Br. J. Pharmac. (1983), **80**, 17–25

The relationship between stimulus-induced antidromic firing and twitch potentiation produced by paraoxon in rat phrenic nerve-diaphragm preparations

A.L. Clark, F. Hobbiger & D.A. Terrar

Department of Pharmacology and Therapeutics, The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB

1 The relationship between stimulus-induced repetitive antidromic firing (ADF) in the motor nerve and twitch potentiation produced by the organophosphate anticholinesterase paraoxon, has been investigated in rat diaphragm preparations.

2 Little or no ADF was produced by paraoxon in preparations bathed in a Tyrode solution containing 1 mM calcium and 1 mM magnesium ions although the preparations showed marked twitch potentiation.

3 Increases in the calcium:magnesium ion ratio produced a ratio-dependent increase in the ADF but had no consistent effect on peak twitch potentiation.

4 Dithiothreitol, a disulphide bond reducing agent which decreases the affinity of acetylcholine for nicotinic cholinoceptors, abolished ADF but only modified the time course of twitch potentiation.

5 It is concluded that the initiation of ADF is a consequence of the prolonged action of acetylcholine within the synaptic cleft following inhibition of acetylcholinesterase, and that ADF is not the only mechanism by which twitch potentiation can be produced.

Introduction

It has been suggested (Masland & Wigton, 1940) that the mechanism underlying twitch potentiation by anticholinesterases is the initiation of repetitive antidromic action potentials (APs) (antidromic firing, ADF) subsequent to the application of a stimulus to the motor nerve and that ADF is also responsible for the muscle fasciculations produced by anticholinesterases (Feng & Li, 1941). Previous studies (Clark & Hobbiger, 1983) have shown that in rat diaphragm preparations which have been treated with the disulphide bond reducing agent dithiothreitol (DTT), the anticholinesterase paraoxon still produced marked twitch potentiation, but failed to initiate muscle fasciculations. The same dissociation between these two effects was obtained in the absence of DTT when the ratio of calcium:magnesium ions in the solution bathing the muscle was reduced. If it is true that ADF is responsible for muscle fasciculations, these observations indicate that ADF might not be the sole mechanism by which twitch potentiation is or can be produced.

In this paper we report results of studies of stimulus-induced ADF in the phrenic nerve and its relationship to twitch potentiation in rat diaphragms treated with paraoxon, together with the effects of DTT and changes in the calcium:magnesium ratio in the bathing solution on this relationship.

A preliminary account of this work has been presented to the British Pharmacological Society (Clark, Hobbiger & Terrar, 1979).

Methods

The preparation and organ bath

Left hemidiaphragms together with their phrenic nerves were obtained from male Sprague-Dawley rats and mounted in one chamber of a two chamber perspex bath. Each chamber had a 50 ml capacity. One chamber had a cork floor and the ribs attached to the muscle were pinned to it. The muscle was kept in a vertical position by a thread which connected the central tendon to a Grass FT-10C force-displacement transducer. Muscle contractions were recorded under a resting tension of 10 g.

The chamber containing the muscle was perfused at a constant flow rate (20 to 30 ml min^{-1}) with one of four Tyrode solutions (Tyrode A, B, C or D) flowing from a reservoir which was gassed with O_2 containing 5% CO_2. The Tyrode solution was removed from the chamber by surface suction. All solutions contained 137 mM NaCl, 2.7 mM KCl, 12.0 mM $NaHCO_3$ and 11 mM glucose together with the following concentrations of calcium and magnesium ions: Tyrode A 2 mM $CaCl_2$, 0.1 mM $MgCl_2$; Tyrode D 2 mM $CaCl_2$, 1 mM $MgCl_2$; Tyrode B 1.4 mM $CaCl_2$, 1 mM $MgCl_2$; Tyrode C 1 mM $CaCl_2$, 1 mM $MgCl_2$.

The perspex bath was mounted in a constant temperature water bath. Tyrode solutions were passed through coils in this bath before entering the muscle chamber, and the muscle chamber itself was fitted with a perspex lid to minimize heat loss to the air, so that the temperature within the muscle chamber was maintained at 36 to 37°C. Drugs were made up to final concentrations in individual reservoirs.

The phrenic nerve was stimulated via a circular electrode located in the muscle chamber and connected to a Farnell physiological stimulator (stimulation parameters: 0.2 Hz frequency, 50 or 200 μs pulse duration and supramaximal voltage). The proximal part of the phrenic nerve was passed through a small hole in the partition between the two chambers of the perspex bath. The hole was then sealed with vaseline, the nerve placed in a horizontal position over two platinum electrodes for recording ADF and the chamber containing the nerve filled with liquid paraffin prewarmed to 37°C.

Recording from the phrenic nerve

The nerve was crushed between the two recording electrodes so that all APs were recorded as monophasic potentials. The recording electrodes were connected to a Grass P16 amplifier (amplification 100 fold) and the signal displayed simultaneously at low and high gain on a Gould OS 4000 digital storage oscilloscope (bandwidth 1 Hz to 10 kHz). The analogue output unit of the oscilloscope allowed traces which had been stored at a fast sweep speed (5 ms cm^{-1}) to be reproduced accurately at a much slower speed (5 s cm^{-1}) with a Servascribe pen recorder.

