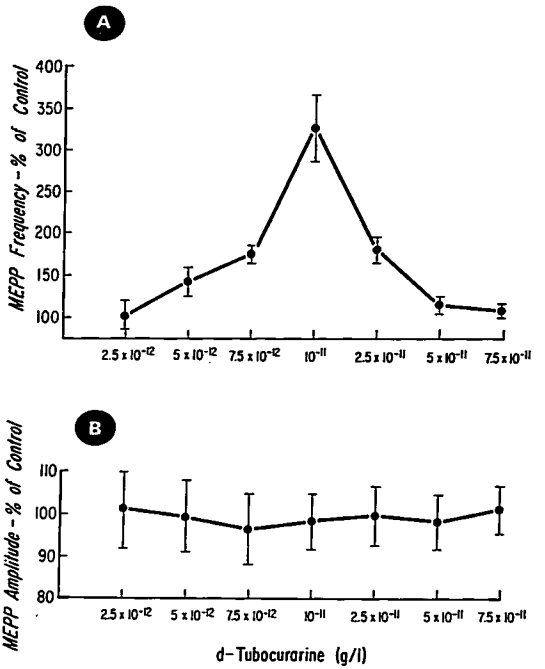

FIG. 1. Effects of *d*-tubocurarine, between 10^{-12} and 10^{-11} g/l, on MEPP frequency and amplitude. *A*, frequency; *B*, amplitude. Values = mean ±SE. n = 10. $P < 0.05$.

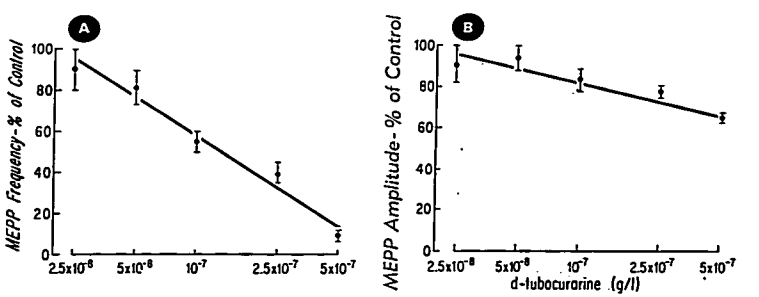

FIG. 2. Effects of *d*-tubocurarine, between 2.5×10^{-8} and 5×10^{-7} g/l, on MEPP frequency and amplitude. *A*, frequency; *B*, amplitude. Values = mean ±SE. n = 10. $P < 0.05$.

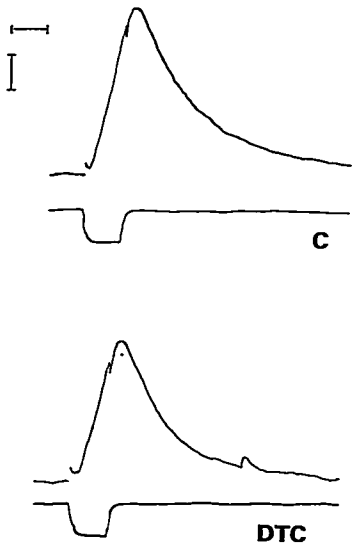

FIG. 3. Typical record of the effect of *d*-tubocurarine, 10^{-7} g/l, on the sensitivity of the end-plate to iontophoretically-applied acetylcholine. The top trace represents change in membrane potential, while the bottom trace represents the current pulse. C, control; DTC, *d*-tubocurarine. Horizontal bar = 10 msec; vertical bar = 10^{-8} amperes.

ACETYLCHOLINE SENSITIVITY

The method of assaying the effect of iontophoretic microapplication of acetylcholine was similar to that employed by del Castillo and Katz.[6] The pipettes were filled with a 4-M solution of acetylcholine which was delivered by current pulses of 10 msec in duration. Diffusion of acetylcholine from the tip was prevented by the application of a steady dc current. Acetylcholine sensitivity was expressed as the ratio of depolarization of the membrane in mV to the charge in coulombs $\times 10^{-9}$ (nC) which flowed through the acetylcholine pipette.

Maximal acetylocholine sensitivity readings were obtained before and after application of the *d*-tubocurarine-containing solution. Changes observed were expressed as per cent of control.

