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The fusogenic substance dimethyl sulfoxide enhances exocytosis in motor nerve endings

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Fusion of synaptic vesicles with the surface membrane of the nerve terminal is a key step in synaptic transmission, which normally requires the entry of calcium ions into the cell. We report that this fusion and the subsequent liberation of transmitter can also be induced by the fusogenic substances DMSO (dimethyl sulfoxide) and PEG (poly(ethylene glycol)). Calcium ions and DMSO exhibit a synergistic effect in the fusion of synaptic vesicles with the axolemma, resembling their action on fusion phenomena in liposomes.

Several moleclar mechanisms have been suggested to explain the triggering of membrane fusion phenomena by calcium ions: (1) Calcium induced separation of acidic phospholipids into rigid crystalline domains, with fusion occurring between domain boundaries on closely apposed membranes [1,2]. (2) Formation of dehydrated complexes connecting separate bilayers, which may serve as nucleation sites for membrane fusion [3–6]. Since the cryoprotectant substance dimethyl sulfoxide (DMSO) can mimic the action of calcium ions in provoking fusion of phospholipid vesicles accompanied by a shift in their phase transition temperature [7–9], and of cells [10–12], we examined whether DMSO can trigger physiologically functional fusion of membranes, such as in exocytosis, which is ordinarily induced by calcium ions.

Quantal release of acetylcholine from the motor nerve terminals involves most probably fusion between the transmitter containing synaptic vesicles and the axolemma [13–15]. At rest, when the axoplasm has a relatively low calcium concentration, individual quanta of the neurotransmitter are liberated from the nerve terminal at low frequency [16,17]. It is only following the massive influx of calcium induced by the axonal action potential, that hundreds of quanta are released simultaneously within several milliseconds, to stimulate the muscle [18,19]. When synaptic activity is monitored by intracellular microelectrodes inserted in muscle, the fusion of each individual vesicle with the plasma membrane can be detected. Any change in the rate of fusion induced by DMSO can therefore be accurately measured and is easily distinguished from other possible effects of this substance, such as alterations in transmitter metabolism [20–23].

The experiments were performed on isolated cutaneous pectoris and sartorius neuromuscular preparations of the frog *Rana ridibunda*, constantly perfused by a frog Ringer solution of either the standard composition (116 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$ and 5 mM Hepes buffer, pH 7.2) or lower than normal concentration of $CaCl_2$ (0.2–0.45 mM) with added $MgCl_2$ (1.0–4.0 mM).

Abbreviations: epp, endplate potential; mepp, miniature endplate potential.

In some experiments, the bathing medium contained no added calcium but rather 1 mM EGTA and 2–4 mM $MgCl_2$. DMSO at concentrations 0.5%–5.0% (v/v) was added to the perfusion fluid. The spontaneous and stimulation-induced electrical synaptic potentials, monitored by conventional intracellular recording techniques, were fed into the PDP 11/23 MINC microcomputer and were analysed by FORTRAN and MACRO assembler programs. In each experiment, the preparation was equilibrated with the Ringer solution for 30–45 minutes before control synaptic activity was recorded. Subsequently, a solution of similar ionic composition with the required concentration of DMSO was perfused through the experimental bath and the synaptic activity was sampled again.

The effect of DMSO on the spontaneous liberation of individual quanta of acetylcholine is illustrated in Fig. 1. In the virtual absence of calcium ions in the Ringer solution, containing 1 mM EGTA and 2 mM $MgCl_2$, the rate of random discharge was doubled by addition of 0.5% DMSO. The frequency of miniature endplate potentials (mepp) was sustained at this higher level as long as DMSO was present and returned to its original value 10–15 minutes following reintroduction of the control solution. The action of DMSO was greater in calcium-containing solution and was dependent on the $[Ca]_{out}$. DMSO was more effective in enhancing transmitter release in the presence of 0.6 mM $CaCl_2$ in the extracellular medium than in the presence of only 0.3 mM $CaCl_2$. When both the calcium and the DMSO concentrations were increased to 1.8 mM and 2.5%, respectively, the mepp frequency was 12.6-times higher than the

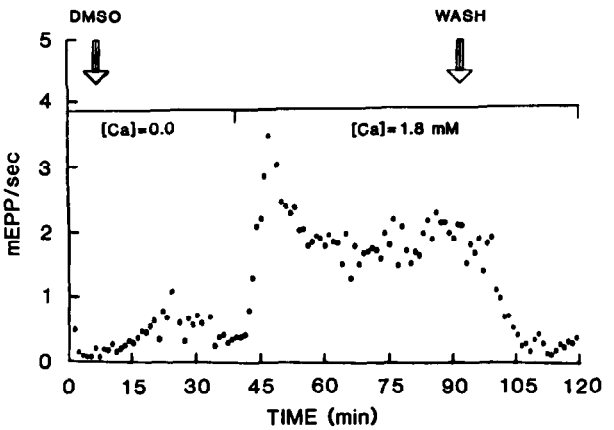

Fig. 2. The effect of DMSO and calcium ions on spontaneous release of transmitter. Addition of DMSO (2.5%) to a Ringer solution containing 1 mM $MgCl_2$ and 1 mM EGTA (left arrow) enhanced the mepp frequency. The rate of release was further increased when the EGTA and $MgCl_2$ were replaced by $CaCl_2$ (1.8 mM). Upon wash out of the DMSO (right arrow) the release decreased to a low level, only slightly higher than that observed in the virtual absence of calcium ions in the initial part of the experiment.

