Effect of Pancuronium Bromide on Acetylcholine Release[1] (36080)

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Pancuronium bromide had originally been shown by Buckett and Bonta in 1966 (1) and by Buckett *et al.* (2) in 1968 to have a nondepolarizing neuromuscular blocking action similar to curare. This was confirmed by Lewis *et al.* (3) in 1967. However, recently Sohn and Aldrete (4) have suggested that the drug might be acting presynaptically. The purpose of this work was to test the hypothesis that this agent did in fact act presynaptically. If it does then it should affect the acetylcholine release from the nerve terminal.

Methods. Bioassay of acetylcholine. The amount of acetylcholine released in the frog sciatic nerve gastrocnemius muscle preparation was bioassayed on the guinea pig ileum according to the method described by Gergis *et al.* (5).

Pancuronium bromide was added to the medium bathing the nerve muscle preparation in different concentrations and the amount of acetylcholine released, with or without nerve stimulation, was measured.

In another group of experiments, the effect of tetrodotoxin, high magnesium, low calcium, and hemicholinium on pancuronium-treated nerve muscle preparation was studied. The first three agents were tested without nerve stimulation, while with hemicholinium the nerve was stimulated until there was a 50% reduction in muscle twitch height, then stimulation was stopped, and pancuronium was added.

In all cases, two preparations were obtained from each animal, one was tested for the particular experimental procedure while the other served as its control.

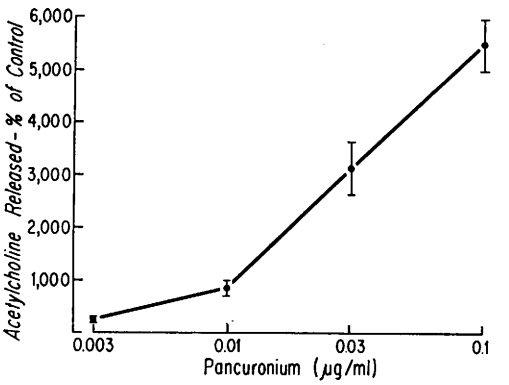

FIG. 1. Effect of pancuronium on acetylcholine release from the frog sciatic nerve gastrocnemius muscle preparation: values = mean ± SE, $n = 5$, $p < .05$.

Statistics. For most comparisons Student's t test or analysis of variance was used (6). In all cases the level of probability was $p \leq .05$.

Results. Pancuronium bromide in concentrations ranging from 0.003 to 0.1 μg/ml produced a dose-related increase in the acetylcholine release in the nonstimulated muscle preparation (Fig. 1). There was no further increase in the acetylcholine release when the drug was added to the indirectly stimulated muscle preparation. At these concentrations, there was no effect on the twitch height. At concentrations of pancuronium which produced muscle blockade, 1 and 5 μg/ml, there was a significant decrease rather than an increase in the presynaptic output of acetylcholine (Table I).

The pancuronium-induced acetylcholine release was not affected by the addition of tetrodotoxin or hemicholinium to the bathing solution. It was, however, significantly reduced when the magnesium ion content was increased or the calcium ion content was re-

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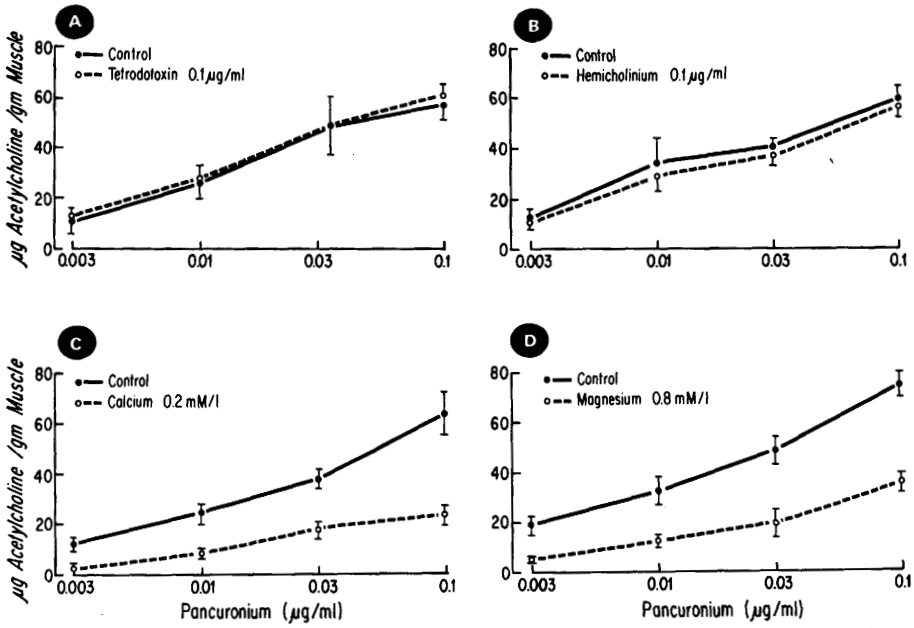

FIG. 2. Effect of various neuromuscular blocking agents on the pancuronium induced release of acetylcholine: (A) tetrodotoxin, 0.1 μg/ml; (B) hemicholinium, 0.1 μg/ml; (C) calcium, 0.2 mmoles/liter in frog Ringer's; (D) magnesium, 0.8 mmoles/liter in frog Ringer's; (—) pancuronium; (- - -) pancuronium plus the blocking agent; values = mean ± SE, $n = 5$, $p < .05$.

TABLE I. Effect of High Concentrations of Pancuronium on Twitch Height and Acetylcholine Release from the Frog Sciatic Nerve Gastrocnemius Muscle.

Pancuronium conc (μg/ml)	Twitch height (% of control)	Acetylcholine content (% of control)
1	84.2 ± 4.4[a]	42.3 ± 6.5[b]
5	3.4 ± 1.8	7.3 ± 3.1[b]

[a] Values = mean ± SE, $n = 5$.

[b] Denotes significance from control, Student's t test, $p < .05$.

duced to one-fourth its original value (Fig. 2).

Discussion. In low concentrations, pancuronium produces a marked increase in the presynaptic release of acetylcholine with no effect on the twitch height. The mechanism does not appear to be related to synthesis of the transmitter or to the nerve action potential since both tetrodotoxin and hemicholinium have no effect. In addition, nerve stimulation is not even required for the action of the drug. However, the entry of calcium into the nerve terminal seems to be essential for this action of the agent since the addition of high magnesium or the reduction in the calcium concentration of the bathing medium affects the drug-induced release of acetylcholine.

In high concentrations, there is a concomitant reduction in the release of acetylcholine with muscle blockade. This would suggest a presynaptic site of action for neuromuscular blockade. This action is similar to that seen with curare and gallamine (5).

In summary, pancuronium has a marked effect on the presynaptic terminal. It releases acetylcholine in low concentrations and blocks the release in concentrations that produce muscle blockade. The mechanism by which the drug can exert these two opposing effects still remains to be elucidated.

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1. Buckett, W. R., and Bonta, I. L., Fed. Proc., Fed. Amer. Soc. Exp. Biol. 25, 718 (1966).

2. Buckett, W. R., Marjoribank, C. E. B.,

Marwick, F. A., and Morton, M. B., Brit. J. Pharmacol. Chemother. **32**, 671 (1968).

3. Lewis, J. J., Martin-Smith, M., Muir, T. C., and Ross, H. H., J. Pharm. Pharmacol. **19**, 502 (1967).

4. Sohn, Y. J., and Aldrete, J. A., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **30**, 557 (1971).

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

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

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