Specific binding was stereoselective, rapid, reversible and saturable. Scatchard analysis demonstrated a single, high affinity, binding site (Kd [^{3}H]-DHA = 0.2 ($\pm$0.02) nM, n = 9; B_{max} = 212 ($\pm$6) fmoles/mg protein, n = 9), while the Hill coefficient (n_H = 1.015 ($\pm$0.06), n = 7) revealed the absence of co-operative interactions.

The relative potencies of β-adrenoceptor agonists and selective adrenoceptor agents in displacing [^{3}H]-DHA from rat erythrocyte membranes is indicative of a β_2-adrenoceptor (Table 1). An indication of the homogeneity of these receptors is also suggested by an analysis of the displacement curves of these highly selective agents. In tissues which possess mixed β_1 and β_2 adrenoceptors, such agents produce non-law of mass action displacement curves with Hill coefficients <1. In rat erythrocytes, however, these agents produce displacement curves with Hill coefficients which are close to 1 (Table 1). Such results suggest that rat erythrocytes possess a homogenous β_2 adrenoceptor population.

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A comparison of the effects of 2-2′-pyridylisatogen, 2-phenylisatogen and papaverine on calcium-stimulated respiration in mitochondria isolated from guinea-pig liver

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The ability of mitochondria to regulate the cytoplasmic level of Ca^{2+} by sequestering Ca^{2+} ions may be of importance in the control of the contractile process in smooth muscle. The demonstration that 2-2′-pyridylisatogen, 2-phenylisatogen and papaverine antagonised Ca^{2+}-induced contractions in isolated taenia of the guinea-pig caecum pointed to a possible involvement of Ca^{2+} in their mechanism of action (Spedding & Weetman, 1978). We have therefore continued our investigations on the mode of action of these smooth muscle relaxant drugs by studying their effects on calcium metabolism in isolated mitochondria.

The uptake of Ca^{2+} by tightly-coupled mitochondria, which is accompanied by a marked stimulation of respiration, can be supported by a variety of respir-

Table 1 Effect of relaxant drugs on Ca^{2+}-stimulated respiration in mitochondria in the presence of either phosphate or acetate

	$IC_{50} \pm$ s.e. mean (μM)	
	Phosphate-activated reaction	*Acetate-activated reaction*
2-2′-pyridylisatogen	12.2 ± 1.6	8.2 ± 0.4
2-phenylisatogen	10.2 ± 0.5	11.0 ± 0.8
Papaverine	5.5 ± 0.4	6.2 ± 0.7

Guinea-pig liver mitochondria (10 mg protein) were incubated at 30° in a medium containing 0.25 M sucrose, 10 mM tris-HCl buffer, pH 7.4, 3.3 mM sodium succinate (or 3.3 mM sodium glutamate plus 3.3 mM sodium malate) and 3.3 mM phosphate buffer, pH 7.4 (or 5 mM sodium acetate) for 2 minutes. The reaction was initiated by the addition of 1 µmol $CaCl_2$, to give a final volume of 3 ml; drugs were added 2 min before the $CaCl_2$. IC_{50} is the concentration of drug that produced a 50% reduction in the stimulation of respiration that followed the addition of $CaCl_2$ to the reaction chamber. The values were obtained using concentration-effect curves for each drug (n = 5). The IC_{50} values for each drug on the two reactions were not significantly different ($P > 0.05$).

atory substrates and can be followed using an oxygen electrode (Chappell & Crofts, 1965). The presence of a permeant anion such as phosphate or acetate further stimulates the process by accompanying the Ca^{2+} across the mitochondrial membrane, thus maintaining electroneutrality in the mitochondrial matrix.

All three compounds inhibited Ca^{2+}-stimulated respiration at low concentrations, in the presence of either phosphate or acetate (Table 1). Since acetate enters the mitochondria by diffusion and phosphate by a carrier-mediated process, the results do not support an action on anion transport systems. A more likely explanation is that the relaxant drugs interfere directly with Ca^{2+} transport, either by combining with the Ca^{2+} carrier or by blocking one of the steps which leads to energy production in the mitochondrion.

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The effect of dithiothreitol on anticholinesterase induced antidromic firing and twitch potentiation

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The inhibition of acetylcholinesterase at the neuromuscular junction leads to twitch potentiation and a failure of the muscle to maintain a tetanic contraction (tetanic fade, Wedensky inhibition). The twitch potentiation is widely thought to arise from antidromic firing (antidromic action potentials in the motor nerve; *see* Hobbiger, 1976). The same explanation has been put forward for the initial phase of the tetanic fade (Blaber & Bowman, 1963). However, there is some doubt about a consistent causal relationship between antidromic firing and twitch potentiation since a number of drugs can preferentially reduce the former (Webb & Bowman, 1974; *see* Hobbiger, 1976). We have previously observed that in the rat isolated nerve-diaphragm preparation treated with the anticholinesterase paraoxon (diethyl-4-nitrophenyl phosphate) the disulphide bond reducing agent dithiothreitol (DTT) modified but did not abolish twitch potentiation whereas it abolished the initial phase of the tetanic fade (Clark & Hobbiger, 1979). This casts further doubt on the interpretation that antidromic firing always plays a major role in twitch potentiation produced by anticholinesterases. We therefore, studied the relationship between antidromic firing and twitch potentiation in the rat isolated nerve-diaphragm preparation suspended in a Tyrode solution containing 2 mM $CaCl_2$ and 0.1 mM $MgCl_2$ (solution A) which is known to enhance antidromic firing (Randić & Straughan, 1964). All experiments were carried out at 37° and the nerve was stimulated at a frequency of 0.2 Hertz. Antidromic firing was recorded by placing the nerve trunk over two platinum recording electrodes connected to a Grass P16 amplifier and the signal displayed on a Gould OS4000 oscilloscope (bandwidth 1 Hz to 10 kHz). Muscle tension was recorded with an FT-10C Grass force-displacement transducer.

In solution A the exposure of preparations to paraoxon (0.5 μM) for 5 to 30 min increased twitch tension by a factor of 4.9 ± 0.5 (mean ± s.e. mean; $n = 18$). This effect was associated with marked antidromic firing (peak amplitude ranging from 60 to 700 μV). Following removal of paraoxon from the organ bath, the addition of DTT (1 mM) to solution A for 15 min reduced antidromic firing below the detectable level (10 to 40 μV) but decreased the enhanced twitch tension by only 28.0 ± 12.5% ($n = 5$). The effects of DTT were reversed by the oxidizing agent 5.5'-dithio*bis* (2-nitrobenzoic acid, 1 mM), added to the organ bath for 10 minutes.

In a Tyrode solution containing 1 mM $CaCl_2$ and 1 mM $MgCl_2$ (solution B), exposure of the diaphragm to paraoxon (0.5 and 2 μM) increased twitch tension by a factor of 3.5 ± 1.0 ($n = 3$) and 2.8 ± 0.5 ($n = 4$), respectively. This effect was not associated with any detectable antidromic firing. following removal of paraoxon from the organ bath, replacement of solution B by solution A initiated detectable antidromic firing but had no consistent effect on twitch tension.

From these experiments it can be concluded that antidromic firing is not the only mechanism by which anticholinesterases can produce twitch potentiation. The mechanism by which DTT causes a dissociation between antidromic firing and twitch potentiation might by resolved by electrophysiological studies of postsynaptic events following nerve stimulation.