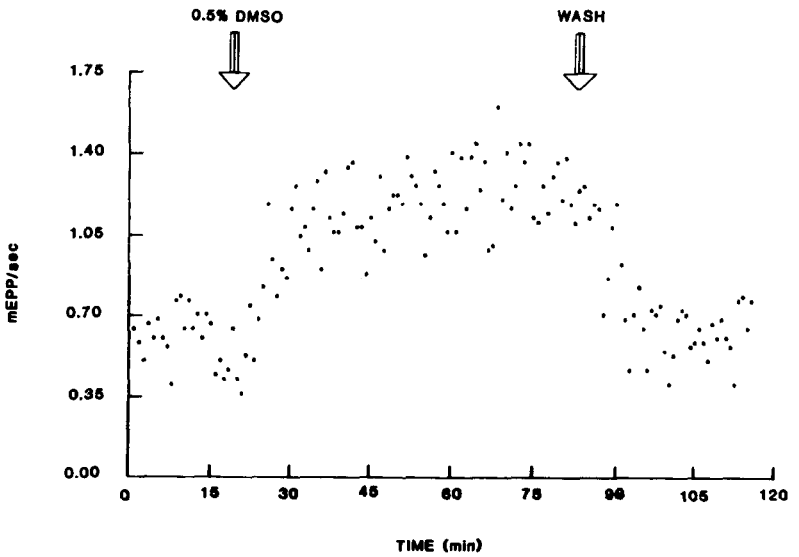

Fig. 1. The effect of DMSO on mepp frequency in a calcium-deficient solution. DMSO (0.5%) was added to a Ringer solution containing 2 mM $MgCl_2$, 1 mM EGTA and no added calcium. Each point represents the frequency of mepps during a one minute period.

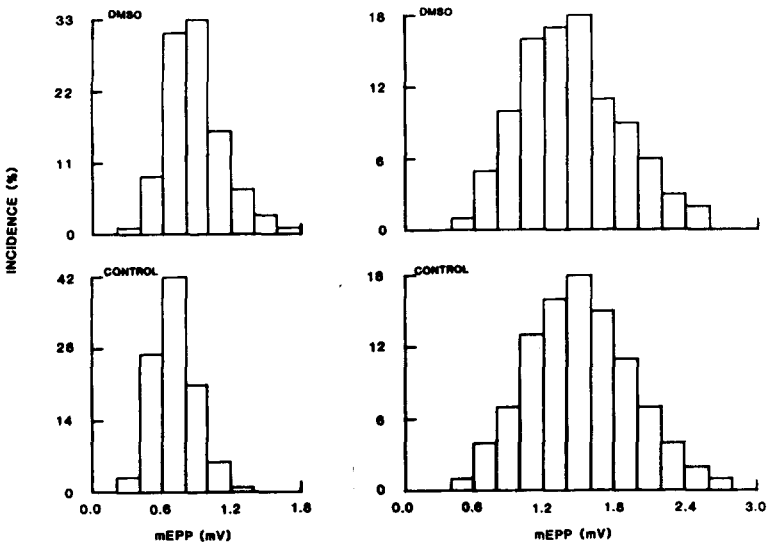

Fig. 3. The effect of DMSO on the mepp amplitude. Addition of 0.5% DMSO to a Ringer solution containing 2 mM $MgCl_2$ and 1 mM EGTA increased the average mepp amplitude by 21.8% (left panel). No change in the mepp amplitudes was observed when the preparation was pretreated by the irreversible blocker of acetylcholine esterase phospholinium iodide (right panel).

control value (Fig. 2). These results resemble the enhancing effect of DMSO on calcium evoked fusion of phospholipid vesicles [4].

Amplitude histograms of mepp with and without DMSO in the Ringer solution are presented in Fig. 3. Addition of 0.5% DMSO to the experimental bath increased the average mepp amplitude by 21.8% (Fig. 3, left panel), most probably due to partial inhibition of the acetylcholine esterase activity [24,25]. This effect was eliminated by pretreating the preparation with the irreversible anticholine esterase agent phospholinium iodide as shown in Fig. 3, right panel.

The higher probability of fusion of the synaptic vesicles with the plasma membrane induced by DMSO is most probably not accompanied by enhanced intervesicular fusion. Had vesicles fused with each other prior to liberation of their content to the synaptic cleft, mepp values of double or triple the control size would have been expected in the presence of DMSO, reflecting the compound quanta produced by such fusion. No change in the bell shaped distribution of the amplitude histogram was observed in the presence of DMSO even when the rate of release of individual quanta was the highest. This indicates that intervesicular fusion is unlikely.

