Br. J. Pharmac. (1973), **49**, 651–657.

Some effects of dimethyl sulphoxide (DMSO) on the frog neuromuscular junction

M. H. EVANS AND PAMELA J. JAGGARD

A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

Summary

1. Dimethyl sulphoxide (DMSO) partially reversed neuromuscular blockade brought about by the action of (+)-tubocurarine or Mg^{2+} on the frog sartorius nerve-muscle preparation.

2. The amplitude and duration of the endplate potential (e.p.p.) were increased by DMSO at concentrations of 70 mM or greater.

3. Miniature endplate potentials were raised in frequency, prolonged in duration and increased in amplitude by DMSO at concentrations of 141 mM or greater, but the increase in amplitude was generally less than in the case of the e.p.p.

4. The resting muscle membrane potential was significantly depolarized by DMSO at 70 mM or greater concentrations, both at the endplate and remote from an endplate.

5. The reversal of neurmuscular blockade by DMSO can be explained in terms of its previously reported ability to inhibit cholinesterase activity, together with the depolarizing action on muscle.

Introduction

During the course of an investigation in which dimethyl sulphoxide (DMSO) was being used as a solvent for some synthetic insecticides, it was observed that a low concentration of DMSO in Ringer solution could itself affect the resting membrane potentials and endplate potentials (e.p.ps) in a sartorius nerve-muscle preparation from a frog. As we could find no previous report of such effects, the present investigation was carried out to elucidate their nature.

Methods

All the experiments were performed on isolated muscles of the frog, *Rana temporaria*. The sartorius muscle was dissected out with its nerve supply; the extensor longus dig. IV muscle was used only to investigate effects on miniature endplate potentials (m.e.p.ps), therefore its nerve supply was not preserved. The preparations were maintained in a Ringer solution containing (mM): NaCl, 110; KCl, 2·5; $CaCl_2$, 1·8; glucose, 5·6 and buffered to pH 7·4 with tris(hydroxymethyl) amino methane HCl 5 mM. When neuromuscular block was required, either (+)-tubocurarine was added to the Ringer solution to give a final concentration in the range 2·5–3·5 mg/l, or $MgCl_2$ 9·5–12 mM replaced an osmotically equivalent quantity of the NaCl in the Ringer solution. The solutions, which were gassed with oxygen, irrigated the preparation at 2X3 ml/minute. All the experiments were

carried out at approximately room temperature, which ranged from 18 to 23° C.

Intracellular electrical potentials were recorded via glass micropipettes filled with KCl, 2·7 M. Their resistances were in the range 10–15 MΩ. They were connected through a calomel half cell to a field-effect transistor in a probe headstage which had unit gain and an input resistance of 500 MΩ. The potentials were displayed conventionally on an oscilloscope (Tektronix 565 with 3A3 twin channel amplifier). Membrane potentials were recorded on a potentiometric chart recorder and were also displayed on a digital voltmeter, from whence they could be printed at intervals by a Kienzle D44 digital printer. The frequency of m.e.p.ps was determined by counting the output pulses from a window gate (Heal, 1972) on a Venner TSA 5536 frequency meter and printing the count with the Kienzle D44 at suitable intervals. In some experiments a Computer of Average Transients (CAT 400 C; Technical Measurement Corporation) was used to obtain the average profiles of a large number of e.p.ps. or m.e.p.ps.

Dimethyl sulphoxide (spectrograde reagent, Fisons) was diluted with Ringer solution from the same reservoir that supplied fluid to the preparation during the control period. The following concentrations of DMSO were used: 0·1, 0·5, 1·0 and 2·0 ml/100 ml, equivalent to 14, 70, 141 and 282 mM respectively.

Results

Effect on resting muscle membrane potential

The resting potential in frog muscle was reduced by DMSO. At a concentration of 14 mM there was no detectable effect, at 70 mM there was a small depolarization, and the higher concentrations produced depolarizations which were approximately proportional to the concentration, although the magnitude varied greatly from fibre to fibre. The mean depolarization at 141 mM was 1·23 mV (range 0·6–2·0) and at 282 mM it was 1·98 mV (range 0·5–3·0). The DMSO depolarized the muscle fibres both at the endplate and at points remote from endplates, as illustrated in Figure 1. The depolarization started almost immediately the DMSO reached the preparation, the apparent latent periods of 0·5—0·8 min seen in the records being due to transit time in the dead space of the apparatus.

