**Effect of Dimethyl Sulfoxide on Excitation-Contraction Coupling in Chicken Slow Skeletal Muscle**

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Abstract. To evaluate the suitability of dimethyl sulfoxide (DMSO) as a solvent for muscle-contraction studies in the chicken, its effect on the slow muscle contracture induced by high-K⁺ solution was explored using the anterior latissimus dorsi (ALD) muscle from one-week-old chicks. Measurements were made of isometric tension and various characteristics of the contractions [peak tension, total tension (area under the curve), duration of contraction, drop in tension from peak to plateau, and resting tension], in the presence and absence of DMSO (20 mM). Exposure to DMSO led to a concentration-dependent reduction in resting tension of up to 9.1771.8% (n = 4) with respect to the control. The threshold concentration was 10 mM, and the maximum effect was reached between 20 and 30 mM. The drop in tension from peak to plateau was three times larger in the presence of DMSO (20 mM) than in its absence. At the same concentration, there was a 10 ± 2.3% increase in the time constant of activation. No significant changes were observed in peak tension or in total tension in the presence of 20 mM DMSO. As a consequence, this type of biological preparation is not suitable for research on muscle contractures involving drugs that must be dissolved in DMSO (at the DMSO concentrations tested here).

Keywords: dimethyl sulfoxide, excitation-contraction coupling, skeletal muscle, slow muscle

**Introduction**

Dimethyl sulfoxide (DMSO) is a dipolar solvent widely used to solubilize small organic molecules. Its use has been subjected to numerous studies over several decades. Some of the early work using DMSO involved clinical evaluation (1, 2), toxicology (3), use in surgery (3), pharmacology, focusing on metabolic effects (4) pharmacological interactions with antibiotics such as gentamicin (5), and studies of neuromuscular synapses (6).

For the last decade, DMSO has been widely used in muscle research, mainly on skeletal muscle, because it seemingly reduces the fatigue induced by the application of electric stimulation at high frequencies. According to some authors (7 – 9), such stimulation leads to elevated concentrations of free radicals from anaerobic metabolism, and these are said to be directly responsible for the muscle-fatigue phenomenon. In fact, DMSO appears to be a suppressor of oxidant agents, and pretreatment of skeletal and smooth muscle with this type of suppressor does help to reduce the experimental muscular fatigue induced by high frequency electrical stimulation (8, 9) and to support the blood reperfusion of a hypoxic tissue (10). Currently, DMSO is commonly used in studies of skeletal muscle as a selective anti-oxidant (particularly, as suppressor of hydroxyl radicals) or as a solvent for numerous drugs (8).
In addition to its action as an anti-oxidant agent, DMSO has proved to have significant effects on excitation-contraction coupling (ECC) in skeletal muscle, a series of events starting with the spread of the action potential over the muscle-fiber membrane and culminating in the development of contraction (11). For example, in rat isolated skeletal muscle preparations, administration of DMSO induces changes in contractile properties (8, 12, 13). In addition, Kurebayashi and Ogawa (14) have shown that at a concentration of 1% (v/v), DMSO depressed contraction in isolated skinned fibers of the frog, suggesting a direct effect on the contractile apparatus. DMSO also modulates isometric tension in mammalian skinned fibers by activating or inhibiting the hydrolysis of ATP by myosin, depending on the cation present (15, 16).

Our main objective in this study was to examine the effects of DMSO on various characteristics of the chicken slow skeletal muscle contracture induced by high-potassium solution. From the results, we hoped to ascertain whether DMSO is suitable for use as a solvent for experimental drugs in muscle-contracture studies in the chicken, since to the best of our knowledge, there are no published reports on this subject in this species.

Materials and Methods

Muscle preparation

The experiments were performed on the anterior latissimus dorsi (ALD), a slow muscle, isolated from 0 – 3-week-old chickens of the Arbor Acres variety, previously slaughtered and beheaded. Thin fascicles of ALD with an approximate diameter of 0.5 mm were dissected free, first by blunt dissection and then under a stereomicroscope.

Preparation for tension recording

The fascicles so obtained were transferred to a continuous-perfusion recording chamber with an adjustable width and a 3-cm-long central channel. At one end of the central channel was a compartment via which perfusates could enter through a three-way tap placed at the channel entrance.