FROG SCIATIC NERVE–SARTORIUS MUSCLE PREPARATIONS

The sartorius muscle with its nerve was carefully removed and placed in a horizontal chamber of 20-ml volume. The same bathing medium was employed as in the microelectrode studies. The tendon was attached to a Grass FT-03 strain gauge by means of a pulley, the origin being firmly fixed. Conditions for nerve stimulation were 0.2 Hz, 0.1-msec duration, and 3 v. Force of contraction was measured on a Grass polygraph. All preparations were subjected to an initial tension of 1 g.

After the muscle twitch had stabilized, *d*-tubocurarine was added to the bath and the amount of blockade determined. Changes were expressed as per cent of control.

In another series of experiments the voltage was adjusted so that twitch height was half maximal. Physostigmine or *d*-isopropylfluorophosphate (DFP) in a concentration of 0.1 μg/ml was added to the Ringer's solution to inhibit the action of acetylcholinesterase. After stabilization of twitch height, *d*-tubocurarine in concentration ranging from 10^{-12} to 10^{-9} was added to the bath.

STATISTICS

Regression analysis of variance (Steel and Torrie[12]) was employed for most comparisons. When the regression was significant, the best-fitting straight line was drawn. In every instance $P \leq 0.05$.

Results

At drug concentrations between 10^{-15} and 10^{-12} g/l there was no effect on MEPP frequency or amplitude. In the range of concentrations from 10^{-12} to 10^{-11} g/l, there was a marked increase in MEPP frequency with no change in amplitude (fig. 1). The increase in frequency was seen within a few seconds following application of *d*-tubocurarine and was maintained until the drug was removed. At 10^{-10} g/l, frequency returned to slightly above control levels. Between 2.5×10^{-8} and 5×10^{-7} g/l there was a linear decrease in frequency to 10 per cent of control (fig. 2A).

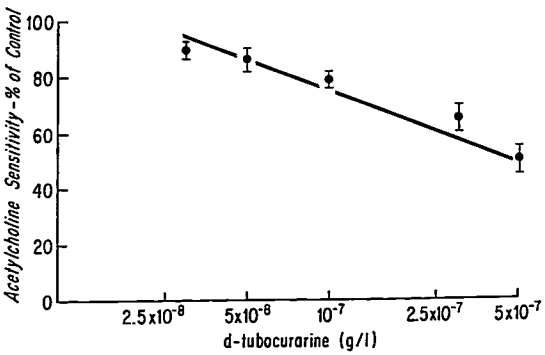

FIG. 4. Effects of *d*-tubocurarine, between 2.5×10^{-8} and 5×10^{-7} g/l, on the sensitivity of the endplate to iontophoretically-applied acetylcholine. Values = mean ±SE. n = 10. $P < 0.05$.

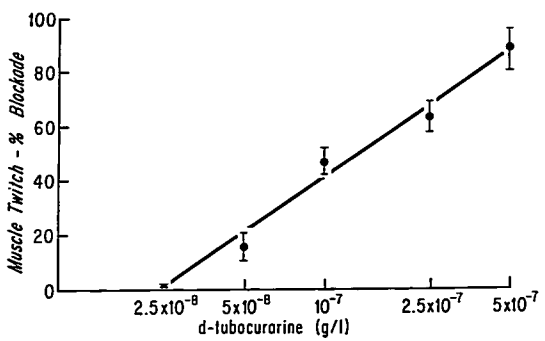

FIG. 5. Effects of *d*-tubocurarine, between 2.5×10^{-8} and 5×10^{-7} g/l, on the twitch height of the indirectly-stimulated sartorius muscle preparation. Values = ±SE. n = 8. $P < 0.05$.

At these concentrations the greatest reduction in amplitude was about 35 per cent (fig. 2*B*).

The sensitivity of the endplate region to focal iontophoretic application of acetylcholine between concentrations of 2.5×10^{-8} and 5×10^{-7} g/l was investigated. A typical record can be seen in figure 3, where the control sensitivity was 53 millivolts per nC and that after *d*-tubocurarine, 10^{-7} g/l, was 44 mv/nC. At the highest drug concentration, endplate sensitivity was reduced by only about 40 per cent (fig. 4). Between 2.5×10^{-8} and 5×10^{-7} g/l there was a close correlation between reductions in endplate sensitivity and MEPP amplitude. The iontophoretic application of acetylcholine did not affect MEPP amplitude or frequency at the drug concentrations studied.

