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.

This work was supported by a grant from the S.R.C.

References

CHAPPELL, J.B. & CROFTS, A.R. (1965). Calcium ion accumulation and volume changes of isolated liver mitochondria: calcium ion-induced swelling. *Biochem. J.*, **95**, 378–386.

SPEDDING, M. & WEETMAN, D.F. (1978). The mechanism of the relexant effect of 2-2'-pyridylisatogen on the isolated taenia of the guinea-pig caecum. *Br. J. Pharmac.*, **63**, 659–664.

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

AMANDA L. CLARK, F. HOBBIGER & D.A. TERRAR

Department of Pharmacology and Therapeutics, The Middlesex Hospital Medical School, London W1P 7PN

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.

References

BLABER, L.C. & BOWMAN, W.C. (1963). Studies on the repetitive discharges evoked in motor nerve and skeletal muscle after injection of anticholinesterase drugs. *Br. J. Pharmac.*, **20**, 326–344.

CLARK, AMANDA L. & HOBBIGER, F. (1979). The effect of dithiothreitol on the action of anticholinesterases on the neuromuscular junction. *Br. J. Pharmac.* **66**, 76–77P.

HOBBIGER, F. (1976). Pharmacology of anticholinesterase drugs. In: *Handbook of Experimental Pharmacology*, **42**, 487–581. ed. E. Zaimis, Berlin-Heidelberg-New York: Springer-Verlag.

RANDIĆ, MIRJANA & STRAUGHAN, D.W. (1964). Antidromic activity in the rat phrenic nerve-diaphragm preparation. *J. Physiol., Lond.* **173**, 130–148.

WEBB, SANDRA N. & BOWMAN, W.C. (1974). The role of pre- and post-junctional cholinoceptors in the action of neostigmine at the neuro-muscular junction. *Clin. exp. Pharmac. Physiol.*, **1**, 123–134.

Homogenates of rat placenta contain a factor(s) which inhibits uterine arachidonic acid metabolism

P.D. HARROWING &. K.I. WILLIAMS

Department of Pharmacology, School of Pharmacy, University of Bath, England

The main arachidonic acid (AA) metabolite of vascular tissue is prostacyclin (PGI_2) (Bunting, Gryglewski, Moncada & Vane, 1976). Despite rich vascularisation the rat placenta does not produce PGI_2 (Williams, Dembinsak-Kiec, Zmuda & Gryglewski, 1978). We have therefore investigated placental tissue to determine whether it contains a PG synthetase inhibitor.

A 25% (w/v) homogenate of rat placentae was prepared in distilled water (pH 7). After centrifugation at 3000 *g* for 30 min the supernatant was ultracentrifuged (100,000 *g* for 60 minutes). The microsomal supernatant (PIF-M) was passed through silicic acid or XAD-2, and then lyophylised. Samples (1–8 mg in 0.05–0.4 ml) in distilled water of PIF-M were incubated with 1 ml samples of decidual or myometrial microsomes (protein concentration 1–2 mg/ml) in 0.1 M. Tris buffer (pH 8) with co-factors (Williams & Downing, 1977). The reaction was terminated with citric acid (0.25 ml 2 M). Products were extracted with 2 × 2 volumes of ether and separated by thin-layer chromatography (TLC), using a solvent system of chloroform:methanol:acetic acid:water (90:9:1:0.65). Similar volumes of boiled PIF-M served as controls. Areas of radioactivity were detected by TLC scanning. After scraping and extraction with methanol the absolute activity was determined by liquid scintillation counting.

PIF-M produced a dose dependent inhibition of PG-synthesis by decidual microsomes, $ID_{50} = 2.95 \pm 0.62$ mg (mean ± s.e. mean, $n = 4$). Inhibition of PG synthesis by rabbit kidney medulla and ram seminal vesicle microsomes was much lower 47% and 32% inhibition per 8 mg PIF-M respectively. PIF-M was found to be unstable in acid solution (pH 3) or when heated at 80°C for 10 minutes. PIF-M was non-dialysable and was precpitated by 10% w/v $(NH_4)_2SO_4$. Incubation of PIF-M with the non-selective, proteolytic enzyme mixture, 'Pronase', 2 mg/ml for 2 h at 37°C resulted in the complete loss of inhibitory activity.

When studying conversion of radiolabelled AA errors can result from dilution by cold precursor giving apparent inhibition of conversion of the labelled substrate. In the present investigations we have precluded this possibility by passage of the placental microsomal supernatant through silicic acid or XAD-2, which eliminated 99% of added [1-^{14}C]-AA. The possibility still remained that PIF-M contained a phospholipase enzyme which could cleave AA from phospholipid contained in the uterine microsomes. This source or error was removed because (1) lipids were removed from uterine microsomes by washing with an acetone-pentane mixture (Wallach & Daniels, 1971) but this did not result in loss of inhibitory activity from PIF-M. (2) No decrease in inhibitory potency of PIF-M was noted when the uterine microsomes were pre-incubated with the phospholipase A_2 antagonist, mepacrine (500 μg/ml).

Human placentae have also been found to contain a PIF-M. The experiments suggest that the placenta contains a protein or polypeptide which even in this impure form is a potent inhibitor of uterine arachidonic acid metabolism.

We thank the Wellcome Trust for a grant, P.D.H. is an S.R.C. (CASE) student.

References

BUNTING, S., GRYGLEWSKI, R., MONCADA, S. & VANE, J.R., (1976) Arterial walls generate a substance (PGX) from PG endoperoxides which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. *Prostaglandins*, **12**, 897–913.