Electrical Properties and Excitation-Contraction Coupling in Skeletal Muscle Treated with Ethylene Glycol

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ABSTRACT The contractility of the frog sartorius muscle was suppressed after treatment with a Ringer solution added with ethylene glycol (EGR). No contraction was elicited by nerve stimulation when the muscle was brought back to normal Ringer solution after having been soaked in 876 mM EGR for 4 hr or in 1095 mM EGR for 2 hr. However, the action potential of normal amplitude was generated and followed by a depolarizing afterpotential. The resting membrane potential was slightly decreased from the mean normal value of −91.1 mv to −78.8 mv when 1095 mM EGR was used, and to −82.3 mv when 876 mM EGR was used, but remained almost constant for as long as 2 hr. The afterpotential that follows a train of impulses and a slow change in membrane potential produced by a step hyperpolarizing current (so-called "creep") were suppressed after treatment with ethylene glycol. The specific membrane capacity decreased to about 50% of the control values while the specific membrane resistance increased to about twice the control values. Therefore, the membrane time constant remained essentially unchanged. The water content of the muscle decreased by about 30% during a 2 hr immersion in 1095 mM EGR, and increased by about 30% beyond the original control level after bringing the muscle back to normal Ringer. The intracellular potassium content did not change significantly during these procedures. Some differences between the present results and those obtained with glycerol are discussed.

INTRODUCTION

It has long been known that the skeletal muscle loses its ability to contract when treated with hypertonic solutions (Fenn, 1936; Hodgkin and Horowicz, 1957; Howarth, 1958; Yamaguchi et al., 1962; Dydýnska and Wilkie, 1963; Caputo, 1968). Recently it was found that the contractility of the muscle was suppressed when it was brought back to normal Ringer solution after having been soaked in a solution made hypertonic with solutes capable of penetrating the muscle membrane; these solutes include glycerol (Howell and Jenden,

1967; Howell, 1969; Gage and Eisenberg, 1967, 1969 *a*), ethylene glycol, propylene glycol, and acetamide (Caputo, 1968; Krolenko and Adamyan, 1967; Krolenko et al., 1967).

After treatment with a Ringer solution added with glycerol at a concentration of 400 mM, the muscle was still able to elicit action potentials by nerve stimulation. The action potentials were normal in amplitude but lacking the depolarizing afterpotential (early afterpotential) which normally follows each spike (Gage and Eisenberg, 1967, 1969 *a*). The resting membrane potential declined steadily after excitation-contraction (E-C) uncoupling (Howell, 1969; Howell et al., 1970; Eisenberg et al., 1971). The late afterpotential that follows a train of impulses (Freygang et al., 1964) and the slow potential change produced by a hyperpolarizing pulse of current (so-called "creep") were also suppressed (Gage and Eisenberg, 1969 *a*). Both the late afterpotential and the creep were related to the existence of an intracellular compartment in which the potassium ions may accumulate during a train of impulses or may be removed by a hyperpolarizing current (Adrian and Freygang, 1962). It was proposed that the intracellular compartment might correspond to the sarcotubular system (Hodgkin and Horowicz, 1960). Furthermore, after returning to normal Ringer following glycerol treatment, the sarcotubular system became vesiculated and could not be reached by electron-opaque markers such as horseradish peroxidase (Graham and Karnovsky, 1966; Eisenberg and Eisenberg, 1968) or ferritin (Krolenko, 1969), suggesting that the normal connection of the T system with the extracellular space was impaired.

Since the synaptic transmission is not impaired in the glycerol-treated muscles, such preparations have been conveniently used for studies of the end-plate membrane (Kordaš, 1969, 1970; Maeno et al., 1971; Deguchi and Narahashi, 1971; Deguchi et al., 1971). However, the glycerol treatment has disadvantages in that the resting membrane potential declines steadily, that internal sodium concentration increases, and that internal potassium concentration decreases (Henderson, 1970). Solutions made hypertonic with substances having a low permeability across the membrane such as sucrose, glucose, or sodium chloride also inhibit the E-C coupling (Hodgkin and Horowicz, 1957; Howarth, 1958) but block the neuromuscular transmission (Fatt and Katz, 1952; Furshpan, 1956). We have found that ethylene glycol inhibits the E-C coupling without blocking the neuromuscular transmission and without causing a great depolarization. Therefore, electrical properties of the muscle treated with ethylene glycol were analyzed.

