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The Actions of Dimethyl Sulfoxide on Neuromuscular Transmission

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SUMMARY

The effects of dimethyl sulfoxide (DMSO) on subsynaptic response and quantal release of transmitter have been studied at the mammalian neuromuscular junction. Subsynaptically, at low concentrations (up to 1% by volume), DMSO prolongs the time course of decay of miniature endplate currents, (MEPCs), with no significant effect on the amplitude of the currents, which is consistent with an action of DMSO to inhibit acetylcholinesterase. At higher concentrations of DMSO (in excess of 1% by volume) the amplitude of MEPCs and the steady state response to carbamoylcholine (carbachol) are significantly reduced, which suggests an additional action of DMSO other than pure anticholinesterase activity. After pretreatment of the preparation with a low concentration of paraoxon, higher concentrations of DMSO decrease MEPC height and cause highly variable changes in the decay time course of the MEPC. The results suggest that DMSO concentrations in excess of 1% by volume have two distinct and opposite actions on the subsynaptic response; a pure anticholinesterase activity to enhance the response and a depressant effect which is similar to that caused by *d*-tubocurarine. Presynaptically, DMSO increased both the spontaneous release (measured as the frequency of miniature endplate potentials, fMEPP) and the evoked release (measured as the quantal content of endplate potentials). Both types of release were increased as an exponential function, with the same slope, of the DMSO concentration, suggesting a common mode of action on these two types of release. This action appeared not to be due to an effect on the disposition or effectiveness of calcium ions inside the terminal but, rather, was due to a fusogenic or global effect. In addition, the increase in fMEPP with DMSO was the same when external calcium was replaced by barium. At the concentrations studied, up to 8% by volume, DMSO did not cause any substantial depolarization of the nerve terminal or any appreciable change in the nerve terminal action potential. In a few experiments facilitation was studied at the frog neuromuscular junction and was unchanged by DMSO at concentrations which considerably enhanced transmitter release.

Previous electrophysiological studies have shown that DMSO acts to inhibit acetylcholinesterase at mammalian and amphibian neuromuscular junctions (1, 2). In preliminary experiments we found, however, that concentrations of DMSO in excess of 1%, by volume, also act to attenuate the height of MEPPs, an effect which is not consistent with a pure anticholinesterase activity (3). A depression of subsynaptic response by higher concentrations of DMSO has previously been reported in several preparations including the neuromuscular junction of the chick (1) and in nerve cells from *Aplysia* (4). At present, however, no voltage clamp data are available to determine the nature of possible direct interactions of the compound with the ion channel-receptor complex. We have used the point-voltage clamp technique to determine the subsynaptic effects of DMSO (at concentrations up to 6% by volume) on MEPCs and on the responses to applications of ACh and carbamoylcholine (carbachol).

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DMSO also affects the release of transmitter. Evans and Jaggard (5) found that DMSO produced an increase in fMEPP, which they suggested might be secondary to a depolarization of nerve terminals; a concurrent depolarization was observed in the muscle fibers. Such a depolarization might arise in principle from a reduction in potassium conductance; indeed, it has been found that, in *Aplysia*, rather high concentrations of DMSO (8–15%) decrease the permeability of ganglionic neurons to K^+ and to Cl^- (4). In frog sympathetic ganglia, DMSO produces an enhancement and prolongation of the fast excitatory postsynaptic potential in a manner similar to that produced by 3,4-diaminopyridine or by cesium, which was attributed to a prolongation of the nerve terminal action potential as a consequence of a reduction in potassium conductance (6).

In this work we find that the action of DMSO to alter transmitter release at the neuromuscular junction is similar to that exerted by ethanol, which causes a parallel multiplication of spontaneous and depolarization-evoked transmitter release (7, 8). Our results show that DMSO does, indeed, increase

ABBREVIATIONS: DMSO, dimethyl sulfoxide; MEPP, miniature endplate potential; EPP, endplate potential; fMEPP, frequency of miniature endplate potentials; MEPC, miniature endplate current; ACh, acetylcholine.

quantal release of ACh; both the "synchronous" release, evoked by an action potential (EPP), or the "asynchronous" release, manifest as spontaneous frequency of MEPPs, are increased by the same factor. The increase in spontaneous transmitter release caused by DMSO is insensitive to concentrations of cadmium (Cd^{2+}) which block depolarization-evoked increases in MEPP frequency and, therefore, is not to be attributed to any depolarization of the nerve terminal that might occur.

Materials and Methods

Experiments were performed using the mouse hemidiaphragm preparation or, in a few experiments, the frog cutaneous pectoris preparation. The preparations were superfused using a system which allows rapid switching of the bathing medium as described in Cooke and Quastel (9).

In the experiments using the mouse diaphragm, the standard bathing solution had the following composition (in mM): NaCl, 125; $NaHCO_3$, 24; NaH_2PO_4, 1; $MgCl_2$, 1; KCl, 5; glucose, 11; $Cacl_2$, 2; the solutions were bubbled with 95% O_2/5% CO_2 to give a pH of 7.4. The frog muscle preparation, described in some detail in Ref. 10, was superfused with a solution containing (in mM): NaCl, 90; Na_2HPO_4, 2; NaH_2PO_4, 1; KCl, 2; $MgCl_2$, 4; glucose, 11; $CaCl_2$, 0.3; the solutions were bubbled with O_2.

In the postsynaptic studies a two-electrode point-voltage clamp technique (11) was used and MEPCs were recorded digitally, using a PDP-12 computer, for off-line averaging and analysis; the procedures used in the determination of the height and time course (the decay time constant was determined between $e^{-0.5}$ and $e^{-1.5}$ of the peak) of the MEPCs are outlined in Refs. 11 and 12. MEPCs were recorded with DMSO concentrations (by %/volume) of 1, 2, 4, and 6, over the voltage range from −40 mV to −100 mV and at ambient room temperature (25–27°). MEPCs were also recorded after application of 4 μM paraoxon (for a period of 5 min before wash-off). In some experiments the responses of the voltage-clamped endplate to superfusion with ACh or carbachol were recorded on a Mingograf ink-jet system. In these experiments, care was taken to select junctions, presumably superficial in location, where response to the agonist commenced within a few sec after switching the perfusion solution and a peak response was obtained in about 15 sec; the peak height of the response was measured before any obvious desensitization had occurred. Sufficient time was allowed between agonist applications (usually 30–60 sec) by ensuring that baseline levels had reached steady state.