The spinal end of the bundle was fixed to the wall of the chamber, while the humeral end was hooked up to the lever of a linear mechano-electrical transducer (400A; Cambridge Technology Inc., Cambridge, MA, USA). The bundle was stretched to 1.3 times its resting length before performing any isometric-tension recording. The mechanical responses generated by high-potassium solution in the control situation and after incubation in DMSO were registered on a pen-recorder (model 220; Gould, Oxnard, CA, USA) and captured through an analog data-acquisition system (CyberAmp 320; Axon Instruments, Foster City, CA, USA) and a digital-analog card (TL-1 DMA, Axon Instruments), then processed and stored in a microcomputer (Printform 386X) for subsequent analysis. The graphics were generated by Sigmaplot software (ver.8.0; Stonehill Corporate Center, Saugus, MA, USA).

Experimental solutions

The normal Ginsborg saline solution for use in the chicken (17) contained 167 mM NaCl, 5 mM KCl, 2 mM MgCl₂, 5 mM CaCl₂, 2 mM imidazole chloride, and 11 mM dextrose. Contractures were induced following rapid substitution (1 to 2 s) of the normal Ginsborg solution by a high-potassium solution (90 mM KCl), produced by equimolar substitution of NaCl by KCl, the other components remaining the same.

DMSO (Sigma Co., St. Louis, MO, USA) was diluted in Ginsborg solution and administered in a series of concentrations (2 to 40 mM) for the analysis of its effect on resting basal tension. A concentration of 20 mM was chosen for the remainder of the experiments. The ALD bundles were incubated in the appropriate solution for five minutes before contractures were induced using high potassium. All solutions were adjusted to a pH of 7.4 with NaOH or HCl, and the experiments were performed at a room temperature of 22 – 24°C.

Analysis of experimental data

The contracture parameters subjected to analysis were as follows: a) resting basal tension, b) amplitude to peak tension, c) total tension (area under the tension-time curve), d) time constant of activation, and e) drop in tension from the peak to the plateau of the contracture.

Fig. 1. Analyzed parameters in recording of contractures in slow skeletal muscle. a, resting basal tension; b, peak tension; c, total tension in each of muscle contracture; d, time constant (τ) of activation; e, drop in tension from the peak to the plateau of contracture.
obtain the activation time constant via the Clampfit sub-routine of pClamp ver. 6.04 (Axon Instruments) software.

**Statistical analysis**

The experimental results are each presented as the mean ± S.E.M. from four experiments (n = 4). The data were subjected to Student’s unpaired t-test, differences being considered statistically significant at P < 0.05.

**Results**

In our experiments, 20 mM DMSO proved to be the concentration needed to obtain 80% of the contracture’s maximum effect on resting tension (see below).

**DMSO effect on resting tension**

As mentioned above, in each experiment, the fascicles were stretched to approximately 1.3 times the resting length before proceeding to the experimental phase. When fascicles had been incubated for 5 min in DMSO, there was a concentration-dependent reduction in resting tension (Fig. 4). The threshold concentration was 10 mM DMSO, as it was for each of the other analyzed parameters (data not shown). The maximum effect, obtained at 20 – 30 mM DMSO, involved a decrease with respect to the control of 9 ± 1.8%, which represented a significant pharmacological effect (P = 0.010).

**DMSO effect on peak amplitude of the contracture**

Incubation of the preparation with 20 mM DMSO gave a value for the peak amplitude of contracture of 101.6 ± 1.16% with respect to the control (100 ± 0%) (P = 0.395). Thus, DMSO did not have a significant effect on the peak amplitude of the high-potassium contracture.

**DMSO effect on total tension**

Likewise, DMSO (20 mM) did not have a significant effect on total tension, a value of 101.6 ± 4.18% being obtained with respect to the control (100 ± 0%) (P = 0.51).

**DMSO effect on time constant of contracture activation**

Application of 20 mM of DMSO did, however, have a significant effect on the time constant of activation (Fig. 2), there being an evident increment of 10 ± 2.3% with respect to the control (100%). This increment is the equivalent of an average increment of approximately 0.22 s in the activation time constant with respect to the control, indicating a slowing of the activation time to peak. The statistical analysis of this data indicated a significant difference between the groups (P = 0.003).

**DMSO effect on sustained component of contracture**

The parameter most clearly affected by DMSO was the sustained component of the contracture (plateau). Figure 3 shows that in the control situation, the drop in tension from the peak to the plateau was 8 ± 0.7%, while in the presence of 20 mM DMSO the corresponding value was 25.4 ± 2.15%. In other words, with DMSO, the drop to the plateau was three times that seen the
control situation, an evident and significant ($P = 0.0003$) pharmacological effect.

