Anesthesiology
V 53, No 3, Sept 1980

Title : EDROPHONIUM: PRE- AND POSTSYNAPTIC ACTIONS

Authors : S. M. Sarracino, Ph.D. and M. D. Sokoll, M.D.

Affiliation: Department of Anesthesia, Univeristy of Iowa Hospitals and Clinics, Iowa City, Iowa 52242

Introduction: Anticholinesterases are widely used as medicines and insecticides. Some 40 years ago Eccles et al[2] demonstrated that anticholinesterases increase both the duration and amplitude of end plate potentials. However, their mechanism of action at the neuromuscular junction has not been sufficiently clarified; in addition, the data on their presynaptic actions is contradictory. The effect of a reversible anticholinesterase, edrophonium, on parameters reflecting both presynaptic and postsynaptic actions was examined. These studies were designed to corroborate earlier observations of its anticholinesterase actions, to examine possible presynaptic actions and to characterize the mechanism at higher concentrations, where it might also affect receptor blockade.

Methods: Either the extensor digitorum longus (EDL) or the left hemidiaphragm and the attached phrenic nerve was exised from Sprague-Dawley rats weighing 200-250 gms. Miniature end plate potential (MEPP) amplitude and frequency were recorded in the EDL using standard microelectrode techniques. In the phrenic nerve-hemidiaphragm preparation, nerve transmission was blocked by cutting muscle fibers, as described by Barstad[1]. End plate potentials (EPPs) were evoked by nerve stimulation at 50 Hz. The mean end plate potential amplitude, the average number of quanta in the tetanic train, the mobilization rate and quantum size were determined from measurements of the amplitudes of EPPs #31-50. Quantal content in the first EPP, the size of the readily releasable store and the probability of release were estimated by the method of Elmqvist and Quastel[3]. All EPPs were corrected for nonlinear summation as described by Martin[6]. The average reversal potential used in the correction (determined experimentally) was 0.0 mV $\pm$ 5.0 mV. Both preparations were perfused with oxygenated (95% O_2 - 5% CO_2) rat ringers. Edrophonium chloride was obtained as a gift from Hoffman-LaoChe, Inc.. Concentrations of drug ranged from 10^{-7}M to $5x10^{-4}$M.

Results: MEPP amplitude was 115% of control at $5x10^{-6}$M, 140% of control at 10^{-5}M, 40% of control at 10^{-4}M and abolished at $5x10^{-4}$M. Concomitantly MEPP frequency was unchanged over the dose range used until a significant decrease of MEPP amplitude was observed at 10^{-4}M. Resting membrane potentials (RMP) ranged from 70-90 mV and were unaffected by the drug. An analysis of histograms of MEPP amplitudes revealed a shift towards larger MEPPs and an increased occurrence of giant MEPPs at 10^{-5}M. For a typical experiment 96% of the MEPPs had amplitudes ranging from .2 mV to .5 mV, with the greatest percentage having an amplitude of .35 mV. After the 10 minute application of 10^{-5}M edrophonium, 64% of the MEPPs had amplitudes in the same range, while 36% of them had amplitudes ranging from .5 mV to 1.7 mV. At drug concentrations up to $5x10^{-5}$M, mean EPP amplitude was not different from control values. At higher drug concentrations, 10^{-4}M and $5x10^{-4}$M, mean EPP amplitude decreased to approximately 25% of control. RMP remained unchanged at all drug concentrations. The time for the EPP to decay to 1/2 maximal amplitude increases with increasing drug concentrations above 10^{-5}M. In a representative experiment the time to 1/2 maximal amplitude increased 190% at $5x10^{-5}$M and 300% at 10^{-4}M. Lastly the effect of edrophonium on presynaptic nerve terminals was examined. Most importantly, quantum size was unchanged at all edrophonium concentrations. Secondly, the average number of quanta released in the series of EPPs, the mobilization rate, the quantal content of the first EPP and the size of releasable store remained unchanged from control values up to approximately 10^{-4}M. At the highest concentrations, all four were decreased. Up to 10^{-4}M, the probability of release was not different from control. At the two highest concentrations of drug, the fractional release was much greater.

Discussion: The increase in MEPP amplitude at lower drug concentrations without a change in MEPP frequency is consistent with inhibition of acetylcholinesterase. The lengthening effect of the drug on EPP decay is also typical of anticholinesterases (Eccles et al[2], Goldner et al[4]). The decrease in EPP amplitude supports the findings of Goldner and Narahashi[4] and those of Katz and Thesleff[5] where high drug concentrations of edrophonium applied for long periods of time made the end plate receptor less sensitive to acetylcholine. The data from the nerve-diaphragm preparation in which nerve terminal effects of the drug were examined suggest that at higher drug concentrations the decrease in MEPP amplitude accompanied by a decrease in the MEPP frequency may result in lowered neurotransmitter stores. The increase in the fractional release at higher drug concentrations suggests that a greater fraction of the already depleted neurotransmitter store is released in the initial EPP. The decrease in MEPP amplitude taken together with the unchanged quantum size at higher drug concentrations is consistent with receptor blockade at those concentrations.

References:

1. Barstad JAB: Presynaptic effect of neuromuscular transmitters. Experientia 18:579-581, 1962
2. Eccles JC, Katz B and Kuffler SW: Effect of eserine on neuromuscular transmission. J Neurophy 5: 211-230, 1942
3. Elmqvist D and Quastel DMJ: A quantitative study of end plate potentials in the isolated human muscle. J Physiol 178:505-529, 1965
4. Goldner MM and Narahashi T: Effects of edrophonium on end plate currents in frog skeletal muscle. Eur J Pharm 25:362-371, 1974
5. Katz B and Thesleff S: The interaction between edrophonium (Tensilon) and acetylcholine at the moter end plate. Brit J Pharm 12:260-264, 1957
6. Martin AR: A further study of the statistical composition of the end-plate potential. J Physiol 130:114-122, 1955