Stimulation-induced release of transmitter is markedly augmented by DMSO. In the experiment illustrated in Fig. 4A, the average amplitude

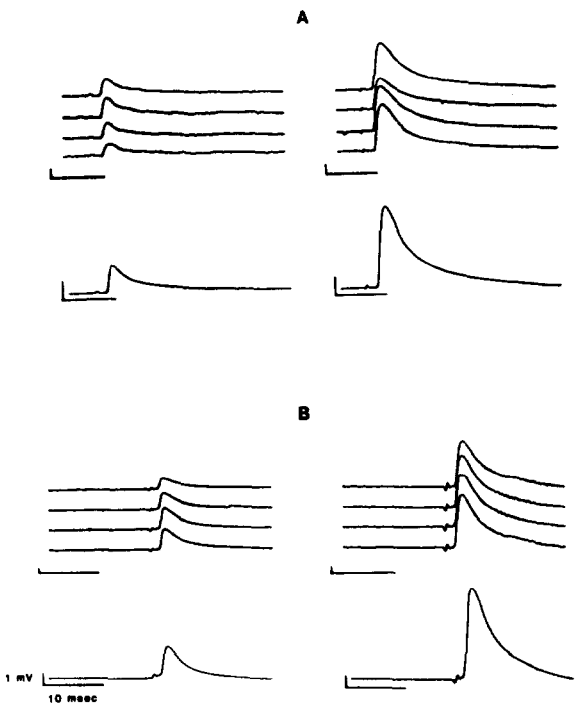

Fig. 4. The effect of the fusogenic agents DMSO and PEG on evoked release of transmitter. Individual (top) and average (bottom) epp values presented. (A) In control Ringer solution containing 0.35 mM $CaCl_2$ and 2 mM $MgCl_2$ (left), and in the presence of 256 mM DMSO (right). (B) Control solution with 0.3 mM $CaCl_2$ and 1 mM $MgCl_2$ (left) and in the presence of 12.5 mM PEG 8000 (right).

of endplate potentials (epp) was 3-times higher with 2% DMSO than in the corresponding control solution. The mean number of neurotransmitter quanta released by nerve impulse (quantal content) was estimated by conventional methods. The quantal content of evoked transmitter release was increased by 199 ± 16% in 0.5% DMSO and by 231 ± 38% in the presence of 2% DMSO in Ringer solution containing 0.35 mM $CaCl_2$ and 2 mM $MgCl_2$. Following exposure to 5% DMSO, the increase in transmitter release was so large that the epp reached the threshold for action potential and the muscle fibres twitched. When 0.35 mM $CaCl_2$ and 4 mM $MgCl_2$ were present in the solution, 5% DMSO caused up to 19-fold increase in the quantal content of release. No failure of nerve activity like that described by Evans and Jaggard [26] was noticed even after 3 h exposure of the preparation to 5% DMSO.

Similar effects on spontaneous and evoked transmitter release were induced by another water soluble fusogenic substance poly(ethylene glycol) (PEG) [10]. Increased amplitude of endplate potentials in the presence of 12.5 mM PEG 8000 is illustrated in Fig. 4B.

Considering the possible cellular mechanisms via which DMSO may exert its action, we favour the direct effect of DMSO on the fusing membranes. We find it difficult to attribute the enhanced release to hyperosmotic neurosecretion [13,28]. A Ringer solution containing 1% DMSO (v/v) has an osmolarity which is higher by 128 mosM than the osmolarity of the normal Ringer. However, DMSO is highly permeable through cell membranes [27] and had hyperosmolarity been its mode of action, only a transient increase in the mepp frequency should have been observed upon its application [28,29]. Moreover, stimulation-induced release of transmitter which has been shown to be unaffected by increased osmolarity of the extracellular medium [28], is markedly augmented by DMSO. DMSO may increase the permeability of the nerve terminal to calcium ions but, since transmitter release is enhanced even in the virtual absence of calcium from the extracellular medium, it most probably has another site of action. The possibility that DMSO causes a release of calcium ions from intracellular organelles cannot be ruled out although we do not know of such an effect. The direct effect of DMSO on fusing membranes of phospholipid vesicles and its fusogenic effect on animal cells have been well documented, however, the concentration of DMSO necessary for cell fusion was much higher than that used in the present study [11,12]. Although both fusogenic substances examined the DMSO, and the PEG 8000 enhanced the release of neurotransmitter, their molecular mode of action may not be the same. Unlike the cell penetrating DMSO, the PEG 8000 large molecules most probably do not permeate the axolemma and do not therefore affect the transmitter carrying vesicles directly.

The present results show that the fusogenic substance DMSO promotes release of a neurotransmitter from the motor nerve endings and demonstrate a synergistic action between DMSO and calcium ions in modulating the physiologically functional fusion between synaptic vesicles and the axolemma.

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