Standard intracellular techniques were used to record the MEPPs and EPPs, which were displayed simultaneously on an oscilloscope and a Mingograf ink-jet recorder. The fMEPP was measured on-line by a PDP-12 computer. This count was corrected from the Mingograf records when the MEPP frequency was high, e.g., such as occurred with high concentrations of DMSO. Stimuli were applied via a suction electrode to the phrenic nerve in the case of the mouse preparation and to the pectoralis proprius nerve in the frog preparation. The estimates of quantal content were done by both the method of failures and the method of variance (13).

Results

Postsynaptic Studies

Height and time course of MEPCs. DMSO concentrations in the range 1–6% were applied to the voltage-clamped endplate. In Fig. 1, MEPCs in the absence and presence of 4% DMSO are shown; at this concentration the compound acted both to prolong the time course of decay and to diminish the height of the current. The time course of MEPC decay remained close to exponential over the range of DMSO concentrations studied; there was no indication of any "split" of the decay into a biphasic form, such as is observed with a variety of anesthetic and local anesthetic compounds. Table 1 includes the heights, relative to control, and the time constants for MEPCs in the presence of DMSO concentrations of 1%, 2%, 4%, and 6%; the compound caused both a progressive diminution of the height and a prolongation of the decay time course. In a few cells the voltage sensitivity of the decay time course of the MEPCs was measured over a range of holding potentials from −40 mV to −100 mV (Fig. 2); DMSO had no significant effect to alter the voltage dependence of the time course. The decrease in the rate of the MEPC decay with DMSO is consistent with an action to inhibit acetylcholinesterase. However, the decrease in height with increasing concentration of DMSO indicates an action of the compound on the receptor-ion channel complex.

Height and time course of MEPCs after poisoning of acetylcholinesterase. The effects of DMSO on MEPC amplitude and time course were also determined after acetylcholinesterase poisoning using short-term treatments of 4 μM paraoxon (see Materials and Methods). This treatment (14) extends the lifetime of ACh in the synaptic cleft and decreases the rate of decay of the MEPC by about 4-fold. It should be noted that the low concentration of paraoxon used does not lead to any apparent effects to directly modify properties of the receptor-ion channel complex such as has been documented for some other organophosphorus compounds (15). Fig. 3 shows MEPCs in a cell before and after perfusion with a solution containing 4% DMSO. The amplitude of the MEPC was diminished by the DMSO as noted previously at junctions with no acetylcholinesterase poisoning, but now the MEPC decay time constant was decreased. The rate of the MEPC decay in the presence of 4% DMSO was highly variable after paraoxon treatment; in 5 of the 12 junctions studied, a decrease in the time constant of the decay was observed. Table 1 indicates the normalized MEPC heights and time courses for DMSO concentrations of 2%, 4%, and 6%. On average, the DMSO had an action to slightly decrease the rate of the decay of the MEPC. In a single cell studied with 8% DMSO, the time constant of the decay was markedly attenuated (a detailed study of the effects on the time constant of decay of MEPCs was not possible at DMSO concentrations in excess of 6% due to the associated increase in the frequency of the currents). The effect of DMSO to decrease the MEPC height is the same with or without cholinesterase inhibition, whereas the consistent prolonging actions of DMSO on decay time course with no paraoxon treatment are not observed after application of the anticholinesterase.

Correlation of the height and time course of individual MEPCs. After poisoning of acetylcholinesterase the decay of the MEPC is slowed by repeated bindings of ACh with receptors as the transmitter diffuses from the synaptic cleft (16). Under this condition individual MEPCs show a large variability in both height and time course (14). Linder *et al.* (14) have found that, after poisoning of acetylcholinesterase with paraoxon or neostigmine, the time constant of decay is significantly correlated with the height of the MEPC for a series of individual MEPCs. In the present work the slopes of the graphs for the log height versus log time constant were determined for two cells (holding potentials of −80 mV) bathed in 4% DMSO. In one cell, where a total of 325 MEPCs were recorded, the slope of the correlation was 0.62 ± 0.04 (mean ± SE) and in the second cell, with a total of 238 MEPCs, the slope was determined to be 0.47 ± 0.05 (mean ± SE). These values are significantly larger than the slope obtained in previous experi-