It was not uncommon for the records of membrane potential to become unstable a few minutes after exposure to DMSO (see Fig. 1b and d and the latter part of the trace in Fig. 5) and occasionally units were lost abruptly.

Effects on endplate potentials

When a nerve-muscle preparation had been treated with doses of tubocurarine or Mg^{2+} which were just sufficient to abolish the indirectly elicited contractions, exposure to DMSO 141 or 282 mM would result in the reappearance of muscle twitches on stimulation of the nerve. In order to study the effects of DMSO on the intracellular e.p.p., it was therefore necessary to use a concentration of tubocurarine or Mg^{2+} high enough to prevent the muscle from twitching when DMSO was present.

Intracellular recording showed that the e.p.p. amplitude was increased by DMSO. At a concentration of 14 mM no effect could be detected; higher concentrations increased the amplitude by 20–300%, there being great variation from one pre-

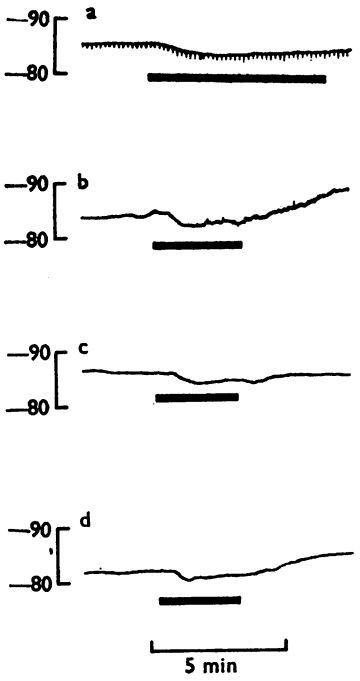

FIG. 1. Potentiometric chart records of intracellular potentials (a) from a frog sartorius muscle in the presence of Mg^{++} 9·2 mM, (c) and (d) from a similar muscle in Mg^{++} 11·7 mM, (b) from a frog toe extensor muscle in normal Ringer solution. Records (a) and (b) were obtained with the microelectrode at an end plate; in (a) the rapid downward deflections are due to endplate potentials which were being evoked by stimulation of the motor nerve every 10 seconds. Records (c) and (d) were taken from a region of the muscle remote from an end-plate, no e.p.p. being detectable. The bars under each record indicate the periods during which dimethyl sulphoxide (DMSO) was allowed to flow into the muscle chamber. The DMSO concentration was 141 mM in (a) and 282 mM in (b), (c) (d). The calibration brackets on the ordinates refer to the intracellular potential in millivolts. When the tip of the micro-electrode was immersed in the bathing fluid, DMSO solutions produced no detectable junction potentials.

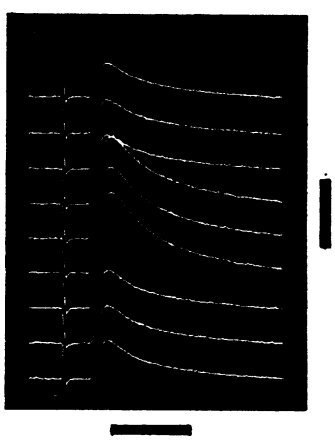

FIG. 2. Endplate potentials recorded intracellularly from a frog sartorius muscle paralysed by the addition of (+)-tubocurarine 2·5 mg/l to the Ringer solution. The top three traces show control responses, the middle three show the responses after being exposed to DMSO 141 mM for 2·5 min, and the bottom three show the responses recovering after washing for 15 minutes. The time trace below the bottom response carries 1, 5 and 10 ms markers. The calibration bars indicate 10 mV and 5 milliseconds.

paration to another. The biggest increases were seen in a preparation which had been paralysed with tubocurarine (and some of the responses recorded during this experiment are shown in Fig. 2), but an insufficient number of experiments have been done with tubocurarine to determine whether this is a general difference from preparations paralysed with Mg^{++}.

In one experiment, on a sartorius preparation, DMSO 70 mM in the presence of Mg^{++} 11·8 mM caused the usual increase in e.p.p. amplitude, but after 3 min the e.p.p. suddenly failed. It reappeared equally abruptly 3·5 min after starting to wash out the DMSO. Some preliminary experiments had shown that compound action potentials in frog and rat nerves were not significantly affected by DMSO at concentrations below 3 ml/100 ml (425 mM), but evidently much lower concentrations can on occasion block conduction near the terminals of motor axons. It is possible that the presence of Mg^{++} may accentuate the tendency to block nerve conduction.

