Effect of Anesthetics on Acetylcholine Release from the Myoneural Junction[1] (36838)

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It has been reported that diethyl ether as well as other intravenous and inhalation anesthetics have varying depressant actions on neuromuscular transmission (1–3). With respect to diethyl ether this has been ascribed to a curare like action on the postjuctional membrane (4, 5). However, in recent years curare as well as other competitive neuromuscular blocking agents have been shown to have depressant action on the motor nerve terminal resulting in reduction of acetylcholine output (6, 7).

This work was conducted to elucidate the effects of some intravenous and inhalation anesthetics on acetylcholine output and thus gain a direct measure of activity on the nerve terminal. If they have a nerve terminal action, then they should reduce the amount of acetylcholine released upon nerve stimulation.

Methods. Frog sciatic nerve gastrocnemius muscle preparation. Frogs weighing between 20 and 25 g of the species *Rana pipiens* were stunned and decapitated. The skin was removed exposing the gastrocnemius muscle. The sciatic nerve was dissected free, ligated and cut. The gastrocnemius 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 100% O_2. The intravenous anesthetics were added directly to the bath. The inhalation agents were vaporized in a copper kettle and delivered to the bath mixed with 100% O_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. The frogs were divided into six groups. Two muscle preparations were obtained from each animal. One of the preparations served as control while the other was tested with a particular anesthetic concentration bubbled through the bathing medium. With those agents which produced neuromuscular blockade two concentrations of each were employed, one producing less than 50% muscle blockade and the other more than 50% blockade. The anesthetics employed in the study were: diethyl ether, halothane, methoxyflurane, Forane, thiopental and ketamine.

After 0.5 hr of stimulation, samples from the bath were assayed for acetylcholine content using the superfused guinea pig ileum and compared with acetylcholine standards, and the height of the muscle twitch was measured. 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 and 350 g were stunned by a blow to the back of the head and a 20–30 mm segment of the ileum was

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TABLE I. Effect of Various Anesthetics on Muscle Twitch and Acetylcholine Release.

Drug and concn	Control acetylcholine released (μg/g muscle)	Acetylcholine released with anesthetic	Muscle contraction (% of control)
Diethyl ether (%)			
3	1.77 ± 0.83[a]	1.69 ± 0.74[b]	74.7 ± 5.0[c]
6	1.24 ± 2.0	1.28 ± 0.15[b]	24.9 ± 7.9[c]
Methoxyflurane (%)			
0.1	1.31 ± 0.18	1.27 ± 0.12[b]	81.0 ± 5.6[c]
0.3	1.16 ± 0.13	1.11 ± 0.15[b]	18.6 ± 6.3[c]
Thiopental (mg/ml)			
10	1.40 ± 0.28	1.32 ± 0.21[b]	74.9 ± 5.4[c]
100	1.23 ± 0.17	1.27 ± 0.16[b]	25.0 ± 3.1[c]

[a] Values represent mean ± SE, $N = 5$.
[b] Not significant, $p \geq .05$.
[c] Significant, $p < .05$.

removed. One end of the ileum was fastened to a stationary rod while the other 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 (8) with eserinized Tyrode's solution (NaCl, 136.87; KCl, 2.68; $CaCl_2 2H_2O$, 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.

Statistics. Comparisons were made with the use of Student's t test, paired comparisons (9).

Results. Thiopental in the range of concentrations which depressed the height of the muscle twitch did not alter the amount of acetylcholine released as measured on the guinea pig ileum (Table I). The volatile anesthetics diethyl ether and methoxyflurane also produced a dose related depression in the muscle twitch height without any effect on the acetylcholine output (Table I).

Ketamine in concentrations up to 100 mg/ml had no effect on either muscle twitch height or acetylcholine release. In concentrations up to 10% halothane and Forane were also devoid of any action on these two parameters.

Discussion. The data indicate that none of the six anesthetic agents tested had any effect on the acethylcholine release and only diethyl ether, methoxyflurane and thiopental produced depression of the twitch height of the indirectly stimulated muscle. Thus it appears that these agents have little or no effect on the nerve terminal.

Somjen (10, 11) and Katz (12) suggested that the muscle relaxation seen with ether anesthesia is not due to a direct action on the neuromuscular junction. However, Karis, Grissen and Nastuk (13) stated that ether depresses neuromuscular transmission by an action on the endplate. The present work also suggests an action on the endplate. Ngai, Hanks and Farhie (14) attribute the muscle relaxation obtained with methoxyflurane anesthesia to depression of spinal cord reflexes; however the present results would indicate a direct action on the myoneural junction as well.

Thesleff (15) has also shown that the barbiturates depress neuromuscular transmission by an action on the postjunctional membrane.

On the other hand, it appears as if halothane and Forane do not affect neuromuscular transmission. Miller *et al.* (16) and Watland *et al.* (1) also found that these agents do not alter the height of the muscle

twitch. Ketamine has been reported to have a slight depressant action on the endplate and not to affect the height of the muscle twitch (17). The present work also agrees with these results.

Some of the antibiotics such as neomycin and streptomycin have been shown to depress the twitch height with no effect on acetylcholine output (18). Thus it would appear as if the antibiotics and anesthetics have similar actions on neuromuscular transmission in that neither type of agents depress acetylcholine output but do depress muscle twitch. This is in contrast to the competitive blocking agents which have been shown to diminish acetylcholine output (6, 7). The interaction of the anesthetics and antibiotics with the latter agents might be due to a combination of the pre- and postsynaptic actions.

1. Watland, D. S., Long, J. P., Pittinger, C. B., and Cullen, S. C., Anesthesiology **18**, 883 (1957).

2. Borgman, R. L., Walker, T. R., Pittinger, C. B., and Long, J. P., Anesthesiology **21**, 150 (1960).

3. Goodman, L. S., and Gillman, A., "The Pharmacological Basis of Therapeutics," p. 613. Macmillan, New York (1970).

4. Secher, O., Acta Pharmacol. Toxicol. **7**, 83 (1951).

5. Secher, O., Acta Pharmacol. Toxicol. **7**, 103 (1951).

6. Gergis, S. D., Dretchen, K. L., Sokoll, M. D., and Long, J. P., Proc. Soc. Exp. Biol. Med. **138**, 693 (1971).

7. Gergis, S. D., Dretchen, K. L., Sokoll, M. D., and Long, J. P., Proc. Soc. Exp. Biol. Med. **139**, 74 (1972).

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

9. Steel, R. G. D., and Torrie, J. H., "Principles and Procedure of Statistics," p. 67. McGraw-Hill, New York (1960).

10. Somjen, G. G., J. Pharmacol. Exp. Ther. **140**, 396 (1963).

11. Somjen, G. G., J. Pharmacol Exp. Ther. **140**, 19 (1963).

12. Katz, R. L., Anesthesiology **27**, 52 (1966).

13. Karis, J. H., Grissen, A. J., and Nastuk, W. L., Anesthesiology **27**, 42 (1966).

14. Ngai, S. H., Hanks, E. C., and Farhie, S. E., Anesthesiology **26**, 162 (1965).

15. Thesleff, S., Acta Physiol. Scand. **37**, 335 (1956).

16. Miller, R. D., Eger, E. I., Way, W. L., Stevens, W. C., and Dolan, W. M., Anesthesiology **35**, 38 (1971).

17. Cronnelly, R., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **31**, 535 Abstr. (1972).

18. Dretchen, K. L., Gergis, S. D., Sokoll, M. D., and Long, J. P., Eur. J. Pharmacol. **18**, 201 (1972).

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