Reduction of the Quantum Content of Endplate Potentials by Atropine

Atropine is best known as a blocker of the muscarinic actions of acetylcholine[1] (ACh). In higher concentrations it also blocks the action of ACh upon the nicotinic receptors[2,3] and recent investigators of this action have uncovered the surprising fact that during atropine blockade of neuromuscular transmission the equilibrium potential for the endplate potential (EPP) is shifted toward the sodium equilibrium potential[4]. The equilibrium potential for ACh and other choline esters applied iontophoretically to the endplate region, however, is not similarly shifted[5].

One explanation for this startling finding[4] could be that other transmitters besides ACh are liberated by nerve impulses and that atropine blocks the release of ACh preferentially. This explanation, although considered by MAGAZANIK and VYSKOČIL[5], was rejected on the ground that investigation[2] had revealed no effect of atropine on the quantum content of EPP's. Unfortunately, however, the experiments upon quantum content[2] were done in the presence of a high concentration of Mg which in itself greatly reduces the quantum content of EPP by a presynaptic action. It could well be that the presence of Mg also blocked the presynaptic action of atropine. Indeed, it has recently been found that D-tubocurarine reduces the number of ACh quanta released by nerve impulses[6] and this action was not demonstrable in the presence of high concentrations of $MgCl_2$[2,7].

The cut muscle preparation[8] offers a means of finding out whether drugs have presynaptic actions without first blocking neuromuscular transmission. In the current investigation we used the rat diaphragm phrenic nerve preparation in vitro with the muscle fibres cut so that the resting potentials were below the level at which action potentials can be generated. As in a previous investigation[6], 40 stimuli at 100/s were given to the muscle nerve and the evoked trains of EPP were recorded intracellularly. The amplitudes of the first 5 and last 20 of the forty EPP were measured and using MARTIN's correction[9] for the large size of the EPP, the variance of the last 20 EPP was calculated and from this mean quantum size (q) was estimated. 5 or more serial estimates of q were pooled and used to calculate the quantal content of the first EPP in each train (m) and the mean quantum content of the last 20 EPP, which when multiplied by the 100/s stimulation rate gave an estimate of the rate of mobilization of transmitter (dm). From the quantal content of the first 5 EPP the available store of quanta (n) was calculated according to ELMQVIST and QUASTEL[10] and this quantity divided by m yielded p, the fraction of the store released by the first impulse.

Figure 1 shows typical EPP responses to a stimulus train in a rat diaphragm cut muscle preparation before, during and 61 min after the end of the exposure to $1.3 \times 10^{-4} M$ atropine sulphate, USP (Sigma Chemical Co.). After 15 min exposure to the drug (Figure 1, B), the EPP pattern is typical of atropinized preparations. The first response is the largest and later responses decline to a plateau level of amplitude which is much smaller than the amplitude of the first EPP. Before and after this exposure, however, the typical pattern seen in cut muscle[6] was found (Figure 1, A and C). EPP amplitudes declined more slowly and the plateau EPP's were relatively much larger. EPP showed a lesser variability in amplitude indicating a higher quantal content.

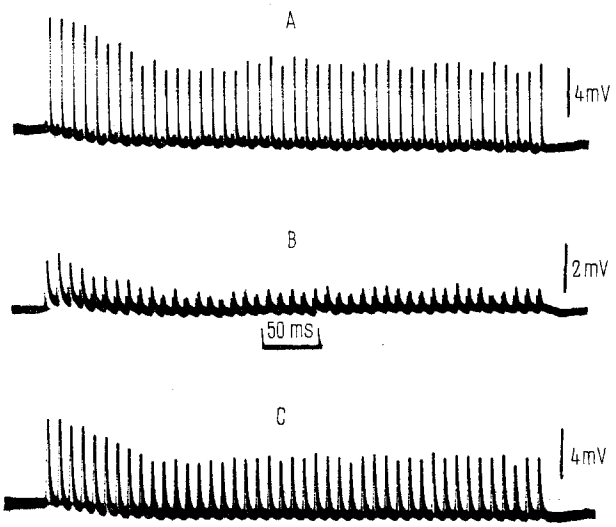

Fig. 1. EPP's intracellularly recorded from an endplate of a rat diaphragm cut muscle preparation following 40 stimuli at 100/s to the phrenic nerve. A, control; B, 15 min after $1.3 \times 10^{-4} M$ atropine sulfate added to the bathing solution; C, 61 min after return to control solution. Temperature 32 °C. A, B, C, recorded from the same endplate.

