The effect of plasticizers on responses mediated by cholinoceptors at the neuromuscular junction

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Reduction by dithiothreitol (DTT, 1mM) of the disulphide linkage associated with the active site of the cholinoceptors at motor endplates has no effect on the maximum response but produces an approximately 4 fold decrease in the effectiveness of acetylcholine (ACh) and carbachol (Bleehen, Clark & Hobbiger, 1978). This result was obtained on the isolated frog (*Rana temporaria*) rectus abdominus muscle suspended in a glass organ bath (not connected to a fluid reservoir) containing oxygenated frog Ringer, pH 8.4 at 21°C (Method 1). When a fluid reservoir was linked to the organ bath by polyvinylchloride (PVC) tubing (Method 2), incubation of the muscle with DTT (1mM) for 30 min reduced the maximum response to ACh and greatly reduced the effectiveness of ACh. Dose ratios after DTT and based on the effects of ACh in the lower effective concentrations ranged between 93–1243 (mean 561 ± 188 (s.e. mean) $n = 6$). The effect of DTT treatment was reversed by the oxidizing agent dithiobisnitrobenzoic acid (DTNB) and DTT treatment had little effect on potassium responses. Comparison of responses to ACh (in the absence of DTT treatment) obtained in the all glass system (Method 1) with those obtained by Method 2 showed there to be a non-parallel shift to the right of the log dose response curve to ACh in experiments using Method 2.

Since plasticizers in PVC tubing are known to contaminate biological fluids and to interfere with responses of tissues (Duke & Vane, 1968; Ono, Tatsukawa & Wakimoto, 1975; Rosseel & Bogaert, 1976), their possible involvement was investigated. Until recently the major components of PVC tubing were the two stabilizers Lankro Q152 and Abrac A and the plasticizer Citroflex A4 (acetyl tri-n-butyl citrate). Testing these substances (kindly supplied by Portex Ltd) showed the first two to have no effect on the frog rectus preparation. However, when Citroflex was added to the Ringer solution in a concentration of 2.7×10^{-5} M (1/100,000 dilution) or greater, the maximum response to carbachol was reduced and the effect of DTT was enhanced. Both effects were reversed by washing. Citroflex, in concentrations which reduced the maximum response to carbachol by 98%, had no effect on caffeine induced contractions. In current PVC tube production, Citroflex has been replaced by a phthalate plasticizer. This has resulted in a product which was found not to interfere with responses to carbachol or the action of DTT upon it.

Plastic tubing is often present in the equipment used for studies on cholinoceptors at motor end plates and this type of interaction might account for variation between results obtained in different laboratories.

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The effect of dithiothreitol on the action of anticholinesterases on the neuromuscular junction

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Dithiothreitol (DTT) by reducing a disulphide bond at the anionic site of the cholinoceptor lowers the affinity of acetylcholine and acetylcholine-like drugs for the cholinoceptor at the neuromuscular junction (Rang & Ritter, 1971; Bleehen, Clark & Hobbiger, 1978). This could be potentially useful in the prevention and treatment of anticholinesterase poisoning. We, therefore, studied the effect of DTT (1mM) on the action of the organophosphate anticholinesterase paraoxon (diethyl-4-nitrophenyl phosphate) on the rat isolated phrenic nerve-diaphragm preparation (Bülbring, 1946) stimulated indirectly at 0.2 or 50 Hz.

In the absence of an anticholinesterase, treatment of the preparation for 30 min with DTT had no significant effect on twitch height ($n = 7$) but reduced tetanic tension to 86.1 ± 2% (s.e. mean) ($n = 15$) of its initial level.

Treatment of the preparation with paraoxon (2μM) produced twitch potentiation which reached a maximum in 15 min (peak tension 352 ± 18% of pre-paraoxon tension; $n = 6$) and then declined (twitch tension after 30 min 221 ± 18%; $= 6$). Following DTT treatment for 30 min the response to paraoxon (2μM) was delayed in onset, the peak effect was reduced (peak tension 272 ± 22%, $n = 7$) and there was no subsequent decline of the potentiation.