# Some actions of dimethylsulphoxide at the neuromuscular junction

During the testing of some water-insoluble neuromuscular blocking drugs, which had been dissolved in the non-polar solvent dimethylsulphoxide (DMSO), we observed that DMSO itself possesses actions on neuromuscular transmission. To elucidate these actions we employed the biventer cervicis muscle preparation of the chick (Ginsborg & Warriner, 1960) mounted in Krebs-Henseleit (1932) solution at 32°, bubbled with oxygen containing 5% $CO_2$. All concentrations of DMSO are expressed as % v/v. The DMSO used was of spectroscopy grade (BDH).

Increasing concentrations of DMSO from 1–10% produced, after a short (1–2 min) initial period of augmentation, a progressive depression of maximal twitches of the biventer cervicis muscle stimulated through its motor nerve with supramaximal rectangular pulses of 0·2 ms duration at a frequency of 0·1 Hz (Fig. 1). The depression of twitch height was reversed by physostigmine (1μg/ml) and partially reversed after a tetanus (50 Hz for 5 s). In slightly higher concentrations, DMSO depressed the responses to direct stimulation of the fully curarized (10μg/ml) muscle (Fig. 1). This effect was readily reversible by washing the preparation. The characteristics of the block produced by DMSO differed from those produced by (+)-tubocurarine in this preparation. (+)-Tubocurarine reduced the responses to both added acetylcholine (50μg/ml) and carbachol (3μg/ml), doses that produced contractures approximately equal in height to that of a maximal twitch. However, DMSO (1–5%), although it reduced the response to carbachol (3μg/ml), augmented that to acetylcholine (50μg/ml). This suggests that DMSO might possess some inhibitory action on cholinesterase, and further evidence that this is so in the chick nerve-muscle preparation was provided by the findings that DMSO (1–5%) in combination with (+)-tubocurarine (2μg/ml) produced a lesser degree of neuromuscular block than did 2μg/ml of (+)-tubocurarine alone, and that the neuromuscular blocking action of (+)-tubocurarine alone could be partially reversed by addition of DMSO (5%). We have made similar observations to those described above using the rat phrenic nerve-hemidiaphragm preparation (Bülbring, 1946).

The dose-response curve for acetylcholine on the chick biventer cervicis preparation was shifted to the left by 5% DMSO (Fig. 2a), the first response being determined after 10 min of contact between DMSO and the tissue. DMSO (5–15%) shifted the dose-response curves for carbachol and for acetylcholine, when determined in the presence of physostigmine (1μg/ml), to the right (Fig. 2b and c). In the presence of 20% DMSO, the maximal responses to both carbachol and acetylcholine in the presence of physostigmine were greatly reduced (Fig. 2b and c). This effect was similar to that reported recently on the guinea-pig ileum preparation (Zetler & Langhof, 1971) and is probably due to the direct depressant action of high concentrations of DMSO on the contractility of the muscle.

A depressant action of DMSO on cholinesterase has been reported (Sams, Carroll & Crantz, 1966; Baker & Gibson, 1971), and was confirmed, in homogenates of chick biventer cervicis muscle, by means of the colorimetric method of Ellman, Courtney & others (1961). At a concentration of 5%, DMSO was found to inhibit 58% of the cholinesterase activity in the homogenates. We conclude that DMSO possesses direct depressant actions on skeletal muscle and on cholinesterase activity. Its greater actions on neurally-stimulated preparations than on directly-stimulated preparations may also indicate that DMSO possesses additional actions on the process of neuromuscular transmission. When used as a solvent, the effects of DMSO are likely to interfere with those of other drugs, and to avoid errors in interpretation, it should

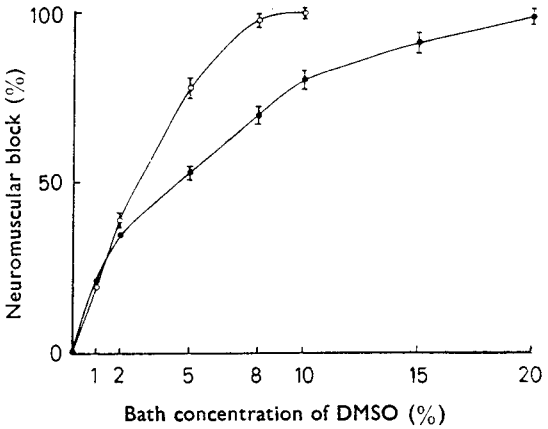


FIG. 1. The effects of DMSO on the responses of the chick biventer cervicis muscle to nerve stimulation (— ○—) and to direct stimulation of the fully curarized muscle (— ●—). The points represent the percentage blockade of twitch height measured after 10 min contact of DMSO with the tissue. Each point represents the mean (± s.e.) of 6 separate determinations.

