The Effect of Glycerol Treatment of Voltage-Clamped Snake Muscle Fibers

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ABSTRACT The effect of glycerol treatment on the membrane currents and tension development was studied in voltage clamped snake muscle fibers. In muscle fibers which were exposed for 1 h to a normal saline containing 400 mM glycerol and then returned to a normal medium, graded depolarizations did not accompany contractile responses. However, when the fiber was depolarized to a certain level, an increment of outward current appeared which partially inactivated with time. The threshold for delayed rectification in glycerol-treated fibers was almost the same as that of intact fibers in spite of the absence of contractile tension. The results suggest that the delayed rectification may be attributed at least in part to the surface membrane and that the contractile activation probably does not depend simply on the inactivating outward currents through the delayed rectification channel.

INTRODUCTION

It has been established that the action potentials are produced by an increase in the conductance of the membrane to sodium ions followed by an increase to potassium ions, both in nerve (Hodgkin and Huxley, 1952) and in muscle (Adrian et al., 1970 a). When active sodium permeability changes are blocked by tetrodotoxin (TTX) in skeletal muscle, depolarization beyond a critical threshold produces an increment of outward current which partially inactivates with time (Heistracher and Hunt, 1969 a). The threshold potential for the appearance of the delayed rectification, evident in earlier studies by the inactivating outward current (Heistracher and Hunt, 1969 a and b), was almost the same as that for the mechanical threshold in standard Ringer's solution (Costantin, 1968; Kao and Stanfield, 1968; Heistracher and Hunt, 1969 a). However, many examples of dissociation between delayed rectification and contractile activation have been shown (Heistracher and Hunt, 1969 a and b; Kao and Stanfield, 1970; Stanfield, 1970). From the study of the kinetics of mechanical activation, Adrian et al. (1969)
also showed a clear-cut difference between the strength-duration relations of the contractile mechanism and of delayed rectification. A method which causes selective disruption of the transverse tubules of skeletal muscle has been described by Howell and Jenden (1967). Later it was found that action potentials could be recorded with no accompanying contraction and that membrane capacity decreased in these fibers (Gage and Eisenberg, 1969 a and b). Thus, these preparations may be used to dissociate the properties of the surface membrane and tubular membrane of muscle fibers.

In the experiments reported here the effect of glycerol treatment in short snake muscle fibers has been studied, using voltage clamp techniques. The object of the work was first to examine the behavior of the outward currents in the absence of mechanical responses by the glycerol treatment, and secondly to localize the site where the delayed rectification takes place.

METHODS

All experiments were carried out at room temperature (20°-24°C), using short (1.0-1.5 mm in length) scale muscle fibers from the garter snake (Thamnophis). Conventional techniques for intracellular recording and stimulation were used for studying electrical properties of muscle fibers. The methods employed in voltage clamp experiments were similar to those used by Heistracher and Hunt (1969 a). Current and recording electrodes were inserted in the center of a fiber. In this situation the distribution of steady-state potential change along the length of a fiber has been shown to be almost uniform (Heistracher and Hunt, 1969 a). The procedure of glycerol treatment used by Howell and Jenden (1967) was followed for the disruption of the sarcotubular system, namely the fibers were exposed for 1 h to the normal snake saline containing 400 mM glycerol and then returned to the normal one. The normal bathing fluid had the following composition (in millimolar): NaCl, 158; KCl, 2.15; CaCl₂, 3.5; MgCl₂, 1.7; Na₂HPO₄, 2.15; NaH₂PO₄, 0.83; and glucose, 9.8. Tetrodotoxin (Sankyo, Tokyo, Japan) in a concentration of 1 X 10⁻⁷ (wt/vol) was added to both glycerol and standard saline to block spontaneous contractures and impulse activities.