METHODS

Material Experiments were carried out with the sartorius muscle of the frog (*Rana pipiens*). To facilitate diffusion and uncoupling, small frogs and muscles (about

25 mg) were used. Only the experiments for determining water and potassium content were carried out with larger muscles (about 150 mg) to make measurements more accurate. In either case muscles were dissected with the nerve (about 1 cm) attached, and the proximal and distal ends were tied off with thin silk threads. Special care was taken not to damage the muscle at the proximal tendon. The muscles were soaked in hypertonic solution at 5°C for a certain period of time, and then mounted in a Lucite chamber containing normal Ringer solution at room temperature (22°C). The low temperature was chosen for soaking in hypertonic solution to prolong the survival time of muscle.

Solutions Frog Ringer solution was used as the normal bathing medium and contained K^+ 2.4 mM, Na^+ 116 mM, Ca^{++} 1.7 mM, Cl^- 122 mM. The pH was maintained at 7.4 by addition of 3 mM HEPES (*N*-2-hydroxyethylpiperazine-N′-2-

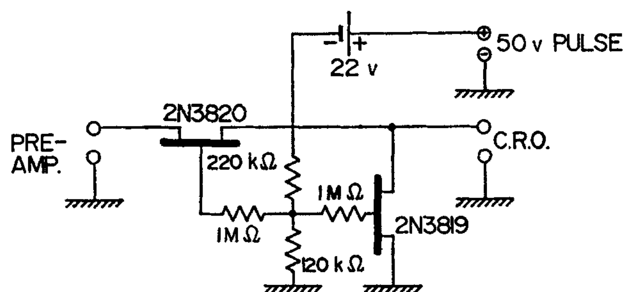

FIGURE 1. Diagram of the electronic switch used for short-circuiting the input of the oscilloscope (*C.R.O.*) to set a base line. Without pulse the N-channel transistor (2N3819) is kept off and the P-channel transistor (2N3820) on, and the pulse applied to the circuit reverses the polarity and the state of the transistors. The drain-source impedance of both transistors is 10^9 Ω in the off state and less than 1000 Ω in the on state.

ethane sulfonic acid). Ethylene glycol, glycerol, or sucrose was added to the Ringer. Two concentrations of ethylene glycol were tried: 1095 mM (called 6× EGR) and 876 mM (5× EGR). The osmolarity of these solutions was determined by measurements of lowering of the freezing point.

Recording Techniques Glass microelectrodes filled with 3 M KCl solution and having an electric resistance of 5–10 MΩ were inserted in the muscle fiber either to inject current or to record the membrane potential. The recording microelectrode was connected to a high input impedance preamplifier, and the time constant of the recording system was estimated to be 33 μsec. The nerve was stimulated by means of a suction electrode.

In order to monitor the base line on the screen of the oscilloscope, the input was short-circuited for less than 2 msec duration at the beginning of each sweep by means of an electronic switch triggered by a square pulse. The diagram of the switch is shown in Fig. 1. It consists of two field effect transistors (FET), one P-channel FET (Texas Instruments Inc., Dallas, Tex.; 2N3820) in series with the preamplifier, and an N-channel FET (Texas Instruments Inc., 2N3819) in parallel with the oscilloscope. If the gates of both transistors are kept negative, the amplifier will be con-

nected through a resistance of a few ohms with the oscilloscope. When a positive pulse is applied to the gates, a resistance of the order of $10^9\ \Omega$ is built up in series with the amplifier and at the same time the oscilloscope input is shunted by a resistance of less than 100 Ω to the ground. The error introduced in the voltage measurements by the use of this circuit with the transistors properly biased was of the order of 0.1%.