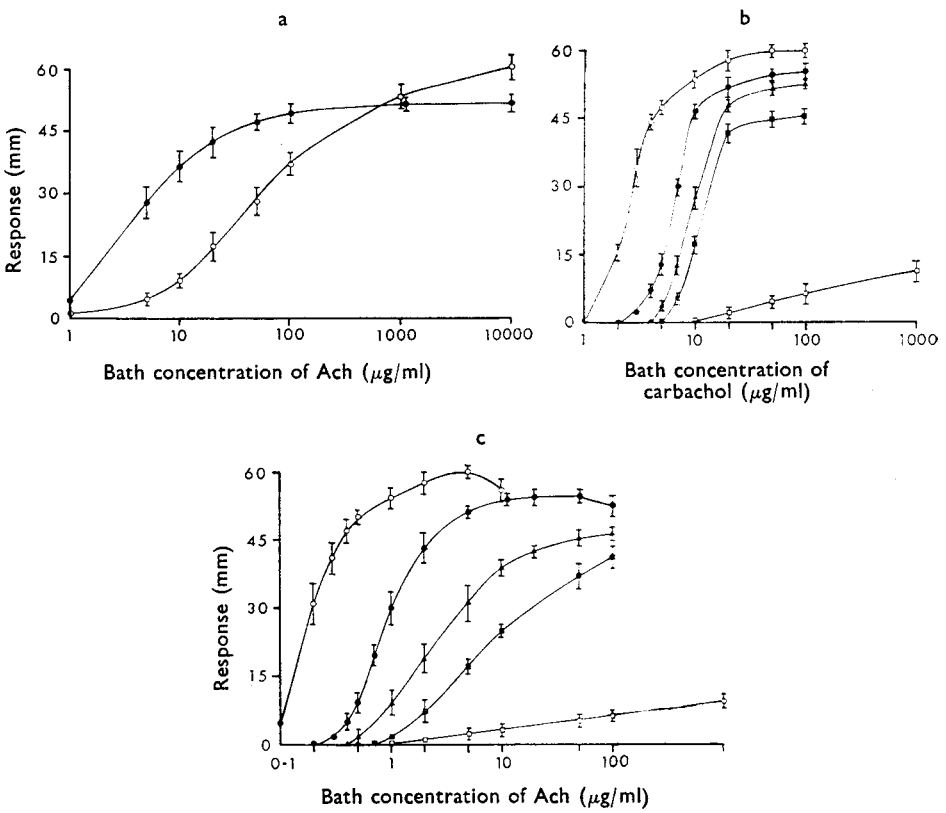


FIG. 2. The effects of DMSO on the dose-response curves for acetylcholine and carbachol on the chick biventer cervicis muscle. (a) Dose-response curves for acetylcholine (— ○—) and for acetylcholine in the presence of 5% DMSO (— ●—). (b) Dose-response curves for carbachol (— ○—) and for carbachol in the presence of DMSO, 5% (— ●—), 10% (— ▲—), 15% (— ■—) and 20% (— □—). (c) Dose-response curves for acetylcholine (— ○—), redetermined throughout in the presence of physostigmine (1 μg/ml), and in the presence of DMSO 5% (— ●—), 10% (— ▲—), 15% (— ■—) and 20% (— □—). Each point represents the mean (± s.e.) of 6 separate determinations.

clearly be used with caution in experiments involving the neuromuscular junction.

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## Dimethylsulphoxide effect on myocardial β-adrenoceptors

An unusual spectrum of actions of dimethylsulphoxide (DMSO) on isolated cardiac muscle has been reported. Sams, Carroll & Crantz (1966) noted that DMSO (0·42 and 0·85 M) produced positive inotropism in the guinea-pig isolated atrium-vagus preparation. Karow, Unal & others (1967) and Karow, Carrier & Clower (1968) reported that DMSO (2·1 M in Ringer solution) perfused through the rat isolated heart Langendorff preparation produced precipitous negative inotropism and negative chronotropism. This so called "pseudotoxic effect" (Karow, 1969), was shown to be reversible with DMSO washout. Shlafer & Karow (1971) have further demonstrated with the rat isolated perfused heart that low concentrations (0·14, 0·70 M) of DMSO produced positive inotropism; moderate concentrations (1·41 M) produced a reversible negative inotropism (pseudotoxicity), and high concentrations (2·82 M) produced irreversible negative inotropism (true toxicity); while all concentrations investigated produced reversible negative chronotropism (pseudotoxicity). These observations and normal animal species variability can reconcile the apparently conflicting reports of Offerijns, Freund & Krijnen (1969), Spilker (1970) and of Feuvray & de Leiris (1971) about the action of DMSO (positive or negative inotropism) on isolated cardiac muscle.

To determine the relation existing between the cardiac effects of DMSO and myocardial β-adrenoceptors, the following experiment was done.

Rabbits (approximately 2 kg) were decapitated and hearts immediately removed and placed in Ringer solution (NaCl, 153·9; KCl, 5·4; $CaCl_2$, 2·4; $NaHC0_3$, 16·8; dextrose, 11 mM; distilled water to 1 litre; pH adjusted to 7·4), gassed with 5% carbon dioxide in oxygen. The left atria were isolated, trimmed of peripheral tissue, tied to a Plexiglas holder containing 2 platinum electrodes, and bathed in oxygenated Ringer at 30°. Tension (1·0 g) was placed on each atrium which was stimulated continually throughout the experiment with a square wave of 5 ms duration (1·7 Hz, 5 V). Contractions were recorded by a direct writing oscillograph. To minimize osmotic stress to the muscle, stepwise increments were used to achieve the desired DMSO concentration. All atria were first soaked for at least 15 min in Ringer; some of these were then soaked in Ringer containing 0·14 M DMSO for 15 min. Of the