At the same concentrations, there was a progressive decline in twitch height of the indirectly stimulated preparation (fig. 5). Maximum blockade was obtained 5 minutes after the introduction of *d*-tubocurarine. Again, there was a close correlation between reductions in twitch height and MEPP frequency.

When a dose of 10^{-10} g/l was applied to five submaximally indirectly-stimulated muscle preparations there was an immediate increase in twitch height, which was sustained until the drug was removed (fig. 6). This effect was

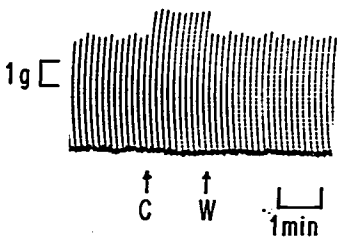

FIG. 6. Typical record of the effect of *d*-tubocurarine, 10^{-10} g/l, on twitch height of indirectly-stimulated sartorius muscle preparation. C, *d*-tubocurarine; W, wash.

not seen at the other small doses used in this study or at this dose without inhibition of cholinesterase.

Discussion

The legitimacy of using iontophoresis of acetylcholine on the endplate to evaluate solely the postsynaptic site of action has been questioned.[5] The concern is that the applied agent might also have effects on the nerve that result in further release of endogenous acetylcholine. However, Albuquerque and McIsaac[12] found no significant difference between the degrees of responsiveness of innervated and chronically denervated rat extensor digitorum longus and soleus muscles to focally-applied acetylcholine. Furthermore, if the motor-nerve-terminal action of acetylcholine proposed by Riker and Standaert[5] were true, the application of acetylcholine might be expected to increase MEPP frequency. This was found not to occur.

It has usually been assumed that the decrease in MEPP frequency seen after the application of *d*-tubocurarine is owing to the decreased sensitivity of the endplate. Thus, the smaller MEPP's would be decreased below the noise level of the recording. That this may not be completely true has been demonstrated by Gergis *et al.*,[7] who observed decreased output of acetylcholine after *d*-tubocurarine. This observation suggests that the decrease in MEPP frequency may be due to decreased release of quanta from the nerve terminal.

The data indicate that there are corresponding decreases in MEPP frequency and twitch height. Even with a dose of *d*-tubocurarine which caused a 95 per cent reduction in twitch height, there was only 40 per cent reduction in MEPP amplitude and sensitivity to iontophoretically-applied acetylcholine. Considering that the size of the normal endplate potential is in the range of 80–90 mv (Fatt and Katz,[2] del Castillo and Katz[14]), then a 40 per cent decrease would leave an EPP in the order of 51 mv. According to Fatt and Katz,[2] an EPP of 36 mv is sufficient to generate an action potential. Therefore, concentrations of *d*-tubocurarine which produce as much as 95 per cent muscle blockade should not block the ability of the postsynaptic membrane to generate an action potential and should be acting presynaptically.

An interesting observation was that small doses of *d*-tubocurarine increased MEPP frequency with no alteration in their amplitude. This would suggest an increased presynaptic release of acetylcholine. This is substantiated by the fact that these small doses were actually able to increase the size of the muscle twitch in the submaximally stimulated preparation.

In conclusion, *d*-tubocurarine markedly affects twitch height and MEPP frequency in doses which do not block the ability of the postsynaptic membrane to generate an action potential. In small doses it even increases the presynaptic output of acetylcholine. Concurring with Galindo,[6] we believe that the primary blocking effect of *d*-tubocurarine in presynaptic.

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

References

1. Bernard D: Analyse physiologique des proprietes des systemes muxculaire et nerveux au moyer du curare. CR Acad Sci [D] (Paris) 43:825–829, 1856
2. Fatt P, Katz B: An analysis of the end-plate potential recorded with intra-cellular electrode. J Physiol 115:320–370, 1951
3. Auerbach A, Betz W: Does curare affect transmitter release. J Physiol 213:691–705, 1971
4. Standaert FG, Riker WF: The consequences of cholinergic drug actions on motor nerve terminals. NY Acad Sci 144:517–533, 1967