Each stimulus applied to the nerve normally triggers a single orthodromically and antidromically conducted AP. Antidromically conducted APs subsequent to this initial AP are referred to as repetitive antidromic firing (ADF) and are initiated by treatment of preparations with paraoxon. ADF was monitored over a period of 50 ms subsequent to nerve stimulation, during which time its intensity built up to a peak and then declined. For quantitative assessment of ADF, its peak voltage was used. In a limited number of experiments, the area under the ADF waveform was also estimated by connecting the output from the oscilloscope to a Time Electronics digital integrator. By setting the zero level so as to exclude activity attributable to instrument noise, a figure was obtained for neuronal activity during a 50 ms sweep triggered by the stimulus applied to the nerve. The value obtained by subtracting from this the count attributable to the initial antidromically conducted nerve AP was taken as the value of ADF activity. Values obtained in this way showed a good correlation with the peak amplitude of ADF. For example, in a typical experiment, the following values were obtained: after brief contact of the preparation with paraoxon peak amplitude of ADF was 165 μV and integrated count of ADF was 30; after prolonged contact with paraoxon, peak amplitude was 50 μV and the integrated count was 1. The peak amplitude of ADF includes a noise level of approximately 45 μV (peak-to-peak).

Drugs

The following drugs were used: diethyl-4-nitrophenylphosphate, paraoxon (Koch-Light), dithiothreitol, DTT (Sigma), 5,5′-dithio*bis*(2-nitrobenzoic acid), DTNB (Sigma).

Results

Relationship between antidromic firing and twitch potentiation produced by paraoxon

In the absence of any drugs, a single supramaximal stimulus applied to the phrenic nerve produced a single muscle contraction and a single antidromically conducted AP with an amplitude up to 20 mV. When paraoxon (0.2 to 2 μM) was added to the perfusion fluid (Tyrode A, B or D solution) twitch potentiation developed and the initial antidromic AP was followed within 2 to 5 ms by a series of repetitive antidromic APs (ADF). The amplitude of the ADF triggered by an individual nerve stimulus built up to a peak within 10 to 15 ms after stimulation of the nerve and then declined (Figure 1). When two closely spaced (5 ms apart) stimuli were applied to the nerve, ADF was greater than that seen after a single nerve stimulus.

On continued exposure of the preparation to paraoxon twitch potentiation and peak ADF associated with consecutive nerve stimuli progressively increased to a maximum and thereafter they both declined. The time course of these changes in twitch potentiation and peak ADF was accelerated by increasing the concentration of paraoxon. The decline could be prevented by stopping the perfusion of the

