Treatment of the preparation with paraoxon (50μM) produced twitch potentiation (peak tension 325 ± 30%; $n = 7$) more rapidly than did 2μM, and the potentiation rapidly declined (twitch tension after 30 min 140 ± 21%; $n = 7$). Following DTT treatment for 30 min the response to paraoxon (50μM) was reduced (peak tension 234 ± 12%; $n = 8$) and the tension after 30 min was 203 ± 9% ($n = 8$).

In the absence of DTT paraoxon (2μM) initially produced a notch in the tetanic response and subsequently a tetanic fade, which is also characteristic of other anticholinesterases (Blaber & Bowman, 1963). After DTT treatment of the preparation for 30 min paraoxon (2μM) failed to produce the notch in the tetanic response. Reactivation of the phosphorylated acetylcholinesterase by N,N-tri-methylene*bis* (pyridinium-4-aldoxime) (TMB-4, 2μM) in paraoxon treated diaphragms produced a recovery of the tetanic response which passed through a phase where the response had a typical notched appearance. This was not the case in preparations treated with DTT before paraoxon.

These results indicate that if a disulphide reducing agent with a sufficiently high selectivity for cholinoceptors at the neuromuscular junction can be found, such a drug might be of some use in the protection against and treatment of anticholinesterase poisoning.

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Comparison of the autonomic effects of some currently-used neuromuscular blocking agents

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Although the tachycardia produced by many neuromuscular blocking agents has been ascribed to a 'vagolytic' action of the drugs (Hughes & Chapple, 1976), there is now some evidence that the tachycardia produced by pancuronium may also involve a sympathetic component (Ivankovitch, Miletich, Albrecht & Zahed, 1975; Docherty & McGrath, 1977). The object of the present studies in isolated electrically driven guinea pig left atria (2Hz, 5ms, 32°C) was to quantitatively assess the cardiac muscarinic receptor antagonist potencies of a series of neuromuscular blockers and in addition to investigate whether they were able to modify the cardiac β_1-adrenoceptor stimulating actions of (−)-noradrenaline and (−)-isoprenaline. The neuromuscular blockers investigated were (+)-tubocurarine, pancuronium, fazadinium, chandonium and NC 45, a monoquaternary analogue of pancuronium (Durant, 1978).

In isolated electrically-driven left atrial preparations taken from reserpinised guinea pigs, all the neuromuscular blockers tested antagonised the negative inotropic actions of pilocarpine and acetylcholine. The mean pA_2 values obtained were: chandonium, 7.4 ± 0.2, pancuronium, 7.0 ± 0.1, fazadinium, 6.3 ± 0.2, (+)-tubocurarine, 5.2 ± 0.4 and NC 45, 4.6 ± 0.2. Analysis of the Schild plots suggested that the antagonism was competitive except in the case of (+)-tubocurarine. Under the same experimental conditions (32°C, 20 min contact time) atropine had a pA_2 of 8.8 ± 0.1. All the neuromuscular blocking agents also antagonised the effects of ACh on guinea pig ileum but had a significantly lower affinity for muscarinic receptors in this preparation than those in the atria. Atropine had a similar pA_2 in ileum (8.6 ± 0.2) to that obtained in atria.

In order to assess effects on the cardiac sympathetic neuro-effector junction, the ability of the neuromuscular blockers to modify the positive inotropic actions of (−)-noradrenaline and (−)-isoprenaline were investigated in guinea pig left atria incubated with atropine (1 μg/ml) and were compared with the standard neuronal uptake inhibitor, cocaine. Cocaine (3 μg/ml) significantly potentiated the effects of (−)-noradrenaline (8–12 fold shift to the left) but left those to (−)-isoprenaline unaffected. Similar effects were observed with pancuronium (0.5–20 μg/ml) chandonium (1–10 μg/ml) and fazadinium (>5 μg/ml). In contrast (+)-tubocurarine (20 μg/ml) and NC 45 (10 μg/ml) did not affect the responses to either noradrenaline or isoprenaline.

These results suggest that some currently used neuromuscular blocking agents can selectively potentiate the cardiac actions of noradrenaline, an action which may be due to blockade of neuronal uptake mechanisms (Docherty & McGrath, 1977). NC 45 would seem to warrant further investigation since it clearly has little effect on either atrial muscarinic receptors or on cardiac responses to noradrenaline.

