A NEW METHOD FOR EXCITATION-CONTRACTION UNCOUPLING IN FROG SKELETAL MUSCLE

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ABSTRACT

The mechanical activity of frog sartorius muscle fibers can be uncoupled from the electrical activity of their surface membranes by immersing the preparation in Ringer solution containing either 1.5 or 2.0 M of formamide for 15-30 min. This uncoupling is not reversed when the muscle is transferred to normal frog Ringer solution. Formamide does not affect the electrical activity of the sciatic nerve branch, and both endplate potentials and miniature endplate potentials may be recorded from the uncoupled muscles. Prolonged exposure to formamide, beyond the time needed to paralyze, causes neuromuscular block.

KEY WORDS formamide · excitation-contraction uncoupling · frog skeletal muscle · neuromuscular transmission

The mechanical activity initiated by membrane depolarization is a serious obstacle to intracellular electrophysiological work in skeletal muscle, as the twitch associated with the action-potential soon dislodges or breaks the intracellular microelectrodes and damages the muscle fiber. To avoid this, two main techniques have been used.

The first technique is to work in hypertonic solutions with 2.5 × the normal osmolarity. Fenn (6) and Hodgkin and Horowicz (7) showed that these solutions abolish contraction but leave the action-potential unaffected, an observation confirmed since then by many authors. However, hypertonic solutions of nonpermeant compounds such as sugars or NaCl block neuromuscular transmission (5).

The second technique depends upon an osmotic shock which disrupts or disorganizes the transverse tubular system. The muscle is first immersed in a hypertonic solution of a solute that penetrates the cell membrane, such as glycerol. If the muscle is left in this solution, the tension developed decreases temporarily and the fibers recover their initial volume and twitch tension (1). However, if the muscle is rapidly transferred from the hypertonic solution to an isotonic medium, the fibers swell and lose their power to generate tension. This appears to be due to the breakage of the transverse tubular system (8, 3), which becomes vesiculated and loses continuity with the extracellular space, as shown by the observation that horseradish peroxidase does not penetrate into its interior any longer. These changes are accompanied by a decrease in membrane capacity (4).

This method has been used successfully by several authors, but it requires relatively long periods of immersion, up to 2 h, in the hypertonic glycerol solution, it works only on small, thin muscles, and many fibers are damaged and have low resting potentials. In addition, for reasons which are not clear, this method does not always work. A new version of this technique using ethylene glycol instead of glycerol was introduced by Seveik and Narahashi (9). Muscles were im-
suspended in ethylene glycol (876 or 1095 mM) for periods of 2-4 h at 5°C and then returned suddenly to normal Ringer’s solution. Twitches occurred spontaneously, and the contraction elicited by nerve stimulation gradually decreased. Muscles uncoupled with this technique are said to be in better condition than those treated with glycerol; the average resting potential of the muscle fibers decreases only by 10-13 mV and remains constant for as long as 2 h. Unfortunately, and also for obscure reasons, the ethylene glycol method does not always work.

Recently, we needed to record endplate currents from the frog sartorius with a double microelectrode voltage clamp technique, and neither the glycerol nor the ethylene glycol method worked on our preparations. We decided to attempt uncoupling the mechanical activity with formamide (FMD), a compound known to exert, at a concentration of 1.75 M, a relaxing action on glycerinated muscle fibers (10) and at concentrations lower than 1 M to block completely and reversibly the mechanical activity of mammalian smooth muscle (2).

We found that immersion of sciatic-sartorius preparations of the frog Rana pipiens for 15-30 min in a 1.5-2.0 M solution of FMD in frog Ringer’s solution produces a complete and long-lasting uncoupling of the mechanical activity of the muscles. These muscles cease to contract in response to either direct or indirect electrical stimulation while they are in the FMD solution. Therefore, in spite of the high concentrations of FMD used, the uncoupling caused by this compound does not depend upon an osmotic shock in the usual sense. When uncoupled muscles are transferred back into normal Ringer’s solution, no spontaneous twitching occurs, no gross swelling is observed, and the muscles retain their normal transparency while remaining paralyzed. The possibility that returning the muscles to normal isotonic Ringer’s solution may cause further changes in their function cannot be excluded, but there is no indication that these changes, if any, contribute to the uncoupling observed.

Using the following procedure, we have obtained the best uncoupling results. The sciatic-sartorius preparation, pinned to a layer of Sylgard plastic (Dow Corning Corp., Midland, Mich.) in the bottom of a petri dish, was immersed in the FMD solution and the muscle twitch was tested every 10 or 20 s with both direct and indirect stimuli (1 ms duration) applied by means of two platinum electrodes. Both the FMD concentration and the time of exposure necessary to achieve uncoupling seem to depend upon the condition of the frogs. Muscles obtained from one batch of frogs stopped twitching after from 5 to 15 min in a 2.0 M FMD solution. However, this same concentration caused damage to muscles taken from frogs of other shipments. These, in turn, were best uncoupled, in about 15-30 min, with a 1.5 M FMD solution. It is, therefore, advisable to determine, by trial and error, the FMD concentrations and times of exposure needed to uncouple the muscles from any particular batch of frogs to avoid causing damage to the fibers.

As soon as contractions stopped in any of the above mentioned conditions, the muscles were transferred to normal Ringer’s solution. These muscles remained uncoupled during the rest of the experiment, usually 6-8 h, with resting potentials ranging from 80 to 90 mV. In a few preparations, exposed to 2.0 M FMD for periods over 20 min, the resting potentials decreased to values lower than 60 mV, but this decrease was reversed if the muscles were left overnight at 5°C in normal Ringer’s solution. Next morning, their resting potentials were again normal, and the muscles were still paralyzed.

Generally, uncoupling did not impair the electrical activity of the nerve, and both endplate potentials and miniature endplate potentials could be recorded. However, prolonged exposure to FMD would also block neuromuscular transmission, though still not affecting the electrical activity of the nerve. For this reason, to do work on neuromuscular transmission the preparation in the FMD solution should be stimulated every few seconds and changed to normal Ringer’s solution while nerve stimulation still elicits weak twitching in a few fibers. Occasionally, the mechanical responses to stimulation may increase too much upon returning the muscles to normal Ringer’s solution; but, if this happens, a second and brief exposure to FMD is usually sufficient to produce uncoupling while neuromuscular transmission remains unaffected. Muscles treated in this manner have remained paralyzed for periods of up to 3 days, at 5°C, with normal resting potentials. FMD is, indeed, a very effective uncoupling agent for both teaching and research purposes.

The mechanism of action of FMD is unclear. Unpublished observations of Córdoba, García-Salazar, and del Castillo showed that FMD even at 1 M concentration failed to remove water from
muscle cells and to inhibit the activity of actomyosin-like ATPases of the guinea pig ileum. However, FMD exerts striking changes in the optical density of suspensions of freshly prepared skeletal muscle microsomes ("relaxing factor") which suggest that the vesicles formed by membranes of the sarcoplasmic reticulum swell under the influence of that compound. It has been suggested by the above mentioned authors that FMD may act by preventing the movement or storage of calcium ions, a mechanism that could also account for the observed block in neuromuscular transmission.

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REFERENCES