RESULTS

It was characteristic that muscle fibers became cloudy and opaque when the fibers were returned to the standard solution from the solution containing 400 mM glycerol as observed on frog muscle by Gage and Eisenberg (1969 a). Experiments on glycerol-treated fibers were started around 20 min after return to the standard saline. Although no systematic study was made on the resting potentials, the resting potential of some fibers was so low (20-30 mV) as to suggest damage. Only fibers having resting potentials of more than 50 mV were used for these experiments.
1. Electrical Properties of Glycerol-Treated Muscle Fibers

In frog skeletal muscle fibers in which the transverse tubular system has been disrupted by treatment of glycerol (Eisenberg and Eisenberg, 1968; Howell, 1969), the slow and progressive increase in membrane potential (creep) produced by hyperpolarizing current pulse has been found to be absent (Gage and Eisenberg, 1969b). A reduction in this creep was found in snake twitch muscle fibers after glycerol treatment. Examples may be seen in the experiment shown in Fig. 1. Long (upper records) and short (lower records) hyperpolarizing current pulses were applied to the glycerol-treated fibers (C and D) and the intact fibers (A and B) in the standard solution. The relation between current and resultant membrane potential in a glycerol-treated fiber is shown in Fig. 2. The membrane potentials were measured at the end of the current pulse of 230 ms duration which was applied with current electrode within a distance of 100 µm from the recording electrode. The curve had a linear intermediate region on the side of hyperpolarization with a slope of about 700 kΩ. When the fiber was depolarized by more than about 10 mV, a decrease in input resistance was observed.

**FIGURE 1.** The effect of hyperpolarizing currents on the membrane potential of the intact fibers (A and B) and the glycerol-treated fibers (C and D) in the standard solution. Long (about 1 s in A and C) and short (about 100 ms in B and D) currents were applied. Upper trace, membrane potential; lower trace, hyperpolarizing current.
2. Relation between Membrane Potentials and Outward Currents in Voltage-Clamped Fibers

Typical responses to graded depolarization of a glycerol-treated fiber are shown in Fig. 3. The fiber was clamped at \(-100 \text{ mV}\) and then graded depolarizing pulses of constant duration were applied. Weak depolarization steps were associated with an outward current which remained nearly constant during the step (Fig. 3 A). When depolarization exceeded a critical threshold, an increment of outward current appeared which inactivated with time (Fig. 3 B-F). This outward current reached a steady value after about 1 s. However, no tension was produced by step depolarizations (see Discussion), even when the membrane potential reached 0 mV or even more positive (Fig. 3 E and F) at which level maximum tension usually occurred in the intact fibers (Heistracher and Hunt, 1969 a). A plot of the relation between membrane potentials and currents is shown in Fig. 4 A of which data were obtained from the fiber in Fig. 3. The delayed rectification appeared when the fiber was depolarized to \(-37 \text{ mV}\) (Fig. 3 B and Fig. 4 A). The threshold for the appearance of delayed rectification in the glycerol-treated fiber was almost the same as that of the intact fiber. The former threshold was at approximately the same level as that for contraction, being about \(-35 \text{ mV}\) in the standard saline (Heistracher and Hunt, 1969 a).
This inactivating current, a component of outward current which inactivated with time, showed some degree of anomalous rectification at more than 0 mV. While it is less obvious, there was a slight tendency for the steady-state current to increase at approximately the same level as the threshold potential for the delayed rectification, without contractile tension. Fig. 4 B shows an example obtained from another glycerol-treated fiber. The delayed rectification appeared at about \(-35\) mV. A slight inflection of steady-state current was also found, but the inactivating current showed an almost linear relation with depolarization, even to levels more positive than 0 mV. The inactivating current was found to be mainly nonlinear with membrane potential, but in a few fibers a linear relationship was also found. Under our experimental conditions it is difficult to interpret quantitatively these current-voltage relationships, because these relations before glycerol-treatment could not be studied in the same fiber. However since the steady-state potential distribution along the fiber is considered to be uniform in our experiment (see Methods) and on the assumption that the delayed inactivating current is carried mainly by potassium ions, it seems likely that the delayed rectification takes place in the surface membrane, although delayed rectification from the tubular membrane cannot be excluded.