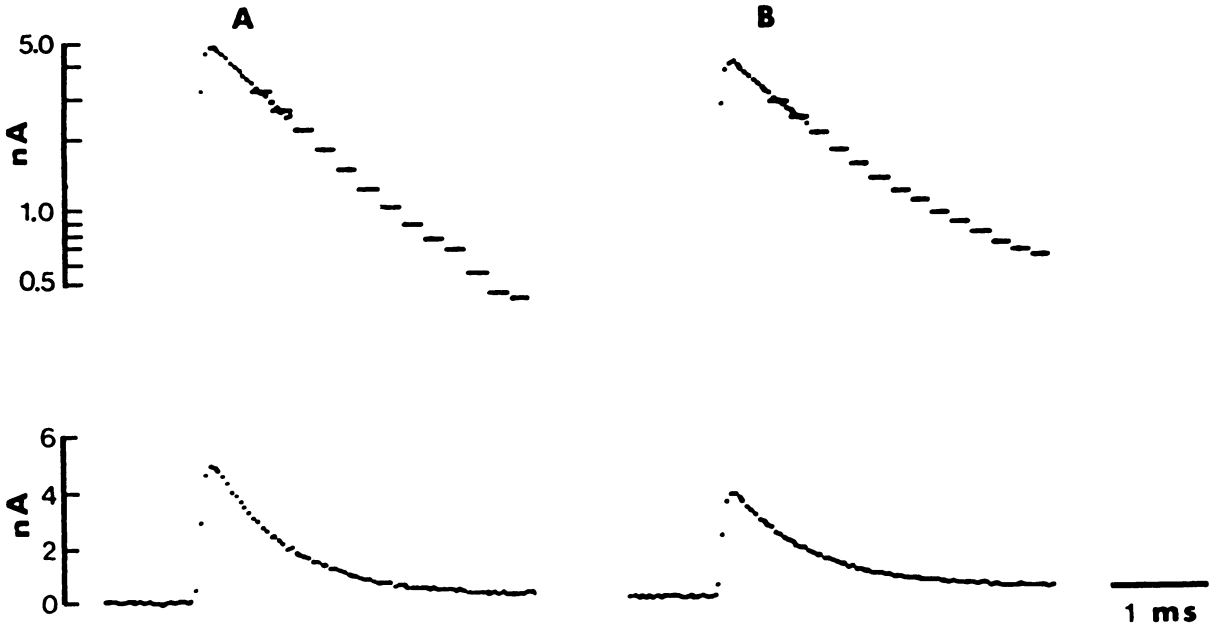

Fig. 1. MEPCs plotted linearly (*lower traces*) and semilogarithmically (*upper traces*) at a holding potential of −100 mV. A, control solution; B, 4% DMSO. Data are from a single junction and each plot represents an average of 30–40 MEPCs.

TABLE 1

Heights and time constants for MEPCs relative to controls for different concentrations of DMSO

Values are expressed as a ratio to the values obtained in control solution at the same junction ± standard error. Holding potential was −80 mV.

DMSO concentration	Percentage	*n*	Height	Time constant
AChE[a] intact	1	4	0.93 ± 0.07	1.15 ± 0.08
	2	5	0.84 ± 0.06	1.44 ± 0.06
	4	12	0.78 ± 0.03	1.62 ± 0.07
	6	6	0.65 ± 0.04	2.12 ± 0.12
AChE poisoned by paraoxon	2	9	0.88 ± 0.04	1.22 ± 0.10
	4	12	0.79 ± 0.02	1.11 ± 0.10
	6	6	0.69 ± 0.04	1.13 ± 0.18

[a] AChE, acetylcholinesterase.

ments of 0.12 ± 0.02 (14) in control solution using a total of 1536 MEPCs and somewhat lower than the slope of 0.77 ± 0.02 (14) obtained after paraoxon inhibition of acetylcholinesterase; in both of these cases data at different holding potentials have been grouped since there was no obvious voltage dependence of the correlations.

Responses to agonist. Several experiments were performed in which the voltage-clamped endplate was perfused with solutions containing either 20 μM carbachol or 15 μM ACh in the absence and presence of 2% DMSO. As shown in Fig. 4, the subsynaptic response to carbachol was diminished in the presence of DMSO, whereas the response to ACh was increased by DMSO. At nine junctions, DMSO depressed responses to carbachol by 47 ± 6% (mean ± SE); with ACh at five endplates, responses were increased to 185 ± 18% (mean ± SE) of the control response.

A similar protocol was used to determine the responses after the endplate had been treated with paraoxon. The results showed that DMSO, at a concentration of 2%, reduced the response to carbachol (20 μM) by 43 ± 5% (mean ± SE) at eight junctions, much the same as is found with acetylcholinesterase intact. With ACh (15 μM) as the agonist, responses with the 2% DMSO were now found to be 88 ± 14% (seven junctions) of the control value.

These data illustrate both the direct actions of DMSO on the receptor-channel complex and the inhibitory action to poison acetylcholinesterase. The direct action is manifest in a substantial decrease in the response to carbachol with 2% DMSO and an effect to poison acetylcholinesterase is evident from the increased current amplitude observed with ACh perfusion compared with that for carbachol application.

Presynaptic Studies

Changes in fMEPP. Fig. 5 shows the results of a multiple sampling experiment, in which the response of fMEPP in a random population of cells in the diaphragm was measured by rapid impalements of the muscle cells (at a rate of about 3 per min). The diaphragm was superfused with either control solution (mM: $5K^+/2Ca^{2+}$) or control solution with 0.5 mM Cd^{2+} added. fMEPP rose exponentially as a function of DMSO concentration over the range tested and the response of fMEPP to DMSO was unaltered by the addition of 0.5 mM Cd^{2+}. We had previously verified that, at this concentration of Cd^{2+}, fMEPP did not respond to nerve terminal depolarization using raised K^+. Fig. 6 shows the results of a similar experiment with the K^+ concentration raised to 15 mM; in $15K^+/2Ca^{2+}$, fMEPP was again an exponential function of the concentration of DMSO over the range tested, with the slope of this relation somewhat less than that obtained in $5K^+/2Ca^{2+}$ (see Fig. 5). In the presence of 0.5 mM Cd^{2+} and 15 mM K^+, although there was a profound block of depolarization/calcium-dependent release evident as the reduction of control fMEPP from more than 20 s^{-1} to about 2.5 s^{-1}, the response to DMSO was not reduced; indeed, the slope of ln fMEPP against DMSO concentration was increased in the presence of Cd^{2+} and approached that seen in 5 mM K^+ solutions.