To inject square pulses of current into the muscle fibers, an operational constant current supply was employed (Gage and Eisenberg, 1969 *b*).

Determination of Intracellular Water and Potassium Content The sartorius muscles were dissected from both legs of a frog and weighed. One of the muscles was soaked in normal Ringer solution as control, and the other was immersed in 6× EGR. After 2 hr both muscles were carefully blotted on a filter paper, weighed, and dried for 2 hr in an oven at 150°C. The difference between the first and second weighings corresponds to the change in water content of the muscle. The dry weight of the muscle was also recorded. To determine the potassium content the dried muscles were digested in a mixture of 17 ml of concentrated nitric acid and 3 ml of 70% (v/v) perchloric acid. To accelerate the process and insure better digestion, it was carried out in an oven at 75°C. Finally the macerate was dissolved in a deionized water to make a final volume of 100 ml. The potassium concentration was determined by means of a Perkin-Elmer 303 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.). Several values have been proposed for the volume of the extracellular space in the skeletal muscle, ranging from 8 to 22.4% (see Ling et al., 1969). In the present study, a value of 12% was used because this value is close to most of the measurements. This value was assumed to remain constant in all solutions used, because Dydýnska and Wilkie (1963) found it unchanged upon increasing the tonicity of solution up to twice the normal value (6× EGR is about 1.5× hypertonic).

RESULTS

Resting and Action Potentials

While being soaked in 5× or 6× EGR, the muscle remained semitransparent, and contractions observed under a ×30 dissection microscope could hardly be distinguished from those of the normal muscle. In a few cases, the isometric twitch tension was monitored and a transient decrease in tension was observed immediately after immersion of the muscle in the hypertonic solution. Neuromuscular transmission was not impaired, but large spontaneous miniature end-plate potentials (up to 5 mv by overlapping) were often observed. The membrane potential in muscles soaked in normal Ringer was estimated to be -91.12 ± 1.41 mv (mean ± standard error of mean of 50 fibers). The membrane was hyperpolarized to -95.4 ± 0.4 mv (149 fibers) while soaking in 6× EGR, the difference being highly significant ($P < 0.01$) as determined by Student's *t* test (Guttman and Wilks, 1965).

Muscles were soaked in 6× EGR for 2 hr or in 5× EGR for 4 hr at 5°C,

and then returned to normal Ringer at 22°C. Vigorous twitches occurred spontaneously several times upon returning to Ringer, and then the contraction elicited by nerve stimulation gradually decreased in amplitude and eventually disappeared in 15–30 min. Such an uncoupling was sometimes incomplete, especially when large muscles isolated from large frogs or 5× EGR was used. The mean resting potential was estimated to be −78.9 ± 0.81 mv (279 fibers) for seven muscles treated with 6× EGR, and −82.1 ± 0.87 mv (214 fibers) for seven muscles treated with 5× EGR, the difference between the

TABLE I
MEMBRANE POTENTIAL OF MUSCLE FIBERS UNCOUPLED WITH ETHYLENE GLYCOL RINGER (EGR)

Experiment number	Membrane potential*	Linear regression		Number of fibers	Recording period
		Slope‡	$V_{(t=0)}$‡		
	mv	*mv/min*	*mv*		*min*
Fibers uncoupled with 6× EGR					
22	−87.5±4.1	−0.140 ±0.29	−76.4± 24.8	16	131
26	−86.1±1.4	−0.003 ±0.08	−86.0± 5.1	48	127
29	−66.2±2.1	0.104 ±0.11	−73.5± 8.6	28	120
31	−78.3±1.3	−0.015 ±0.12	−77.4± 9.1	48	141
77	−83.1±1.2	0.010 ±0.06	−83.4± 4.94	65	142
79	−86.6±1.7	−0.096 ±1.73	−80.1± 47.18	38	114
24	−59.2±1.8	−0.453 ±0.28	−40.4± 12.0	37	56
Fibers uncoupled with 5× EGR					
15	−84.1±1.5	0.139 ±1.45	−99.2±156.4	14	112
16	−80.1±2.1	−0.0002±0.42	−80.3± 20.8	12	15
17-A	−83.3±3.7	−0.204 ±1.03	−80.3± 17.7	12	24
19	−73.5±2.2	+0.202 ±0.22	−82.9± 11.1	40	74
23	−86.8±1.1	−0.018 ±0.23	−87.3± 7.2	22	48
25	−78.0±1.6	−0.012 ±0.10	−77.0± 9.3	66	148
27	−92.4±1.3	−0.035 ±0.09	−90.1± 6.8	48	100