Mean quantal content of endplate potentials and the effect of blocking drugs

Drug	Cut muscle				Normal muscle
	None	D-tubocurarine (4×10^{-7} g/ml)	Atropine ($6 \times 10^{-5} M$)	Atropine ($1.3 \times 10^{-4} M$)	D-tubocurarine (1.1×10^{-6} g/ml)
No. of endplates	(24)	(14)	(11)	(13)	(16)
1st EPP (quanta)	343	148[a]	234	245[a]	171[a]
Store (quanta)	6,096	1,637[a]	4,559	2,247[a]	1,090[a]
Mobilization (quanta/sec)	21,221	6,521[a]	14,591	9,992	5,020[a]
Fractional release	0.06	0.10[a]	0.06	0.12[a]	0.16[a]

[a] Significant difference ($P \leq 0.05$) between results in the presence of a drug and its absence (Column 2).

The Table shows the mean *dm*, *m*, *n* and *p* found at neuromuscular junctions in normal curarized muscle and in cut muscle after exposure to either D-tubocurarine or 1 of 2 concentrations of atropine for at least 20 min. As shown by the stars, *m*, *dm* and *n* were significantly smaller (*t*-test) in the presence of $1.3 \times 10^{-4} M$ atropine and in the curarized preparations than in the preparation in the absence of drugs.

Figure 2 shows the time course of changes in *q*, *m*, *dm* and *n* for the experiment partially illustrated in Figure 1. During the 15 min control period and the 30 min exposure to $1.3 \times 10^{-4} M$ atropine sulphate, records were taken every minute. Records were taken at the same intervals during the prolonged wash period. It will be noted that, as might be expected from the well-known postsynaptic competition of atropine with ACh[2,3], *q* (mV) is reduced during the exposure to atropine and recovers on washing. The post-exposure increase was found in all our experiments with atropine. As in our previous experiments with D-tubocurarine[6], its significance is unknown.

In conformity with the Table, but at marked variance with expectations based on a purely postsynaptic action of atropine, *m* (quantum content) also fell, more rapidly than *q*, and did not fully recover after 80 min of washing. The time course of this action was always much longer than the time course of the change in *q* and there was a lag period in the offset of the effect when compared with the effect on *q*. *Dm* (mean quantal content of tail × stimulation rate) and *n* were reduced in parallel with the change in *m* and also did not fully recover to the control amplitude after 80 min washing. As would be expected from the comparison of *m* and *n* (Figure 2), *p* was increased during the exposure to atropine and this effect was slowly reversed on washing.