Effect of DMSO on quantal content in high Mg^{2+}/low Ca^{2+} solutions. Fig. 7 shows the results obtained from an experiment on the mouse diaphragm in which the phrenic nerve was stimulated with 3-sec trains of 50 Hz stimuli to elicit low

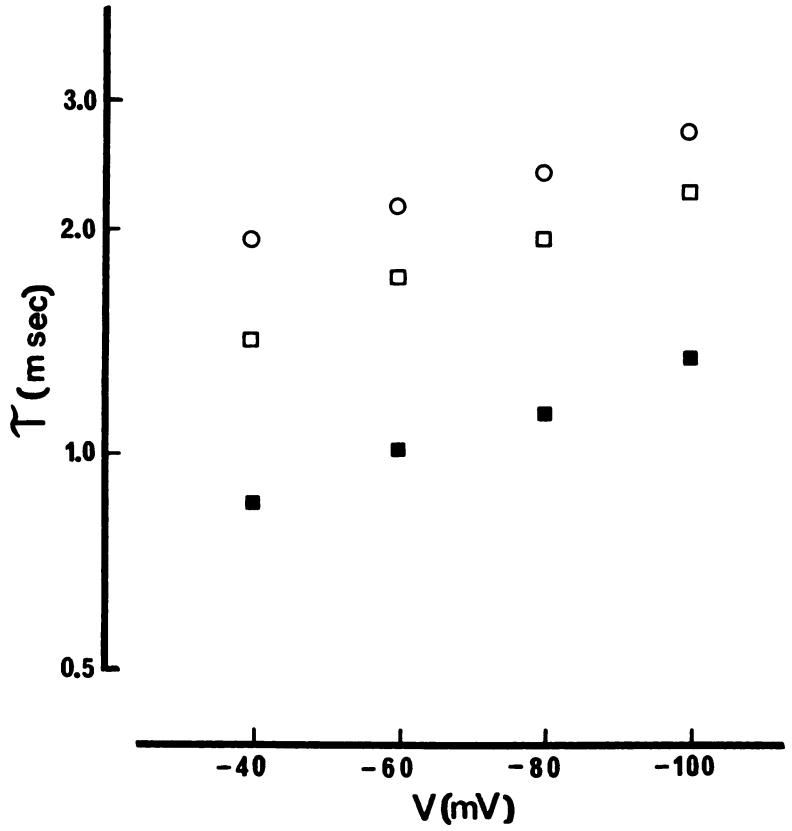

Fig. 2. Voltage dependence of the time constant (see Materials and Methods for measurement) for MEPC decay. ■, control solution; □, 4% DMSO; ○, 6% DMSO. All data were recorded from a single junction.

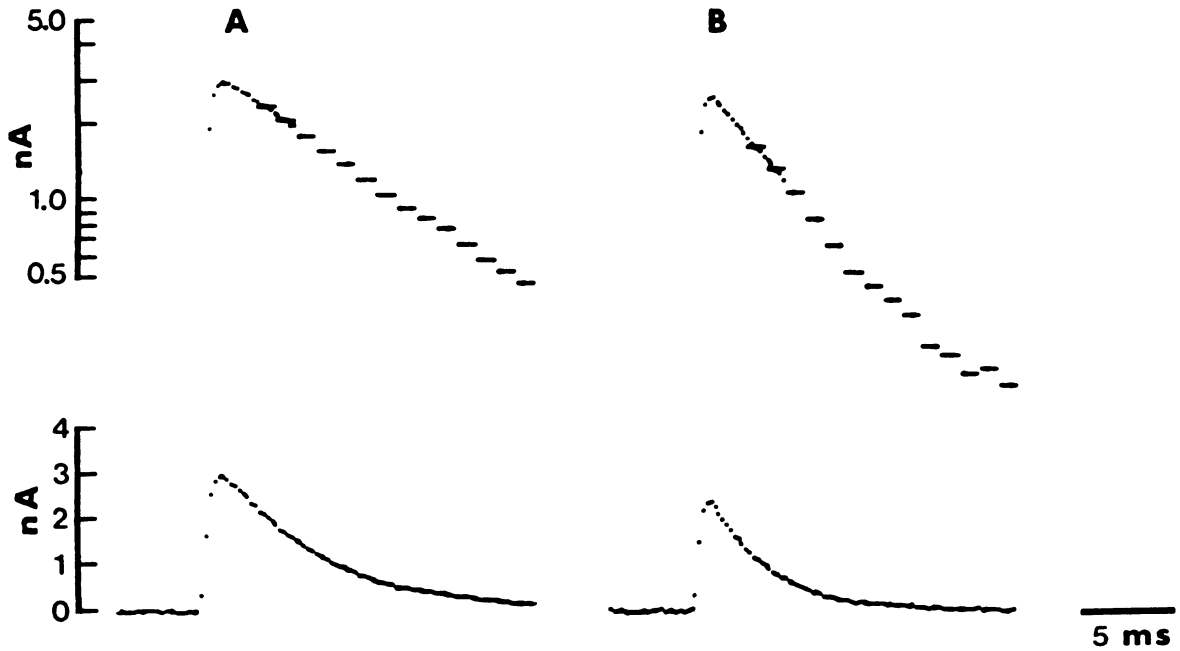

Fig. 3. MEPCs plotted linearly (*bottom*) and semilogarithmically (*top*) at a holding potential of −60 mV. A, control solution; B, 4% DMSO. The preparation was treated with 4 μM paraoxon for 5 min and then washed.

quantal content EPPs in a solution containing $5K^+/0.2Ca^{2+}$ and 4 mM Mg^{2+} with different concentrations of DMSO. Quantal content was estimated by failures and fMEPP was estimated by counting the number of MEPPs occurring in a suitable time period immediately before the train. The results are shown in Fig. 7 as fMEPP and mean quantal content (m) plotted semilogarithmically against DMSO concentration. For the full range of increase of the DMSO concentration, up to 8%, m increased from ≃0.02 to ≃1.0, i.e., 50-fold, whereas the simultaneously recorded fMEPP was also increased 50-fold, from ≃0.3 to 15 s^{-1}. Note that both log m and log fMEPP were linearly related to the DMSO concentration with the same slope, i.e., fMEPP and m are raised by the same factor by a given concentration of DMSO. Table 2 summarizes the effects of DMSO on fMEPP and quantal content.