* Mean ± SEM.
‡ Mean ± 0.95 confidence limits.

means being highly significant ($P < 0.001$). Table I gives the results of experiments in which the decline of the resting potential was measured as a function of time. The slope of decline after treatment with 6× EGR was estimated to be −0.044 mv/min (279 fibers) and that after treatment with 5× EGR −0.008 mv/min (214 fibers), the difference between the slopes being insignificant ($P > 0.05$). Sometimes variability between fibers in the same muscle is large, as can be seen in the 0.95 confidence limits.

After treatment with 6× EGR in another series of experiments, the mean amplitudes of the action potential and resting potential were estimated to be 116.8 ± 0.84 and −84.5 ± 0.56 mv, respectively (271 fibers). The spike was

followed by a depolarizing afterpotential provided that the membrane was not greatly depolarized. Fig. 2 shows the action potential recorded from a fiber after treatment with 6× EGR, and the action potential from another fiber still immersed in the hypertonic solution. The early depolarizing afterpotential was clearly present in both cases.

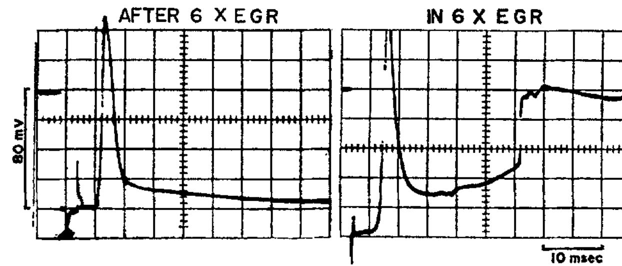

FIGURE 2. Action potentials elicited by nerve stimulation in a muscle fiber soaked in 6X ethylene glycol Ringer (EGR) (right) and in another muscle fiber in normal Ringer after having been soaked in 6X EGR (left). The action potential in 6X EGR is followed by a depolarizing afterpotential and contraction that dislodges the microelectrode and that, after 6X EGR, is also followed by a depolarizing afterpotential but lacks contraction.

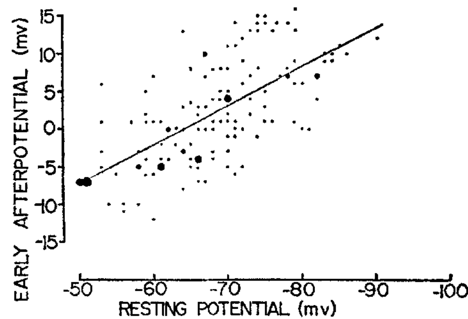

FIGURE 3. Relationship between the initial amplitude of the early afterpotential and the resting potential in muscle fibers uncoupled with 6X ethylene glycol Ringer. The initial amplitude was measured by extrapolating the afterpotential back to the moment when the action potential attained the peak. The regression line calculated by the least square method follows the equation $y = -33.05 - 0.52x$. Concentric circles indicate the number of observations with the same value.