5. Standaert F: The action of *d*-tubocurarine on the motor nerve terminal. J Pharmacol Exp Ther 143:181–191, 1964
6. Galindo A: Prejunctional effect of curare, its relative importance. J Neurophysiol 34: 289–301, 1971
7. Gergis SD, Dretchen KL, Sokoll MD, et al: The effect of neuromuscular blocking agents on acetylcholine release. Proc Soc Exp Biol Med 138:693–695, 1971
8. Beani L, Bianchi C, Ledda F: The effect of tubocurarine on acetylcholine release from motor nerve terminals. J Physiol 174:172–183, 1964
9. Hubbard JI, Wilson DF, Miyamoto M: Reduction of transmitter release by *d*-tubocurarine. Nature 223:531–532, 1969
10. del Castillo J, Katz B: On the localization of acetylcholine receptors. J Physiol 128:157–181, 1955
11. Akerman B, Sokoll MD: The effect of a pair of enantiomers with local anesthetic activity on action potential generation and acetylcholine sensitivity in muscle. Eur J Pharmacol 8:331–336, 1969
12. Steel R, Torrie JH: Principles and Procedures of Statistics. First edition. New York, McGraw-Hill Book Co., 1960, pp 67–109
13. Albuquerque EX, McIsaac RJ: Early development of acetylcholine receptors on fast and slow mammalian skeletal muscle. Life Sci 8:409–416, 1969
14. del Castillo J, Katz B: Quantal components of the end-plate potential. J Physiol 124:560–573, 1954

The Anesthesiologist's Bookshelf

Control Theory and Physiological Feedback Mechanisms. By Douglas S. Riggs. Baltimore, Williams and Wilkins Co., 1971. Pp. 599. $7.50.

Don't let the title fool you, for this is no dull ordinary biomathematics treatise. It is a lively and very readable text, written by a bioscientist for bioscientists. The author's unorthodox background, revealed in the introduction, tells much about what is to come. Dr. Riggs is a physician who resigned his post as chairman of a department of pharmacology to become a graduate student in mathematics, where his introduction to control theory resulted in this excellent volume addressed to the systems analysis of biomedical problems.

The great value of this book to anesthesiologists is that it applies control theory in understandable fashion to topics as disparate as uptake, distribution and excretion, chemical control of respiration, mechanics of circulation, and tracer kinetics. Control theory provides novel approaches to input–output relations, feedback and control in complex network systems. This is the science of the future; better get aboard now or be left behind.

In Chapter 2, the beauty of Riggs' approach begins to emerge. By unifying such seemingly unrelated physical systems as electrical, hydrodynamic and concentrational (fluid and gas flow), he brings identical formulas to bear on problems as diverse as inertia in a rotational system and volume of distribution in pharmacology. Further, he simplifies the units of flow, pressure, concentration, etc., into two simple categories: A(across)-variables such as voltage and concentration, and T(through)-variables such as current or gas and solute flow. From these two variables he builds up other system units such as drug clearance, resistance and distribution volume. Once you get the hang of this unified approach, the whole concept falls into place. Now one may tie engineering problems (they have come a lot further than we) to biological situations; for example, velocity in an engineering system is mathematically identical to alveolar concentration and also to systolic pressure.

Nearly all illustrative examples are pertinent to clinical pharmacology and physiology, for they deal with drug, gas and tracer kinetics, respiration, circulation, and so on. Here is a chance to absorb the fundamentals of biological control theory without having to become an engineer in the process. See, for instance, how cleverly the author introduces transfer function analysis in a nonlinear system with the following "Horrible Example" (page 190): "Bartender! A quadruple martini for the reader! . . . That's as close to an impulse input of alcohol as you're ever likely to get."

Yet, this is no easy book to digest. It needs chewing and rechewing. It presupposes minimum college calculus, sufficient to solve simple differential equations. (For those wishing to refresh their calculus, I can recommend the very readable programmed text, "Quick Calculus," by Kleppner and Ramsey; New York, Wiley, 1965.) While some review of essentials such as solving simultaneous linear equations and rationalizing complex numbers is provided in the appendix, there are times when the author might have proceeded a bit more slowly. If one isn't familiar with Laplace transforms, the going in Chapter 3 is as rough as the title, "On Crossing the Atlantic in a Sailing Dinghy," promises. Yet the author's de-

(Continued on page 611)