Effects of DMSO on high quantal content EPPs. Fig. 8

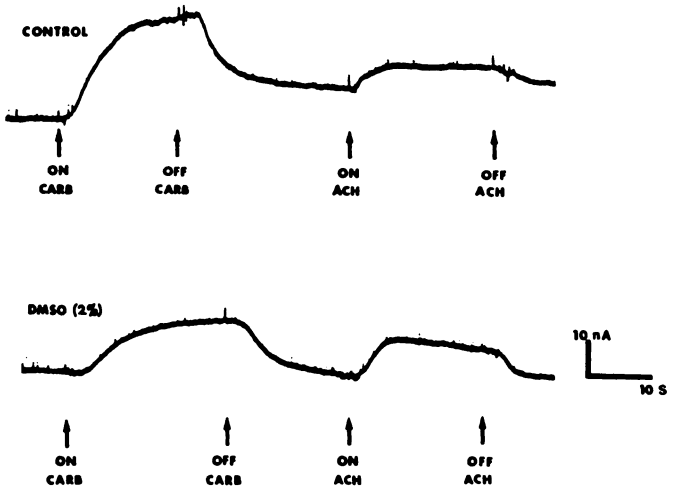

Fig. 4. Responses of a voltage-clamped endplate for perfusion of 20 μM carbachol or 15 μM ACh in the absence (*top traces*) or presence (*bottom traces*) of 2% DMSO. The small projections barely visible from the baseline are MEPCs.

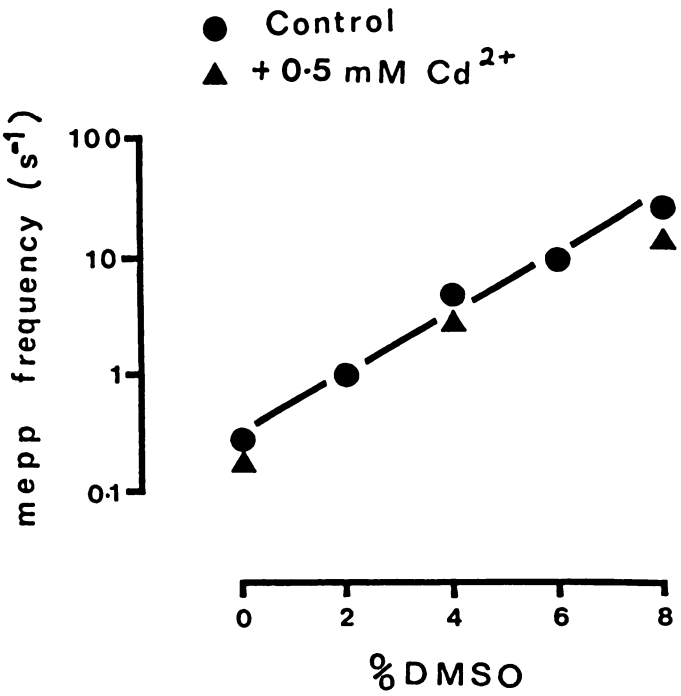

Fig. 5. Effect of DMSO in 5 mM K^+ solution. Response of fMEPP to DMSO was assessed by multiple sampling in a solution containing 5 mM K^+ and 2 mM Ca^{2+}. Each *point* is a mean of 15–20 cells, and the standard error is typically smaller than the *symbols* used. fMEPP rose exponentially with increasing DMSO concentration; this response was essentially unaffected by 0.5 mM Cd^{2+}. The *line* shown was fitted by eye to the data.

shows the effect of DMSO on EPPs produced by a train of stimuli at 50 Hz in a solution containing $5K^+/2Ca^{2+}$ and 500 nM *d*-tubocurarine. Each record represents the computer average of 10 trains. Each individual train was normalized by computer to a membrane potential of −80 mV before averaging. The 2% DMSO produced an increase in the magnitude of the first EPP in the train, to about double the control. In addition, the "rundown" of EPP height appeared faster in the presence of DMSO. However, because of the change in the quantal unit size caused by the postsynaptic action of DMSO, and nonlinear

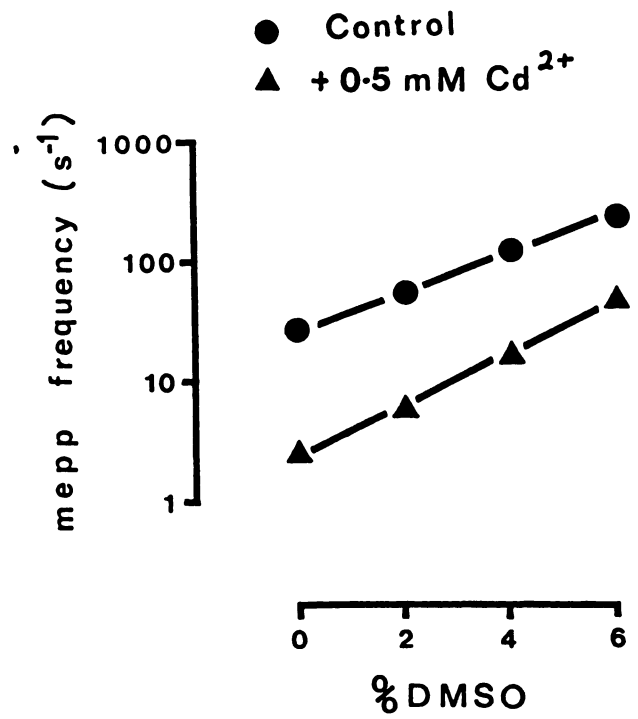

Fig. 6. Effect of DMSO on fMEPP in 15 mM K^+ solution. Response of fMEPP to DMSO was assessed by multiple sampling in a solution containing 15 mM K^+ and 2 mM Ca^{2+}. Each *point* is a mean of 15–20 cells; the standard error was typically smaller than the *symbols* used. fMEPP rose exponentially with increasing DMSO concentration; 0.5 mM Cd^{2+}, although it caused a substantial depression of Ca^{2+}-dependent release, had no substantial effect on the response to DMSO. The *lines* shown were fitted by eye to the data.