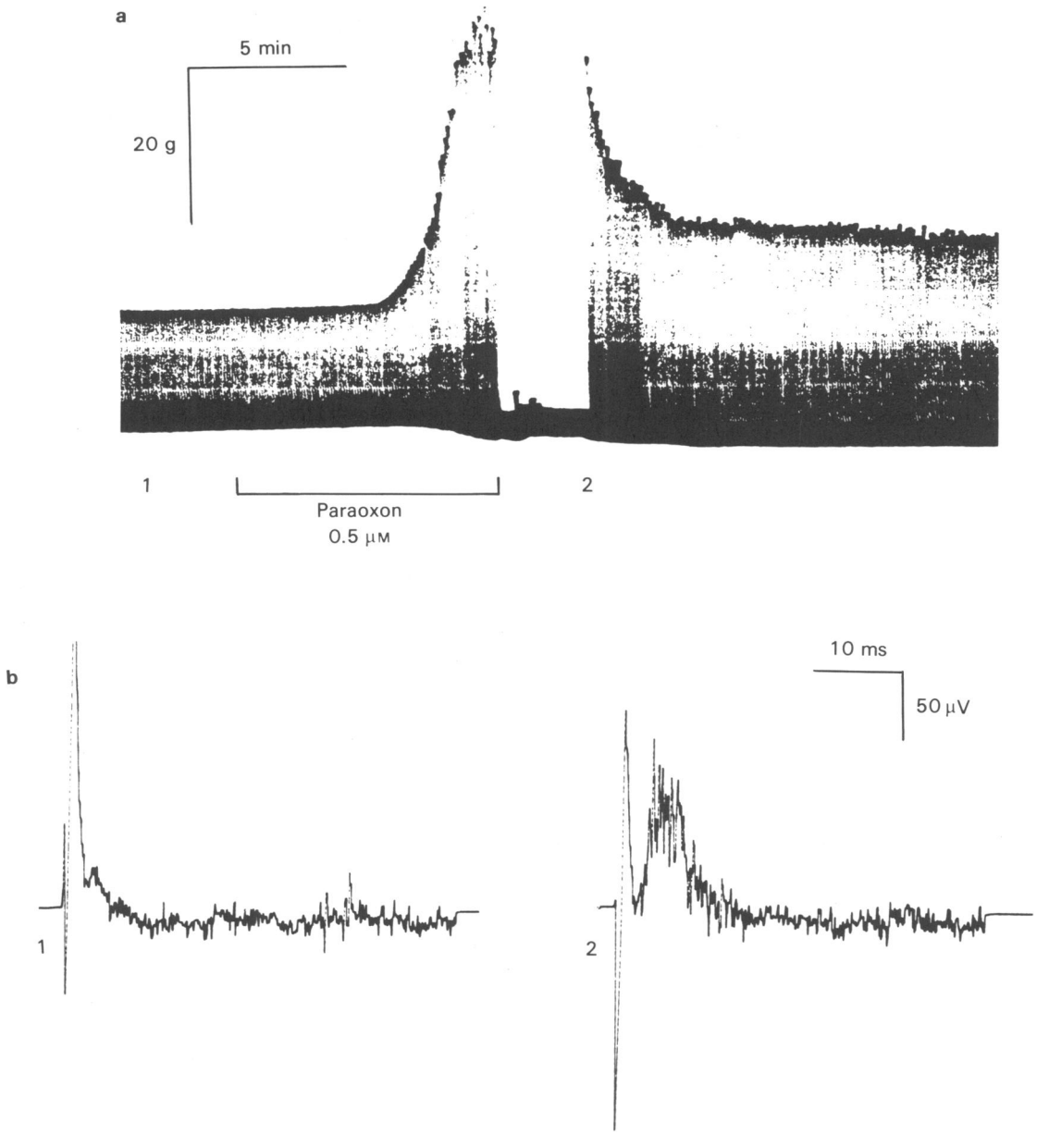

Figure 1 Paraoxon-induced twitch potentiation and antidromic firing (ADF) in a rat diaphragm preparation. In this and subsequent figures, following exposure of the preparation to paraoxon, nerve stimulation was discontinued while the organ bath was emptied and then refilled with Tyrode solution not containing paraoxon. Nerve stimulation and perfusion with drug-free Tyrode solution were then restarted. (a) Twitch responses to indirect supramaximal stimulation at 0.2 Hz frequency, recorded isometrically. Paraoxon (0.5 μM) was added to the perfusion fluid (Tyrode D) for the period indicated. (b) Electrical activity recorded from the phrenic nerve (1) before addition of paraoxon and (2) following removal of paraoxon from the perfusion fluid. In all records of electrical activity, the large upward deflection at the beginning of the trace represents the antidromically conducted action potential (AP) initiated by the stimulus applied to the nerve. This AP is too large to be recorded accurately at the amplification shown here.