It can be seen from Fig. 2 that as well as increasing the amplitude of the e.p.p., DMSO also prolonged the duration. Even at 14 mM it appeared to have a slight effect, although it was not statistically significant. At 70 and 141 mM the duration was prolonged to a highly significant extent. The effect was not studied at a higher concentration.

By using a CAT 400 C to average large numbers of e.p.ps the effect on the duration could be examined in more detail. An e.p.p. recorded at the endplate rose to its peak amplitude in about 1 ms and then declined more slowly. Soon after passing the peak the rate of decline was exponential with a time constant in the range 2·1—3·4 ms, but after a few ms an inflection occurred and the subsequent rate of decline was slower. The rate of this slower phase of repolarization could not be measured accurately in the present experiments but appeared to have a time constant of about 5–8 milliseconds. If the duration of the e.p.p. was measured between the rising phase and the falling phase at 50% of the peak amplitude, then DMSO 70 mM prolonged this time by 16–25%, and 141 mM prolonged it by 25–50%. The averaged responses revealed that the DMSO was prolonging the duration by increasing the time constant of the early rapid phase of repolarization, and seemed to have no effect on the late slower phase.

Effects on miniature endplate potentials

The amplitude and duration of m.e.p.ps were increased by DMSO, which also raised their frequency. These effects can be seen in the oscillograms shown in Figure 3. Normal m.e.p.p. activity can be seen in Fig. 3a, taken shortly before the application of DMSO. The records in Fig. 3b were taken after 1–2 min exposure to DMSO 282 mM. It can be seen that the individual m.e.p.ps were larger, with occasional very large potentials, and were also much more frequent. Figure 4 shows a series of histograms of m.e.p.p. amplitudes measured from recordings made later from the same preparation. The mean amplitude of the m.e.p.p. increased, from control values in the range 360–400 μV, to over 500 μV during exposure to DMSO 282 mM.

The frequency of the m.e.p.ps during the same experiment is shown in Fig. 5, which also shows another example of the depolarization of the muscle membrane potential following the application of the DMSO. It can be seen that the frequency

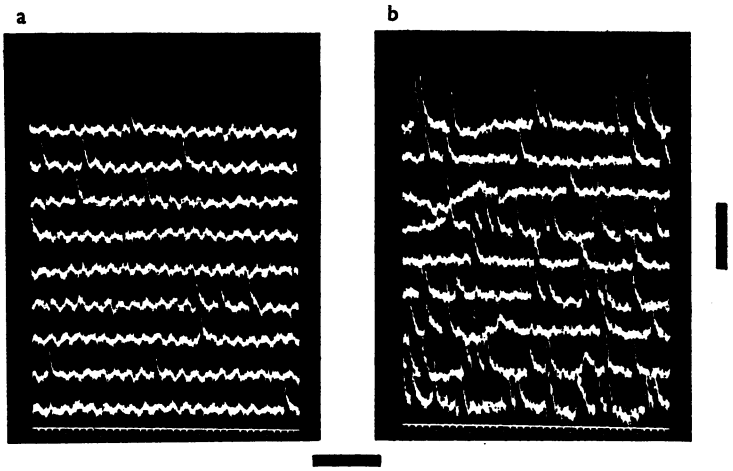

FIG. 3. Miniature endplate potentials recorded from a frog toe extensor muscle. In (a), taken during the control period, up to 3 m.e.p.ps can be seen on each of the oscilloscope sweeps. The records shown in (b) were taken between 1 and 2 min after starting to expose the muscle to dimethyl sulphoxide 282 mM. The m.e.p.ps are now much more frequent and are somewhat larger. The time trace at the bottom of each set of records carries 10, 50 and 100 ms markers. The calibration bars indicate 1 mV and 100 milliseconds.