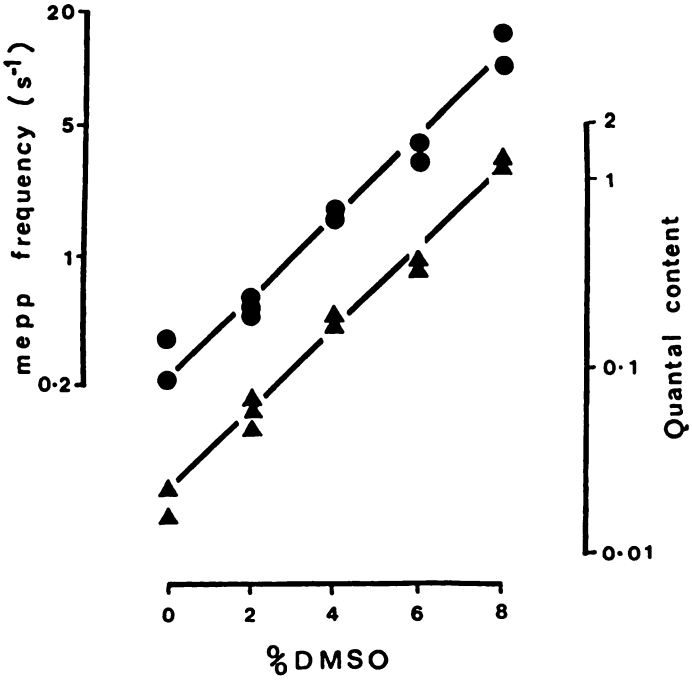

Fig. 7. Effect of DMSO on low quantal content EPPs and fMEPP. ●, fMEPP, measured immediately before the train, on *scale* to the *left*; ▲, quantal content, on the *scale* to the *right*, during a 3-sec train of stimuli at 50 Hz in various concentrations of DMSO. Results shown are from one cell; similar results were obtained in other cells. *Lines* were fitted by eye. fMEPP and quantal content rose by the same factor in response to each concentration of DMSO.

TABLE 2

Effect of DMSO on *m* and fMEPP

The two columns to the right indicate the change in ln fMEPP and ln *m* for the change in DMSO concentrations indicated on the left. The total change for the range of DMSO concentrations from 0 to 8% was 4.07 for fMEPP and 3.96 for m, not significantly different. Errors shown are standard errors; the number in parentheses indicate the number of cells studied.

DMSO	Percentage of ln fMEPP	Percentage of ln *m*
%		
0–2	0.873 ± 0.05 (5)	0.962 ± 0.05 (5)
2–4	1.170 ± 0.03 (5)	1.30 ± 0.16 (5)
4–6	1.060 ± 0.13 (5)	0.625 ± 0.27 (5)
6–8	0.962 ± 0.16 (4)	1.07 ± 0.07 (4)

summation, the EPP height does not directly reflect changes in quantal content. Table 3 shows, in three cells, the estimate of fractional release, calculated from the height of the first EPP in μV, as a fraction of the immediately available store of transmitter expressed in μV. The available store was calculated from the intercept of the run-down in EPP height of the first seven EPPs. The change in the estimated fractional release was small but was consistently increased by 2% DMSO. The parameter, second EPP height/first EPP height, is included as a measure of EPP rundown. Estimates of the quantal size by the method of variance and consequent estimation of increase in quantal content by DMSO are subject to relatively large errors; however, DMSO always increased the estimated quantal content.

Effect of DMSO on facilitation. Since preliminary studies had shown that the frog neuromuscular junction shows a much greater degree of frequency facilitation than does that of the mouse, experiments on facilitation were performed using the frog cutaneous pectoris preparation. Fig. 9 shows the result of an experiment in which 50-Hz trains of stimuli, lasting 10 sec, were given every minute. The quantal content was estimated for each second of the train by the method of failures (see Materials and Methods) and the simultaneous fMEPP was counted. The stimulation paradigm was repeated in the presence of 4% DMSO. As in the mouse (Fig. 8), DMSO raised quantal content and fMEPP by the same factor; the rises in log fMEPP and quantal content during the train due to facilitation were unaffected by DMSO. In addition, the raised fMEPP which persists after the cessation of stimulation was multiplied by the same factor as the other forms of release.

Effect of DMSO on barium-induced release. In Fig 6 (fMEPP measured in solution containing 2 mM Ca^{2+} and 15 mM K^+), the control fMEPP was 27 s^{-1} and the change in ln fMEPP produced by the addition of 6% DMSO was 2.1 ± 0.1. In an experiment in which the diaphragm was superfused with a solution containing 15 mM K^+, 0 Ca^{2+} and 0.3 mM Ba^{2+}, the control fMEPP, presumably primarily due to Ba^{2+} rather than Ca^{2+} under these conditions, was 38 s^{-1} and the change in log fMEPP produced by the addition of 6% DMSO was 2.3 ± 0.1, i.e., the effects of DMSO were not significantly different from that produced when transmitter release was due to Ca^{2+}.

Discussion

Subsynaptically, DMSO exerts two clear and distinct actions on MEPCs at the mammalian neuromuscular junction. The progressive prolongation in the time course of MEPC decay with increasing concentration of DMSO is evidently related to its action to poison acetylcholinesterase (1, 2). The average slope of the correlation between log time constant and height with 4% DMSO for a large series of MEPCs was found to be 0.55 ± 0.05, much larger than the value of 0.12 ± 0.02 previously determined for control solution (14), indicating that in the presence of DMSO the time constant of decay is governed by diffusion and rebinding with receptors as ACh diffuses from