The amplitude of the early afterpotential (EAP) in normal muscle fibers is a linear function of the resting membrane potential (Persson, 1960, 1963; Gage and Eisenberg, 1969 *a*). Fig. 3 shows the relationship between the initial amplitude of EAP's and the resting membrane potential in the uncoupled muscles. The initial amplitude was measured by extrapolating the EAP to the time when the action potential reaches its peak. The regression line was calculated by the least square method. It shows a reversal potential of −64

mv, which is close to the value obtained by Persson (1963) for normal muscle fibers. In the uncoupled muscle the measurements of the amplitude of the EAP at a 5 msec interval starting at the time when the action potential reaches its peak fell on a straight line rather than on an exponential curve. This is interesting because in the normal muscle the EAP decays exponentially with a time constant similar to that of the membrane (Ishiko and Sato, 1956).

After a train of impulses, the membrane potential does not return to the resting level immediately but is followed by a slow repolarizing phase. The half-decay time of the late afterpotential (LAP) is about 300 msec and its amplitude is a function of the duration and frequency of repetitive stimuli (Freygang et al., 1964). Freygang et al. (1964) attributed this afterpotential to an accumulation of potassium ions in the sarcotubular system, and Gage

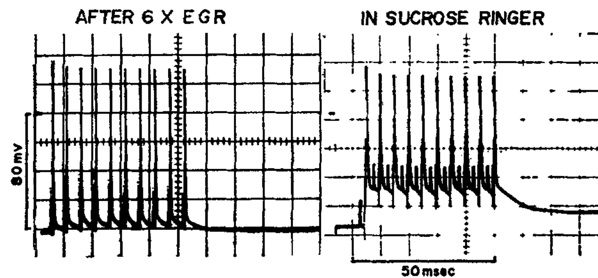

FIGURE 4. Action potentials and afterpotentials produced by repetitive nerve stimuli (100 cps) in a muscle fiber uncoupled with 6X ethylene glycol Ringer (EGR) and by repetitive direct stimuli in another muscle fiber uncoupled with a Ringer added with 200 mM sucrose. In sucrose Ringer, each action potential is followed by an early afterpotential and a train of impulses is in turn followed by a late afterpotential with a slower time-course. After 6X EGR a train of action potentials is followed only by an early afterpotential.

and Eisenberg (1969 *a*) found that the LAP was suppressed after the functional disruption of the T system with 400 mM glycerol Ringer. A train of action potentials produced in an ethylene glycol uncoupled muscle at a frequency of 100 pulses/sec is shown in Fig. 4. A similar record from another muscle immersed in a Ringer solution added with 200 mM sucrose is also illustrated in Fig. 4. The LAP is present in the muscle immersed in sucrose Ringer which possesses a functional sarcotubular system but is absent in the muscle uncoupled with ethylene glycol.

Another electrical property of the muscle fiber that disappears after uncoupling with glycerol (Eisenberg and Gage, 1967; Gage and Eisenberg, 1969 *a*) is the so-called creep. When a strong inward step current lasting for about 1 sec was applied to the normal muscle fiber, the resultant potential change, after having risen with a time-course determined by the membrane time constant, further increased more slowly and eventually attained a steady state

(Fig. 5, control). A similar record from a fiber immersed for 2 hr in 6× EGR and that from another fiber after uncoupling with 6× EGR are also shown in Fig. 5. The creep was absent in the last case. Anodal break excitation was produced in about 10% of the fibers uncoupled either with ethylene glycol or glycerol (Fig. 5). None of the normal control fibers or the fibers soaked in the hypertonic Ringer produced anodal break excitation.

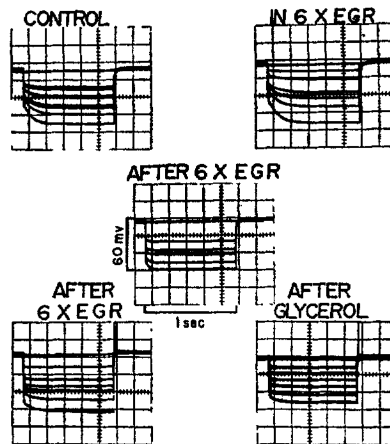

FIGURE 5. Creep and anodal break excitation. The initial phase of the potential change produced by a strong and long-lasting inward step current is followed by a slowly rising phase (creep) in normal control muscle (upper left). The creep is present in a muscle fiber soaked in 6X ethylene glycol Ringer (EGR) (upper right), but disappears after uncoupling with 6X EGR (middle) or with 400 mM glycerol (lower right). Anodal break excitation is observed in about 10% of the muscle fibers uncoupled with either EGR or glycerol (lower left and right).