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Modes of action of gallamine at the neuromuscular junction

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Gallamine is normally classified as a competitive neuromuscular blocking agent (e.g. Rang & Ritter, 1969). We have studied gallamine by means of voltage-jump measurements. Figure 1a shows the normal relaxation of end-plate current induced by acetylcholine (3μM), following step changes of membrane potential from −70 mV to −130 mV, and back. Figure 1b shows a similar experiment but carried out in the presence of gallamine (5 μM). On hyperpolarization there is initially a rapid *decrease* in current, followed by opening of channels that is slower than the normal rate shown in Figure 1a. When the internal potential is reduced to −70 mV again there is, paradoxically, a rapid *increase* in current at first, followed by the usual decrease which is again slower than normal. Similar observations have been made at end-plates of both mouse (Figure 1) and frog.

The effects shown in Figure 1 strongly resemble those found with procaine at the neuromuscular junction by Adams (1977). This suggests that gallamine can produce a potential-dependent block of open ion channels, as tubocurarine appears to do (Colquhoun, Dreyer & Sheridan, 1978), though much faster. These observations are consistent with those made recently by Katz & Miledi (1978). The relative importance of competitive block and ion-channel block by gallamine is being investigated.

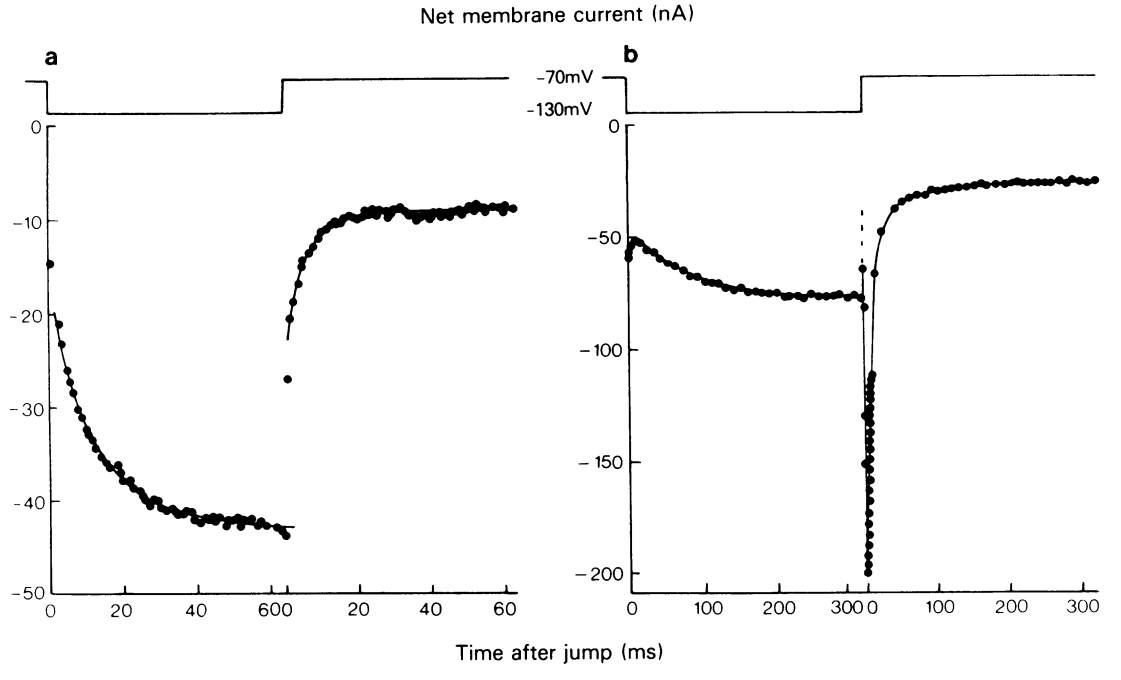

Figure 1 Mouse omohyoid muscle, at 8°C. Cholinesterase inhibited with methane sulphonyl fluoride. Membrane potential clamped as shown schematically above (a) and (b). Inward (negative) current is plotted against the time after the voltage jump. (a) Net currents induced by acetylcholine (3μM). (b) Net currents induced by acetylcholine (10μM) in the presence of gallamine (5μM). The dashed line is extrapolated to the expected instantaneous current immediately following repolarization. Note slower time scale in (b).