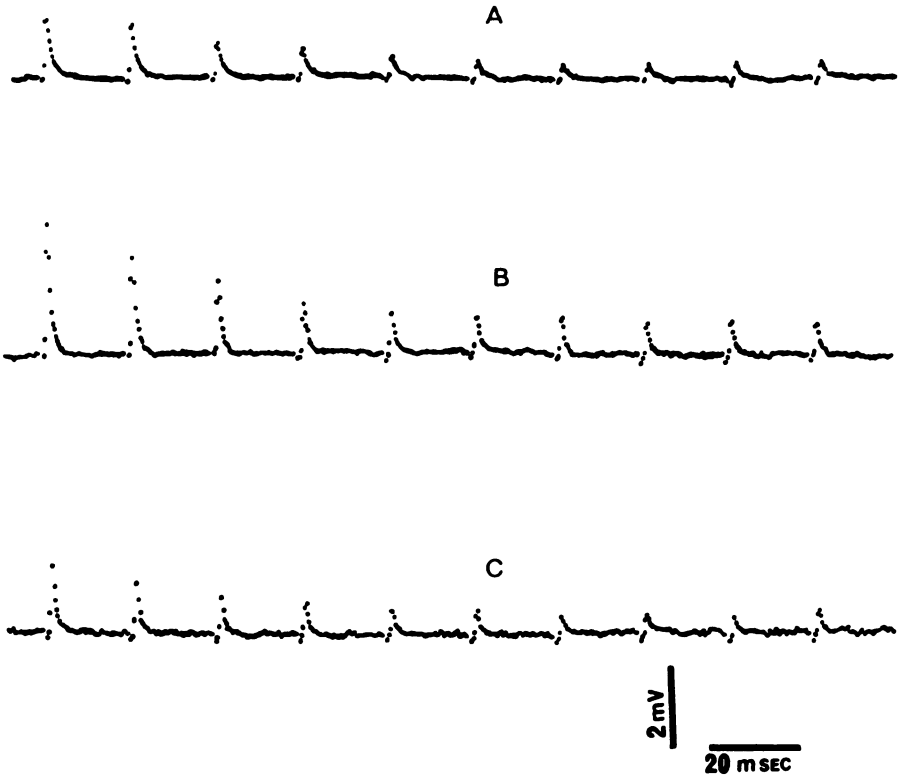

Fig. 8. Effect of DMSO on high quantal content EPPs. Computer averages were taken of 10 trains of EPPs each for control (A), 2% DMSO (B), and re-control (C). The bathing solution contained 5 mM K^+, 2 mM Ca^{2+}, and 5×10^{-7} M *d*-tubocurarine. Each record was corrected for variation in membrane potential before averaging. Stimulation was at 50 Hz. DMSO increased the height of the first EPP (quantal content was approximately 100–200) and also increased the apparent "run-down" of EPP height.

TABLE 3

Effect of DMSO on high quantal content EPPs

Quantal size ± standard error, in column 1 was estimated from the variance/mean ratio of the first EPP in each train (see Materials and Methods). The large error in this estimate is also reflected in the estimate of quantal content (column 3); however, 2% DMSO consistently increased the estimated quantal content. The immediately available store of transmitter (column 4) was calculated from the rundown of the first seven EPPs and is expressed in μV. Fractional release in column 5 was calculated as the ratio of the height of the first EPP in μV to the store, also in μV. The ratio, height of second EPP/height of first EPP, is included as an additional measure of the EPP rundown. Cell 1 is the same as that shown in Fig 4.

	Quantal size (μV)	Height of first EPP	m	Store	Fractional release	Height of 2nd EPP / Height of 1st EPP
	μV	μV		μV		
Cell 1						
Control:	4.0 ± 1.4	720	150	3320	0.22	0.90
2% DMSO:	4.8 ± 1.6	1440	300	5680	0.25	0.75
Re-control:	7.5 ± 2.7	771	103	3092	0.25	0.72
Cell 2						
Control:	9.1 ± 1.0	1300	141	6240	0.21	0.88
2% DMSO:	12.9 ± 1.8	1800	145	7440	0.25	0.80
Re-control:	8.6 ± 0.8	920	105	5290	0.17	0.94
Cell 3						
2% DMSO:	10.2 ± 1.7	1740	150	6300	0.28	0.57
Control:	5.8 ± 2.0	558	100	2470	0.23	0.76

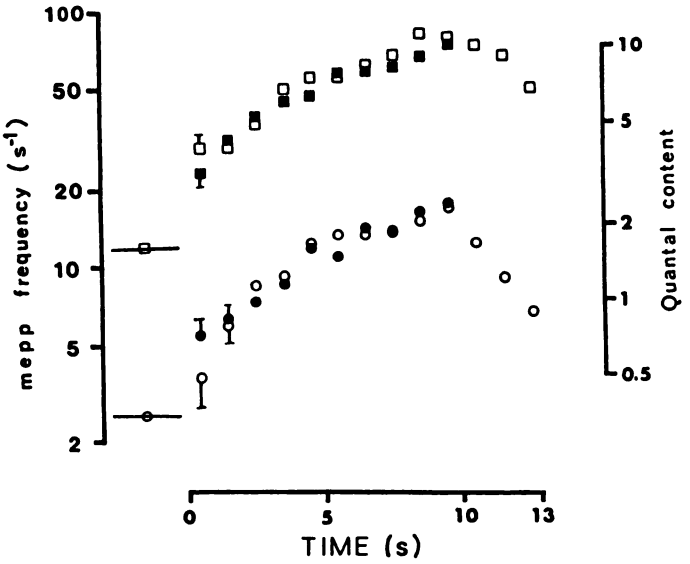

Fig. 9. Effect of DMSO on facilitation. Nerve stimulation was applied to the pectoralis proprius nerve of the frog cutaneous pectoris preparation as 10-sec trains of 50 Hz. Quantal content and fMEPP were measured in each 1-sec time period of the train. ○, control fMEPP (*left axis*); □, fMEPP in the presence of 4% DMSO. ●, control quantal content (*right axis*); ■, quantal content in the presence of 4% DMSO. ○, control, and □, 4% DMSO fMEPPs measured immediately before the train are depicted with the *horizontal bars*. fMEPP was also measured in the three time bins from 10 sec to 13 sec, after the end of the train. For clarity, *error bars* (± standard error) are shown on initial points only; they are similar or smaller for the other points.

the cleft, rather than reflecting the time constant of channel lifetime. The slope of this correlation, however, is significantly lower than the value of 0.77 previously determined after paraoxon treatment (14); this suggests that DMSO, at a concentration of 4% by volume, is far from completely effective as an inhibitor of acetylcholinesterase. An action of DMSO to inhibit acetylcholinesterase is also evident from the current recorded after perfusion of ACh and carbachol to the voltage-clamped endplate: the amplitude of the current with 20 μM ACh was significantly greater than that recorded during perfusion with 15 μM carbachol.