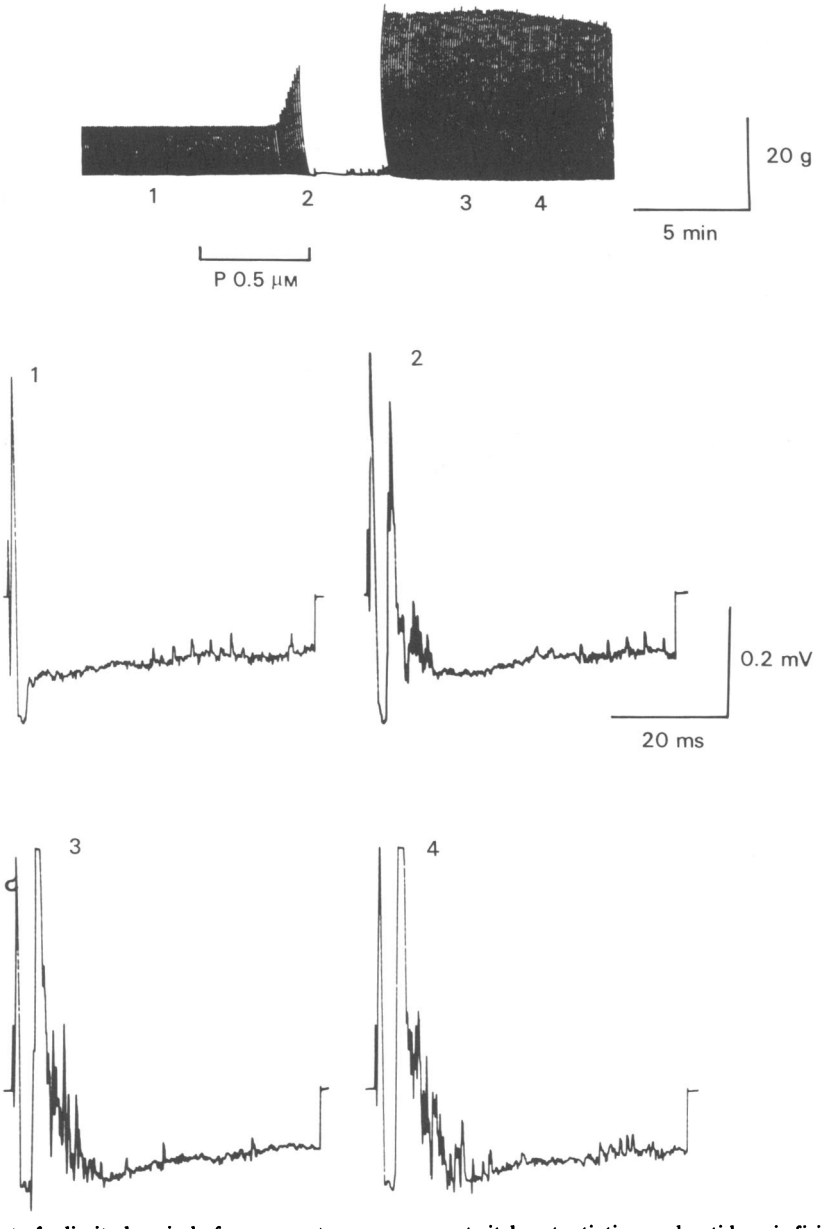

Figure 2 Effect of a limited period of exposure to paraoxon on twitch potentiation and antidromic firing in a rat diaphragm preparation bathed in Tyrode A solution. The preparation was exposed to paraoxon (P, 0.5 μM) for 5 min as indicated. Traces 1 to 4 of electrical activity in the phrenic nerve were recorded at times corresponding to points 1 to 4 on the tension trace.

organ bath with the paraoxon-containing Tyrode solution at a point where twitch potentiation and ADF were pronounced but not yet maximal, replacing the fluid in the organ bath with a Tyrode solution without paraoxon and restarting perfusion of the organ bath with this Tyrode solution. Under these conditions, both ADF and twitch potentiation were fairly well maintained for periods of 30 to 60 min after removal of paraoxon from the organ bath (Figure 2).

Effect of changing the ratio of calcium: magnesium ions in the Tyrode solution on paraoxon-induced antidromic firing

As in the case of twitch potentiation (Clark & Hobbiger, 1983), the rates of onset and decline of paraoxon-induced ADF were related to the ratio of calcium: magnesium ions in the bathing solution. Alterations of the calcium: magnesium ratio had little or no effect on peak twitch potentiation. On the other hand, the peak amplitude of ADF was increased with increasing calcium: magnesium ratios (Table 1).

In experiments in which the period of exposure of preparations to paraoxon was limited, as described above, and preparations were bathed in Tyrode C solution (containing 1 mM $CaCl_2$, 1 mM $MgCl_2$), paraoxon (0.5 μM) produced twitch potentiation without any detectable ADF in 4 out of 5 experiments. In the one experiment where ADF did occur (peak amplitude 63 μV) it quickly fell below the detectable level (i.e. 30 μV) after removal of paraoxon from the organ bath. When in these experiments Tyrode C solution was replaced by Tyrode A solution (containing 2 mM $CaCl_2$, 0.1 mM $MgCl_2$) ADF appeared in all preparations. The effect of replacing Tyrode C solution by Tyrode A solution on twitch potentiation was variable. In two experiments twitch potentiation was enhanced. In another two experiments there was an enhancement followed by a decline. In the fifth preparation only a decline in twitch potentiation was seen. The effects of Tyrode A solution on ADF and twitch potentiation were reversed by changing back to Tyrode C solution (Figure 3).

Effect of dithiothreitol on paraoxon-induced antidromic firing

The effect of DTT on ADF was studied using Tyrode A solution and a limited exposure of the preparation to paraoxon, as described previously. Following removal of paraoxon (0.5 μM) from the organ bath, steady levels of ADF (peak amplitude 146 ± 39 μV) and twitch potentiation (3.3 ± 0.9 times control twitch tension) were seen ($n = 6$). Addition of DTT (1 mM) to the Tyrode solution caused the ADF to decline below the detectable level (10 to 40 μV) but reduced twitch potentiation only by 28.0 ± 12.5%. Removal of DTT from the Tyrode solution was not associated with a return of ADF but partly restored the twitch potentiation to its pre-DTT value. When the oxidizing agent DTNB (1 mM) was added to the Tyrode solution, ADF reappeared (peak amplitude 181 ± 36 μV), and was maintained at a steady level of 132 ± 30 μV when DTNB was removed. DTNB also restored twitch potentiation to pre-DTT levels, after a transient phase of enhancement (Figure 4).

In 5 experiments in Tyrode A solution the effect of treatment of the preparation with DTT (1 mM for 15 min) before addition of paraoxon (0.5 μM for 10 min) was investigated. Under these conditions, ADF was not detectable in 2 out of 5 preparations. In one preparation, ADF was only transient and in the remaining two preparations a steady level of ADF (peak amplitude 125 μV and 300 μV) was seen. Twitch potentiation was slower in onset than in preparations not treated with DTT and peak twitch potentiation was 4.2 ± 0.3 times control twitch ten-

Table 1 Effect of the ratio of calcium: magnesium ions in the perfusion fluid on twitch potentiation and antidromic firing (ADF) produced by paraoxon (0.5 μM)

	$CaCl_2$ (mM)	*$MgCl_2$* (mM)	*Peak ADF amplitude**	*Peak twitch tension†*
Tyrode A	2	0.1	405 ± 92 μV ($n = 13$)	5.4 ± 0.6 ($n = 13$)
Tyrode D	2	1	262 ± 67 μV ($n = 5$)	3.3 ± 0.2 ($n = 5$)
Tyrode B	1.4	1	134 ± 19 μV ($n = 3$)	3.0 ± 0.4 ($n = 3$)
Tyrode C	1	1	49 ± 6 μV ($n = 5$)	3.4 ± 0.6 ($n = 5$)

Figures are mean ± s.e.mean (n = number of experiments).