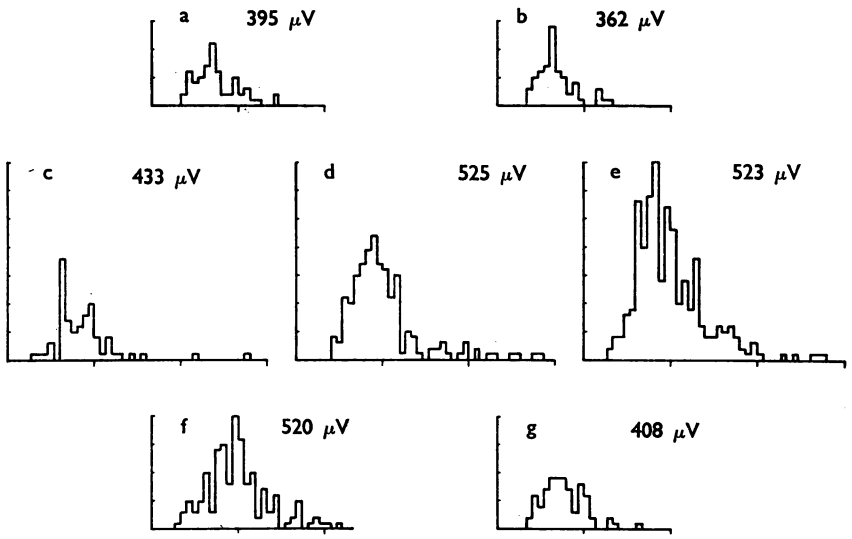

FIG. 4. Histogram of the amplitude distribution of miniature endplate potentials before, during and after exposure to dimethyl sulphoxide (DMSO) 282 mM. The ordinates are marked in divisions of 5 units, the abscissae are marked at 500 μV intervals. The figures at the top right hand corner of each histogram are the means of the amplitudes of all the responses in that histogram. Histograms (a) and (b) are from the control period, 2 and 0·3 min respectively before addition of DMSO. Histograms (c), (d) and (e) are from recordings made in the presence of DMSO, 0·5, 2·0 and 3·2 min after irrigation began. Histograms (f) and (g) show recovery during washout after 2·0 and 5·5 min respectively from the start of washing.

of the m.e.p.p. rose from around 20–25/s during the control period, to over 70/s in the presence of DMSO 282 mM. In similar experiments the same concentration of DMSO caused the m.e.p.p. frequency to increase 3- to 8-fold.

Individual m.e.p.ps could be made to trigger the oscilloscope sweep if the internal triggering level was adjusted to avoid triggering by background noise impulses.

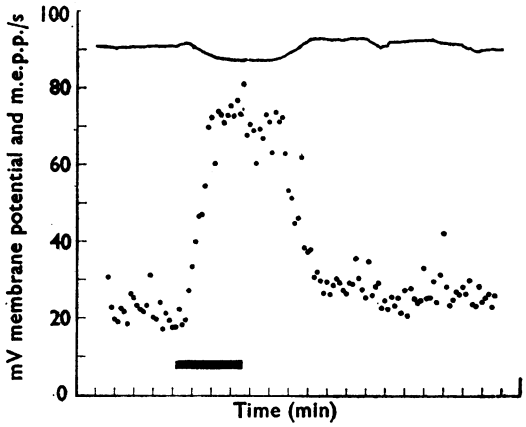

FIG. 5. Graph of the frequency of miniature endplate potentials recorded from a frog toe muscle, plotted below the chart record of the resting muscle membrane potential. The bar below the graph indicates the period during which dimethyl sulphoxide 282 mM was flowing into the muscle chamber. These results were recorded simultaneously with the m.e.p.p. amplitude recordings shown in Figure 4.

By using the oscilloscope's sweep gating pulse to trigger the CAT 400 C, very large numbers of m.e.p.ps could be averaged and the waveform studied in detail. The initial 50–100 μV of the rising (depolarizing) phase could not be recorded because it was below the oscilloscope's trigger level, but the peak and the declining (repolarizing) phase of the averaged m.e.p.ps were recorded satisfactorily. As in the case of the e.p.p., the m.e.p.p. rose to a peak in 1 ms or less and then declined, initially at an exponential rate with a time constant of about 2–3 ms, and then at a slower rate. The duration of the averaged m.e.p.p. at 50% of the peak amplitude was in the range 2·2–2·7 millisecond. In DMSO 141–282 mM this duration was increased by 35–82% mainly, and perhaps entirely, as a result of slowing the first phase of the re-polarization.

Discussion

It was unexpected to find that DMSO had significant excitatory effects at the neuromuscular junction at concentrations of 1 ml/100 ml (141 mM) or less, when previous reports suggested that higher concentrations had either no effect or depressant actions. The toxicity of DMSO to intact animals is low, even when given i.v. (Caujolle, Caujolle, Cros & Calvet, 1967; Smith, Hadidian & Mason, 1967) and *in vitro* studies on nerve have shown effects only at concentrations of 6 ml/100 ml (845 mM) or greater (Davis, Davis & Clemons, 1967; Sams, 1967). Sams, Carrol & Crantz (1966) investigated the effects of DMSO ranging from 0·6–6·0% final bath concentration. They found no effect on guinea-pig hemidiaphragm *in vitro* at 0·6%. A concentration of 3% diminished the contractions both to direct and to indirect stimulation; since the direct and indirect responses were depressed to an equal extent there was no evidence that the DMSO was affecting neuromuscular transmission. There was some biochemical evidence of cholinesterase inhibition at 0·78% (0·1 M). More recently, Gandiha & Marshall (1972) obtained evidence of actions at the neuromuscular junction of the chick biventer cervicis muscle with DMSO concentrations in the range 1–20 ml/100 ml. They attributed

some of the effects to inhibition of cholinesterase activity, but also noted that DMSO had a direct action on the muscle.