3. Effect of Hypertonic Glycerol Solution

To test the possibility that the hypertonic glycerol solution might directly inhibit the contraction, a few fibers still bathed in 400 mM glycerol solution...
were studied. Fibers were kept in the normal saline containing 400 mM glycerol for 60 min before impalement of electrodes. An example of the experiment is shown in Fig. 5 where the fiber was clamped at $-100$ mV and depolarized to graded levels for about 1 s duration. Depolarization of the fiber above a threshold level produced contractures which were graded by the magnitude of the depolarization steps just like the intact fiber in the standard solution. The threshold potential for mechanical activation was about $-35$ mV in this fiber. Also the behavior of the membrane current was found to be similar to that of the intact fibers. This result makes it seem unlikely that 60 min in glycerol Ringer's solution caused any uncoupling
between excitation and contraction. A transient reduction of contractile tension has been noted just after the application of hypertonic glycerol solution (Yamaguchi et al., 1962; Caputo, 1968). However, it was also observed that the ability to twitch recovered while the muscle continued to be bathed in this hypertonic solution.

**DISCUSSION**

The present study clearly showed that the increment of outward current which inactivated with time appeared with almost the same threshold as that of the untreated fibers after glycerol treatment when the depolarization was maintained. Since this increment of outward current during the depolarization seems to be carried mainly by potassium ions (Heistracher and Hunt, 1969a), our results suggest that the outward current which inactivated with time passes through the delayed rectification channel, which is located at least in part in the surface membrane. Hutter (1969) has reached the same conclusion as to the location of the delayed rectification in experiments on frog skeletal muscle fibers treated with formaldehyde.

Now much information has been accumulated on the effect of glycerol treatment on muscle fibers. Eisenberg and Eisenberg (1968) have found that the sarcotubular system was essentially absent in glycerol-treated fibers, only 2% of tubular system remaining connected to the extracellular space. Present results on the absence of contractile responses may be explained by these structural changes with glycerol treatment. It might be worthwhile to point out that maximum depolarization of glycerol-treated fibers in our voltage clamp experiments resulted in a very small tension development, not more than 3 mg (Fig. 3 E and F). Since the maximum tension is between 60 and 90 mg in intact fibers, the tension developed after glycerol treatment is only 3–5% of the maximum tension. This finding implies that glycerol-treated fibers are unable to respond mechanically to depolarization produced by electric currents as well as to elevated external concentration of potassium ions (Howell, 1969). It was found that fibers which continued to be bathed in hypertonic glycerol Ringer's solution did not display any uncoupling between excitation and contraction. The result is in good agreement with earlier findings (Yamaguchi et al., 1962; Caputo, 1968). In
glycerol-treated fibers, however, caffeine produced contractures and these fibers relaxed when caffeine was removed (Howell and Jenden, 1967; Howell, 1969). Thus, the transverse tubular system appears to be an essential link between contraction and excitation of the surface membrane (Howell, 1969; Gage and Eisenberg, 1969). Gage and Eisenberg (1969) observed the absence of the early and late afterpotentials in glycerol-treated frog muscle fibers. On the other hand, Adrian et al. (1970) found three components of the potassium currents using a three electrode voltage clamp method in sartorius muscle fiber: current in the delayed rectifier channel, a slow component through a channel in which depolarization increases potassium conductance, and current in the inward rectifier channel. In their paper they suggested that the delayed rectifier might be mainly in the surface membrane and the other two channels in the tubular system. The constancy of the equilibrium potential for the delayed current also suggested that no large delayed currents might flow through the tubular membrane (Adrian et al., 1970). More recently, however, Almers (1972) has shown a large contribution of tubular membrane to potassium permeability in skeletal muscles in accordance with earlier works (Adrian and Freygang, 1962; Eisenberg and Gage, 1969). In this situation it is possible that both surface and tubular membrane share the site of delayed rectification.

In contrast with our result, Ildefonse and Rougier (1969) reported that no delayed rectification was detectable by pretreatment with glycerol under their voltage clamp conditions, using sucrose-gap method (Rougier, Vassort, and Ildefonse, 1968). At present, the reason for this discrepancy is not clear.
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