* Peak ADF amplitude was measured as the maximum deflection on the oscilloscope record of electrical activity in the phrenic nerve during the 50 ms period following a stimulus to the nerve.

† Peak twitch is expressed as a multiple of that observed at the time of addition of paraoxon to the organ bath, which is taken as 1.

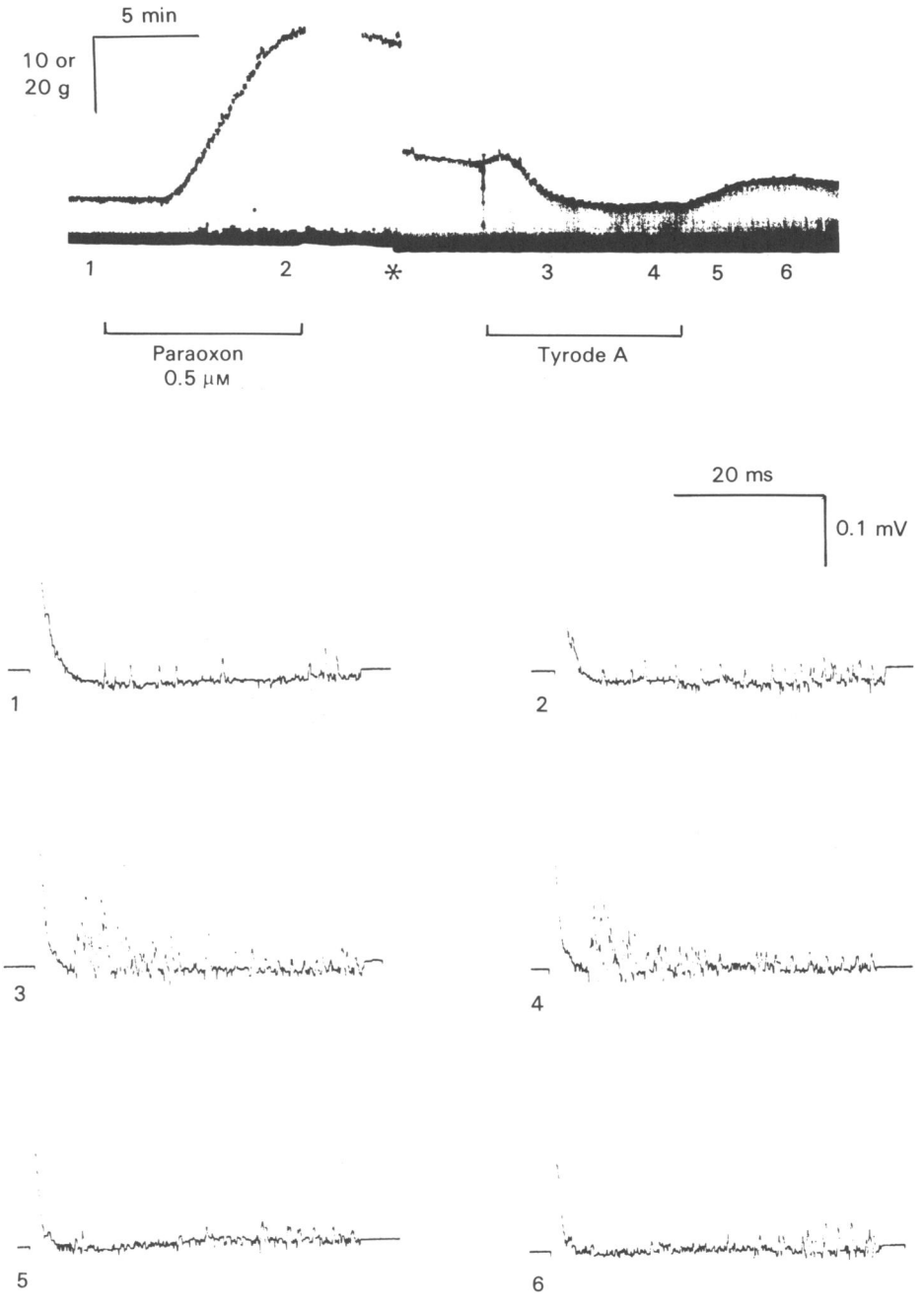

Figure 3 Effect of changing the ratio of calcium : magnesium ions in the perfusion fluid on paraoxon-induced twitch potentiation and antidromic firing. The organ bath was perfused with Tyrode C solution (containing 1 mM $CaCl_2$, 1 mM $MgCl_2$) and the preparation exposed to paraoxon (0.5 μM) for the period indicated. At * the amplification of the tension trace was reduced by half and the perfusion fluid then changed to Tyrode A solution (containing 2 mM $CaCl_2$, 0.1 mM $MgCl_2$) for the period indicated.