The experiments reported in the present paper show that DMSO affects neuromuscular transmission in frog muscle at lower concentrations than any previously reported, and also reveal something of the mechanisms of these effects. Some of the effects are consistent with inhibition of cholinesterase, but other effects are best explained by the depolarizing action of DMSO on the muscle membrane and probably also on motor nerve terminals.

The increased amplitude and duration of the e.p.p. produced by DMSO concentrations of 0·5 ml/100 ml (70 mM) or more, can be attributed to an anticholinesterase action at these concentrations. The fact that similar effects were seen on the m.e.p.p. indicates that the effect was, at least in part, due to an action distal to the nerve terminals. The increased duration of the e.p.p. and m.e.p.p. resulted from a slowing of the first phase of the (repolarizing) decline in potential, suggesting a slower rate of transmitter destruction due to cholinesterase inhibition. The possibility that the increased duration resulted from a prolongation of transmitter release seems unlikely in the case of the m.e.p.p. effects. The later phase in the decline of the e.p.p. and m.e.p.p., during which the charge is redistributed along the muscle membrane (Fatt & Katz, 1951), was unaffected. Hence DMSO seemed not to affect the passive capacitance characteristics of the muscle membrane at these concentrations.

The depolarization of the muscle membrane potential produced by DMSO is probably unconnected with any anticholinesterase activity, because DMSO depolarized the membrane remote from the neuromuscular junction as effectively as at the endplate. Any effect localized to the endplate would be greatly attenuated a few mm away from the endplate. The ability of DMSO, at concentrations of 1 ml/100 ml or greater, to increase the frequency of the m.e.p.ps suggests that it may also have been depolarizing the motor nerve terminals significantly, since depolarizing agents such as KCl are known to increase greatly m.e.p.p. frequency.

Therefore the effect that DMSO has in partially reversing a neuromuscular block can be attributed to two fundamental actions: (a) an increase in the effectiveness of the transmitter through an inhibition of cholinesterase at the endplate, thereby increasing both the amplitude and duration of the e.p.p., and (b) a small depolarization of the resting muscle membrane potential, which might facilitate neuromuscular transmission by summing with the e.p.p.

REFERENCES

CAUJOLLE, F. M. E., CAUJOLLE, D. H., CROS, S. B. & CALVET, M.-M. J. (1967). Limits of toxic and teratogenic tolerance of dimethyl sulphoxide. *Ann. N.Y. Acad. Sci.*, **141**, 110–125.

DAVIS, H. L., DAVIS, N. L. & CLEMONS, A. L. (1967). Procoagulant and nerve-blocking effects of DMSO. *Ann. N.Y. Acad. Sci.*, **141**, 310–325.

FATT, P. & KATZ, B. (1951). An analysis of the end-plate potential recorded with an intra-cellular electrode. *J. Physiol., Lond.*, **115**, 320–370.

GANDIHA, A. & MARSHALL, I. G. (1972). Some actions of dimethylsulphoxide at the neuromuscular junction. *J. Pharm. Pharmac.*, **24**, 417–419.

HEAL, J. W. (1972). A window-type pulse-height discriminator with limit monitoring. *Med. & Biol. Eng.*, **10**, 697–700.

SAMS, W. M. (1967). The effects of dimethyl sulphoxide on nerve conduction. *Ann. N.Y. Acad. Sci.*, **141**, 242–247.

SAMS, W. M., CARROLL, N. V. & CRANTZ, P. L. (1966). Effect of dimethylsulphoxide on isolated-innervated skeletal, smooth, and cardiac muscle. *Proc. Soc. exp. Biol.*, **122**, 103–107.

SMITH, E. R., HADIDIAN, Z. & MASON, M. M. (1967). The single- and repeated-dose toxicity of dimethyl sulphoxide. *Ann. N.Y. Acad. Sci.*, **141**, 96–109.

(*Received June* 22, 1973)