The progressive decrease in the amplitude of the MEPCs with increasing DMSO concentration, with or without anticholinesterase inhibition, presumably represents a direct action of the compound on the receptor-ion channel complex. It seems likely that the basis for the diminished height is a curare-like action of DMSO on the receptor. After irreversible poisoning of cholinesterase with paraoxon, DMSO (at concentrations of 4% and 6%) caused highly variable changes in the time constant of the MEPC decay (a net small increase was found), whereas a significant decrease in the decay rate was found in the absence of the anticholinesterase treatment. This observation is consistent with DMSO occupation of ACh-binding sites which would then have the effect of hastening the diffusion of ACh from the cleft region (16). The small net decrease in the decay rate observed after paraoxon treatment would then reflect opposing actions of DMSO as both an anticholinesterase and a curare-like compound. This explanation would require that the paraoxon treatment used did not fully inhibit acetylcholinesterase. Two points support this possibility. First, the average time constant of MEPC decay after paraoxon treatment was 3.1 msec, which is lower than the 4-fold increase in the time constant usually observed (from a control value of about 1.0 msec, at a holding potential of −80 mV, to 4.1 msec after paraoxon treatment) (14). In addition, in one experiment, 2 μM neostigmine was applied after paraoxon application; the time constant of the MEPC decay was further increased by 25% with the neostigmine indicating that the paraoxon treatment used did not fully abolish the acetylcholinesterase activity. It should be noted that the use of higher concentrations of paraoxon can cause modifications in MEPC shape which are not related to anticholinesterase poisoning.[1] The diminished response of the ACh or carbachol response in the presence of DMSO is consistent with a curare-like action of the agent. Pennefather and Quastel (17) have previously found that the height of the steady state response with bath-applied agonist is attenuated to a much greater degree by curare than is the height of the MEPC; this behavior seems entirely appropriate in describing the actions of DMSO in this study since, with 2% DMSO, the MEPC height is decreased by 16%, whereas the carbachol response is decreased by 47%.

[1] J. G. McLarnon, D. A. Saint, and D. M. J. Quastel, unpublished observation.

The results reported here clarify some of the possible mechanisms by which DMSO could enhance transmitter release. First, it is clear that the suggestion that DMSO increases fMEPP via a depolarization of the nerve terminal (5) is not tenable in view of the result that the response of fMEPP to DMSO is similar in 5 or 15 mM K^+ and is unaffected by addition of sufficient Cd^{2+} to cause a substantial block of Ca^{2+} depolarization-induced release. It also seems unlikely that DMSO causes any appreciable alteration in the presynaptic action potential, for example, by a change in potassium conductance, as suggested in Ref. 6, since both fMEPP and EPP quantal content (of low quantal content EPPs) were always affected equally by all concentrations of DMSO investigated. The most plausible explanation, therefore, is that DMSO exerts some nonspecific, or "global" effect on transmitter release. Such a global effect could consist of either (a) a "fusogenic" effect (18), i.e., a reduction of an energy barrier which must be overcome before membrane fusion and hence transmitter release can occur, as suggested for alcohol (8), or (b) an effect involving Ca^{2+} ions, which would depend upon the relationship of release to intracellular Ca^{2+}. If release is exponentially related to Ca^{2+}, [i.e., log(release) proportional to Ca^{2+}], then the results presented here are compatible with the addition of a constant increment of internal Ca^{2+} by DMSO. Alternatively, if release is proportional to $(Ca)^n$, the effect of DMSO is compatible with an increased effectiveness of Ca^{2+} inside the terminal, either by an increase in affinity of the receptors for Ca^{2+}, or by an increase in the effectiveness of the Ca-receptor complex to cause release. Note that the increase of fMEPP by DMSO in zero calcium solution (18), or in solutions containing a high concentration of Cd^{2+}, are not incompatible with either of these effects on Ca^{2+}, if one supposes that the low control fMEPP under these conditions merely reflects a low internal Ca^{2+} concentration. However, the experiments in which fMEPP was raised by Ba^{2+}, instead of Ca^{2+}, indicate that interference with ion effectiveness or disposition is probably not the mechanism by which DMSO increases transmitter release. Inside the terminal, Ba^{2+} exhibits a higher degree of cooperativity for the release of transmitter than does Ca^{2+} (an n of about 4 as opposed to one of about 2 for calcium) (19, 20); thus, for either of the above models relating release and ion concentration inside the terminal, the change in ln fMEPP produced by a given concentration of DMSO should be higher (at least double) when transmitter release is primarily due to Ba^{2+}, rather than Ca^{2+}. The results quoted for experiments in which transmitter release was induced by exposure to Ba^{2+} in raised K^+ solution indicate that, in fact, the effect of DMSO is essentially the same whether transmitter release is due to Ca^{2+} or to Ba^{2+}. Thus, it appears likely that DMSO enhances transmitter release by a "fusogenic" effect, rather than an effect which is ion dependent. It should be noted, however, that the synergism between calcium and DMSO proposed by Geron and Meiri (18) is not implied if one expresses fMEPP on a logarithmic scale, i.e., a given concentration of DMSO merely increases all types of release by the same factor. This idea of a global effect of DMSO is further reinforced by the finding that facilitation-induced release is also affected in the same way as other forms of release and, incidentally, removes the necessity of supposing that facilitation is caused by a residual elevated ion concentration inside the terminal.

The effect of DMSO on high quantal content EPPs (Fig. 8) appears inconsistent with such a proposed global effect on transmitter release, in that the effect of a given concentration of DMSO on high quantal content EPPs is much less than that expected from the multiplication of transmitter release measured as fMEPP or the quantal content of EPPs in high Mg^{2+}/low Ca^{2+} solutions. It appears plausible that this is a consequence of the saturation of release due to limitations on the available store and the mobilization rate of vesicles. Such a proposal is also compatible with the lower slope of ln fMEPP against DMSO concentration when fMEPP is raised by solutions containing 15 mM K^+. The lower slope is not due to the effect of the 15 mM K^+ per se, since the addition of Cd^{2+} to the solution results in a decrease in fMEPP and an increase in the slope to a value near to that found for the control.

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