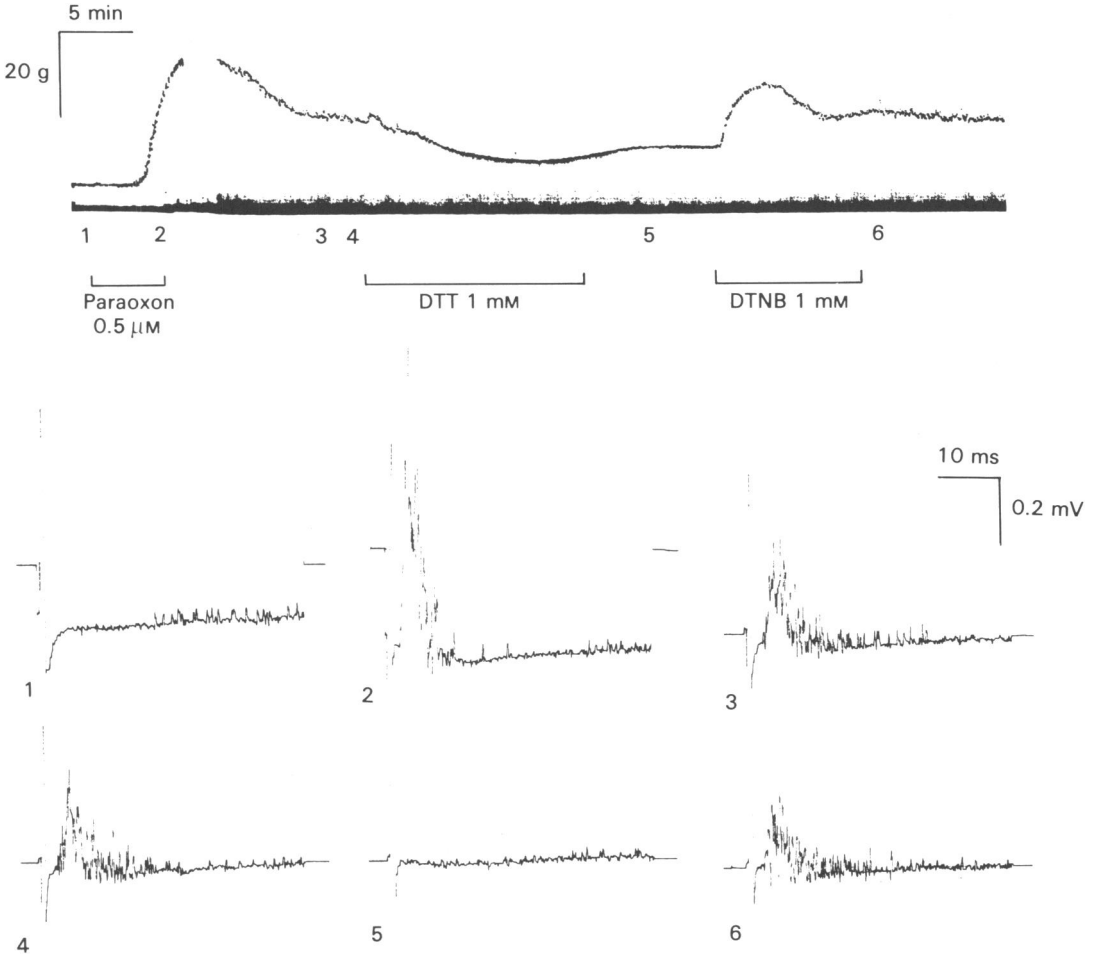

Figure 4 Effect of dithiothreitol (DTT) and 5,5′-dithio*bis*(2-nitrobenzoic acid) DTNB on paraoxon-induced twitch potentiation and antidromic firing. The organ bath was perfused with Tyrode A solution and the preparation exposed to paraoxon (0.5 μM), or DTT (1 mM) or DTNB (1 mM) for the periods indicated.

sion. Addition of DTNB (1 mM) to the Tyrode solution led to the appearance of ADF in the three preparations where it was absent and a marked enhancement of it in the other 2 preparations. The mean peak amplitude of ADF in the presence of DTNB was 382 ± 92 μV and ADF was maintained after its removal from the organ bath. Twitch potentiation was transiently enhanced on addition of DTNB, but then declined to 70 ± 4% of its pre-DTNB level.

Discussion

The experiments described in this paper show that, in the rat phrenic nerve diaphragm preparation exposed to paraoxon, repetitive antidromic nerve activity (ADF) is dependent on the concentrations of calcium and magnesium ions in the bathing solution. This is in agreement with the findings of other workers (Randić & Straughan, 1964; Laskowski & Dettbarn, 1979). In our experiments, ADF was barely detectable with Tyrode C solution, which contained 1 mM $CaCl_2$ and 1 mM $MgCl_2$, but was pronounced with Tyrode A, B and D solutions, which had higher ratios of calcium:magnesium ions. Although raising the ratio of calcium:magnesium ions produced a ratio-dependent increase in ADF, it did not cause corresponding changes in the peak level of twitch potentiation relative to that obtained with Tyrode C solution. The main effect of raising the ratio of calcium:magnesium ions was an increase in the rate of onset of twitch potentiation. Similarly, in experi-

ments in which the calcium:magnesium ion ratio was changed following removal of paraoxon from the organ bath, increasing the ratio consistently increased ADF whereas lowering it had the opposite effect. Under these conditions changes in the calcium:magnesium ion ratio had no consistent effect on twitch potentiation.