**Discussion**

The conversion of chemical energy into mechanical movement at the muscle level is an important subject in muscle physiology studies. The energy contribution made by ATP, the interaction of the contractile proteins within the sarcomeres, and the central role played by Ca$^{2+}$ from the sarcoplasmic reticulum and from outside the fiber are physiological events under constant scrutiny in attempts to explain the process of ECC in skeletal muscle. In muscle physiology among different species, this process is perhaps one of the most widely studied.

Several hypotheses at the skeletal muscle level have been proposed in attempts to explain ECC. These include allosteric regulation (Rios et al., 18), chemical transmission involving inositol triphosphate (Vergara et al., 19), electric continuity (Mathias et al., 11), and intramembrane charge movement (Schneider and Chandler, 20). The involvement of calcium influx is definitive for cardiac muscle (Kurebayashi and Ogawa, 14), but it does not play an important role in ECC in skeletal muscle because the fiber still contracts even in the absence of external calcium (González-Serratos et al., 21).

However, far from proposing a new hypothesis to explain ECC in skeletal muscle, our objective in this work was centered on an effort to describe the effects that DMSO has on the contractile properties of a chicken slow skeletal muscle and, by so doing, to determine whether DMSO is a suitable solvent for experimental drugs in muscle contraction studies on this type of preparation in the chicken.

DMSO is a chemical compound that has been studied for several decades for its effects on multiple physiologic processes. In recent years, it has been used more as an anti-oxidant both in studies of muscle fatigue (8, 9) and in vitro studies as a vehicle when any of a number of drugs needs to be dissolved.

According to our literature survey, in skeletal muscle research the concentration range used is very wide: 0.001 – 50%. We used 0.15%, which is well within this range. We could find no previous report of its effects in chicken slow skeletal muscle, so that was the main objective of our study. Our work reveals that in chicken slow skeletal muscle, DMSO itself has a significant effect on some characteristics of the contracture induced by high potassium. The induced changes in these characteristics, which had not previously been observed with this kind of activation, were as follows: a decrease in the tension of the sustained component (plateau), a reduction in resting tension, and an increment in the time constant of the contracture activation. Probably such changes occurred because DMSO affected ECC in this chicken slow muscle (ALD), as it does in other animal species (8, 12). In 1994, Reid and Moody (8) in an in vitro study performed on bundles of rat diaphragm muscle, showed that DMSO induces a dose-dependent reduction in the tension developed during brief 30–60 Hz and tetanic electrical stimulation. However, the dose range (0.6 to 640 mM) used by these authors extended to much greater concentrations than those...
used in our experiments (2 to 30 mM), which might suggest a greater sensitivity of our preparation. Be that as it may, like those authors we propose an effect at the ECC level.

Making use of the same biologic preparation as Reid and Moody (8), Supinski et al. (9) demonstrated that DMSO is able to reduce the percentage of fatigue induced by high frequency stimulation (40 – 50 Hz), probably because of the elimination of superoxide radicals, which are related to muscle fatigue.

While working on vesicles from rabbit fast skeletal muscle, Mayahara et al. (22) found evidence that DMSO decreases the total intracellular calcium concentration, an effect achieved more by reducing its exit from the sarcoplasmic reticulum than by increasing its recapture; this effect would explain the muscle relaxation seen in the presence of DMSO.

On the other hand, the depressed contraction observed in isolated skinned fibers of the frog with a fairly low concentration of DMSO (1% v/v) by Kurebayashi and Ogawa (14) suggests a direct effect on the contractile apparatus, since DMSO also inhibits isometric tension in mammalian skinned fibers (16). However, in an earlier work Mariano and Sorenson (15) suggested that depression of contraction occurs because DMSO activates or inhibits the hydrolysis of ATP by myosin, an effect that depends on the cation present. In addition, they suggested that DMSO increases the population of that depends on the cation present. In addition, they inhibits the hydrolysis of ATP by myosin, an effect achieved more by reducing its exit from the sarcoplasmic reticulum than by increasing its recapture; this effect would explain the muscle relaxation seen in the presence of DMSO.

On the basis of the above discussion, we believe that DMSO in skeletal muscle (in a chicken slow skeletal muscle) occur mainly because of its effect on the excitation-contraction coupling process, although there may be species differences, either qualitative or quantitative, depending on the metabolic and energy-releasing processes inherent to each species.

References