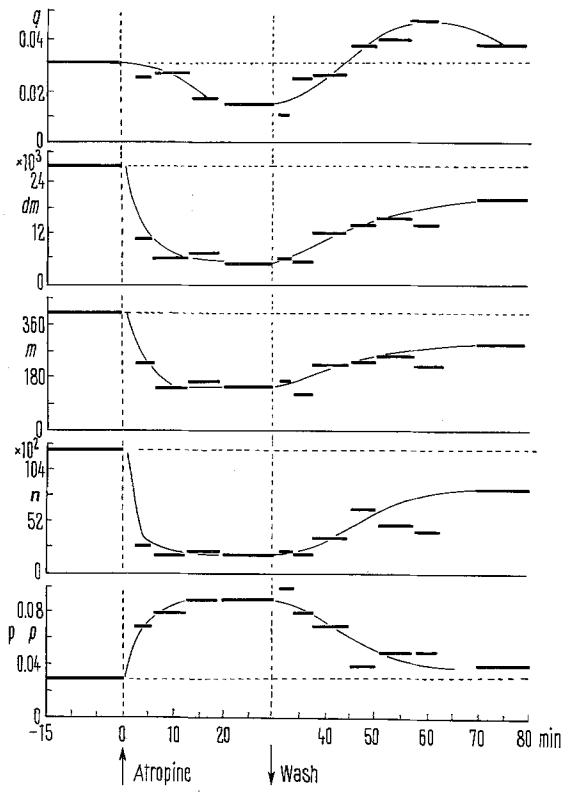

Fig. 2. The time course of atropine action. All data were derived from the same endplate from trains of EPP evoked at 1 min intervals by 40 stimuli at 100/s. Length of lines indicate the time interval over which data were pooled. Arrows and vertical bars indicate changes of bathing solution. *q*, average quantum size (mV) calculated from analysis of variance of EPP 20–39 of the trains evoked in the time period shown by the bars; *m*, quantal content of the first EPP of trains; *p*, fractional release for the first EPP of trains; *dm*, the mean quantal content of EPP 20–39 of a train multiplied by stimulation rate (100/s) to give mobilization rate; *n*, the immediately available store of quanta according to ELMQVIST and QUASTEL[10]. Dotted lines indicate control average for each parameter. The time scale is the same for all parameters. The atropine concentration was $1.3 \times 10^{-4} M$.

Our results indicate (Table 1, Figure 2) that atropine, like D-tubocurarine, has both pre- and subsynaptic actions. As the Table indicates, atropine is at least a hundred times less effective than D-tubocurarine in reducing quantal content. Similar ratios have previously been noted when the subsynaptic actions of the 2 drugs were compared[2]. Clearly the possibility[5] that the change in the EPP equilibrium potential induced by atropine[4] could be related to a fall in the quantum content of EPP cannot be ignored. Unequivocal identification by chemical means of the transmitter(s) released from motor nerve terminals appears the best way of settling this issue.

Why transmitter release should be blocked by atropine we do not know. It is known that atropine is a powerful inhibitor of choline transport in some systems[11] and as this process is an important source of choline for ACh synthesis[12] such an inhibition might explain our results.

Zusammenfassung. Postsynaptische Potentiale der Muskelfasern (EPP) des isolierten Rattenzwerchfells wurden intrazellulär abgeleitet. Die statistische Analyse zeigt, dass der Mengenanteil von EPP bei Anwesenheit von Atropin vermindert ist und zwar simultan mit der postsynaptischen Blockade. Die Erholung des presynaptischen Effektes ist viel langsamer als diejenige des postsynaptischen.

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[1] H. H. DALE, J. Pharmac. *6*, 147 (1914).
[2] R. BERÁNEK and F. VYSKOČIL, J. Physiol. *188*, 53 (1967).
[3] R. BERÁNEK and F. VYSKOČIL, J. Physiol. *195*, 493 (1968).
[4] T. V. POTAPOVA, Biofizika *14*, 757 (1969).
[5] L. G. MAGAZANIK and F. VYSKOČIL, Experientia *25*, 618 (1969).
[6] J. I. HUBBARD, D. F. WILSON and M. MIYAMOTO, Nature *223*, 531 (1969).
[7] J. M. BOWEN and E. H. MERRY, J. Pharmac. exp. Ther. *167*, 334 (1969).
[8] J. A. B. BARSTAD and G. LILLEHEIL, Arch. int. Pharmacodyn. Thér. *175*, 373 (1968).
[9] A. R. MARTIN, J. Physiol., Lond. *130*, 114 (1955).
[10] D. ELMQVIST and D. M. J. QUASTEL, J. Physiol., Lond. *178*, 505 (1965).
[11] K. MARTIN, Br. J. Pharmac. *36*, 458 (1969).
[12] L. T. POTTER, J. Physiol., Lond. *206*, 145 (1970).
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