Little or no ADF was recorded when preparations bathed in Tyrode A solution, which contained the highest calcium:magnesium ion ratio, were first treated with DTT and then paraoxon, although under these conditions twitch potentiation proceeded to a peak which was comparable to that seen in preparations which were treated with paraoxon only. Similarly, in preparations in which paraoxon had produced a marked degree of ADF, the addition of DTT to the bathing solution abolished ADF without a consistent effect on twitch potentiation. The effect of DTT was reversed by the oxidizing agent DTNB.

It can therefore be concluded that ADF is not the only mechanism by which paraoxon-induced twitch potentiation can be obtained. A number of authors (Barstad & Lilleheil, 1968; Webb & Bowman, 1974; Bowman, 1980a,b) have suggested that although ADF and twitch potentiation may both arise as a result of the prolonged action of ACh in the synaptic cleft, the two events are independent and not causally related. ADF would be expected to initiate repetitive endplate potentials and it is difficult to see why these would not trigger additional muscle APs under the appropriate conditions. This could be at least partly responsible for the more rapid onset of twitch potentiation in Tyrode solutions with higher calcium:magnesium ion ratios.

Stimulus-induced ADF is distinct from the ephaptic back response, which is triggered by action currents generated in the muscle (Lloyd, 1942; Brown & Matthews, 1960). The ephaptic back response has a very short latency and terminates usually within 5 ms after stimulating the nerve (Werner, 1960), whereas ADF has a much longer latency and lasts considerably longer.

As far as the mechanisms involved in ADF are concerned, our observations are consistent with a number of hypotheses which have been proposed to account for it. It has been postulated that ADF can be initiated by depolarization of the nerve membrane either at the nerve terminal (Masland & Wigton, 1940) or at the first node of Ranvier (Hubbard, Schmidt & Yokota, 1965). This depolarization might be caused either by the action of ACh released from the nerve terminal and feeding back onto prejunctional cholinoceptors (Webb & Bowman, 1974; Bowman, 1980a,b), or by potassium ions released from the muscle as a consequence of the postjunctional action of ACh (Katz, 1962; Hohlfeld, Sterz & Peper, 1981). In either case, increasing the concentration of ACh in the synaptic cleft, by increasing its output from nerve terminals, would be expected to increase ADF. Consistent with this view is our finding that ADF is increased when the calcium:magnesium ratio is increased or pairs of stimuli are applied to the nerve, since under these conditions the amount of transmitter released is increased (Jenkinson, 1957; Hubbard, Jones & Landau, 1969; Ginsborg & Jenkinson, 1976). In the case of the experiments where the concentration of magnesium was decreased there may be an additional contribution from an increased excitability of the nerve membrane (Frankenhaeuser & Hodgkin, 1956).

If there are prejunctional cholinoceptors, DTT could reduce the action of ACh at this site and consequently diminish ADF directly by reducing the affinity of ACh for these receptors, if they are of the same type as those on the postjunctional membrane (Ben-Haim, Landau & Silman, 1973; Terrar, 1978) or like those in ganglia (Brown & Kwiatkowski, 1976; Bleehen, Clark & Hobbiger, 1983; see Webb & Bowman, 1974). Alternatively, DTT might reduce the prejunctional effect of ACh indirectly by an action on postjunctional cholinoceptors. In the presence of an anticholinesterase, the diffusion of ACh from the synaptic cleft is delayed by its binding to postjunctional cholinoceptors (Katz & Miledi, 1973; Magleby & Terrar, 1975). Following exposure to DTT the affinity of ACh for these receptors is reduced (Ben-Haim, 1973; Terrar, 1978) and thus ACh would be expected to leave the synaptic cleft more rapidly (Terrar, 1976).

If an action of potassium ions on nerve terminals was important for the initiation of ADF, DTT would reduce ADF by reducing the amplitude of endplate potentials and currents (Ben-Haim *et al.*, 1973; Terrar, 1978) and consequently the release of potassium from the postsynaptic site.

Under conditions where twitch potentiation occurs with little or no detectable ADF, presumably multiple muscle APs are initiated from a single, prolonged endplate potential (Eccles, Katz & Kuffler, 1942; Morrison, 1977). When ADF occurs, multiple endplate potentials would be expected to accompany it. To obtain more information on this, further studies are necessary and one possible approach would be to use intracellular microelectrodes to investigate the effect of paraoxon at individual motor endplates in preparations with and without DTT treatment.

References

BARSTAD, J.A.B. & LILLEHEIL, G. (1968). Transversely cut diaphragm preparation from rat. *Archs int. Pharmaco-*

BEN-HAIM, D., LANDAU, E.M. & SILMAN, I. (1973). The role of a reactive disulphide bond in the function of the actylcholine receptor at the frog neuromuscular junction. *J. Physiol.*, **234,** 305–325.

BLEEHEN, T., CLARK, A.L. & HOBBIGER, F. (1983). Effect of dithiothreitol on agonist and antagonist actions in frog muscle. *J. Pharm. Pharmac.*, (in press).

BOWMAN, W.C. (1980a). *Pharmacology of Neuromuscular Function.* John Wright and Sons Ltd: Bristol.

BOWMAN, W.C. (1980b). Prejunctional and postjunctional cholinoceptors at the neuromuscular junction. *Anesth. Analg. (Cleve),* **59,** 935–943.

BROWN, D.A. & KWIATKOWSKI, D. (1976). A note on the effect of dithiothreitol (DTT) on the depolarization of isolated sympathetic ganglia by carbachol and bromoacetylcholine. *Br. J. Pharmac.*, **56,** 128–130.

BROWN, M.C. & MATTHEWS, P.B.C. (1960). The effect on a muscle twitch of the back response of its motor nerve fibres. *J. Physiol.*, **150,** 332–346.

CLARK, A.L. & HOBBIGER, F. (1983). Twitch potentiation by organophosphate anticholinesterases in rat phrenic nerve diaphragm preparations. *Br. J. Pharmac.*, **78,** 239–246.

CLARK, A.L., HOBBIGER, F. & TERRAR, D.A. (1979). The effect of dithiothreitol on anticholinesterase induced antidromic firing and twitch potentiation. *Br. J. Pharmac.*, **67,** 481–482P.

ECCLES, J.C., KATZ, B. & KUFFLER, S.W. (1942). Effect of eserine on neuromuscular transmission. *J. Neurophysiol.*, **5,** 211–230.

FENG, T.P. & LI, T.H. (1941). Studies on the neuromuscular junction XXIII. A new aspect of the phenomena of eserine potentiation and tetanic facilitation in mammalian muscles. *Chin. J. Physiol.*, **16,** 37–56.

FRANKENHAEUSER, B. & HODGKIN, A.L. (1957). The action of calcium on the electrical properties of squid axons. *J. Physiol.*, **137,** 218–244.

GINSBORG, B.L. & JENKINSON, D.H. (1976). Transmission of impulses from nerve to muscle. In *Handb. exp. Pharmac.*, vol.42, ed. Zaimis, E., pp.229–364. Berlin, Heidelberg, New York: Springer-Verlag.

HOHLFELD, R., STERZ, R. & PEPER, K. (1981). Prejunctional effects of anti-cholinesterase drugs at the endplate. Mediated by presynaptic acetylcholine receptors or by postsynaptic potassium efflux? *Pflügers Arch.*, **391,** 213–218.

HUBBARD, J.I., JONES, S.F. & LANDAU, E.M. (1968). On the mechanism by which calcium and magnesium affect the release of transmitter by nerve impulses. *J. Physiol.*, **196,** 75–86.

HUBBARD, J.I., SCHMIDT, R.F. & YOKOTA, T. (1965). The effects of acetylcholine upon mammalian motor nerve terminals. *J. Physiol.*, **181,** 810–829.

JENKINSON, D.H. (1957). The nature of the antagonism between calcium and magnesium ions at the neuromuscular junction. *J. Physiol.*, **138,** 434–444.

KATZ, B. (1962). The transmission of impulses from nerve to muscle, and the subcellular unit of synaptic action. *Proc. R. Soc. (Biol.),* **155,** 455–477.

KATZ, B. & MILEDI, R. (1973). The binding of acetylcholine to receptors and its removal from the synaptic cleft. *J. Physiol.*, **231,** 549–574.

LASKOWSKI, M.B. & DETTBARN, W.D. (1979). An electrophysiological analysis of the effects of paraoxon at the neuromuscular junction. *J. Pharmac. exp. Ther.*, **210,** 269–274.

LLOYD, D.P.C. (1942). Stimulation of peripheral nerve terminations by active muscle. *J. Neurophysiol.*, **5,** 153–165.

MAGLEBY, K.L. & TERRAR, D.A. (1975). Factors affecting the time course of decay of end-plate currents: a possible cooperative action of acetylcholine on receptors at the frog neuromuscular junction. *J. Physiol.*, **244,** 467–495.

MASLAND, R.L. & WIGTON, R.S. (1940). Nerve activity accompanying fasciculation produced by prostigmin. *J. Neurophysiol.*, **3,** 269–275.

MORRISON, J.D. (1977). The generation of nerve and muscle repetitive activity in the rat phrenic nerve-diaphragm preparation following inhibition of cholinesterase by ecothiopate. *Br. J. Pharmac.*, **60,** 45–53.

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

TERRAR, D.A. (1976). The effect of dithiothreitol on the time course of endplate currents in the presence of neostigmine. *J. Physiol.*, **263,** 119P–120P.

TERRAR, D.A. (1978). Effects of dithiothreitol on endplate currents. *J. Physiol.*, **276,** 403–417.

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

WERNER, G. (1960). Generation of antidromic activity in motor nerves. *J. Neurophysiol.*, **23,** 453–461.

(Received October 26, 1982. Revised March 17, 1983.)