Cable Properties

As described in the preceding sections, the membrane potential is higher and more stable in the ethylene glycol uncouple muscle than in the glycerol uncoupled muscle, and the early afterpotential is not changed in amplitude and duration. This raises a question as to whether these two treatments produce uncoupling by different mechanisms. It has been shown that the glycerol treatment is able to disrupt functionally the sarcotubular system (Eisenberg and Eisenberg, 1968; Krolenko, 1969) and that the uncoupled fibers have a smaller membrane capacity and shorter time constant (Eisenberg and Gage, 1967; Gage and Eisenberg, 1969 *b*; Howell, 1969). The magnitude of the change in membrane capacity is in good agreement with the membrane capacity of the sarcotubular system (Falk and Fatt, 1964), suggesting that the sarcotubular space and its membrane are disrupted by the glycerol treatment.

To compare further the two methods and to clarify the mode of action of ethylene glycol, the cable properties of muscle fibers were studied. Square pulses of inward current of about 10^{-8} amp in strength and 25 msec in duration were applied through a microelectrode inserted in a fiber while another microelectrode was successively inserted in the same fiber at various distances from the current microelectrode (50–1500 μ, four to five impalements). The time for the resultant potential to reach 85% of the steady state at an interelectrode distance of less than 50 μ (Hodgkin and Rushton, 1946) was taken as a measure of the membrane time constant. The potential change at the zero interelectrode distance and the length constant were estimated from the regression line relating the potential to the interelectrode distance according to the following equation:

$$\ln V_x = \ln (V_o) - x/\lambda \qquad (1)$$

where V_o is the steady-state potential at the point of current injection, V_x is the steady-state potential at the distance x, and λ is the length constant of the fiber. Then the input resistance, R_e, is given by

$$R_e = V_o/I \qquad (2)$$

where I is the intensity of applied current. It follows that

$$R_x = V_x/I = 0.5\sqrt{r_m \cdot r_i} \exp(-x\sqrt{r_i/r_m}) = R_e \exp(-x/\lambda) \qquad (3)$$

where R_x is the input resistance at the distance x, r_m is the membrane resistance of a unit length of fiber, and r_i is the longitudinal resistance of the cytoplasm in a unit length of fiber. Thus we obtain

$$r_i = \frac{2R_e}{\lambda} \qquad (4)$$

and

$$r_m = 2R_e \cdot \lambda. \qquad (5)$$

If we assume that the fiber is a cylinder of a uniform diameter, the cytoplasm resistivity, R_i, and the specific resistance of the membrane, R_m, are given by

$$R_i = r_i \pi d^2/4 \qquad (6)$$

$$R_m = r_m \pi d \qquad (7)$$

where d refers to the fiber diameter. Finally, from the membrane time constant, τ, we obtain the specific membrane capacity

$$C_m = \tau/R_m. \qquad (8)$$

The above equations may be solved only when the fiber diameter is measured or R_i is known. The measurement of the fiber diameter involves some uncertainty owing to polygonal cross-section of the fiber and low magnification of the microscope ($\times$ 60). Therefore, we first calculated data assuming a constant value for R_i (200 Ω cm) (Falk and Fatt, 1964). The results of calculations are given in the upper three rows of Table II. It is seen that the diameter of the fibers significantly ($P < 0.001$) increases when the muscle is soaked for 2 hr in the hypertonic solution. This is opposite to what would be expected. The result may be due to the erroneous assumption that the internal resistance is kept constant while the muscle fibers are soaked in 6$\times$ EGR. Therefore, calculations were made using a value of 100 Ω cm for the internal resistivity while the muscle fibers are soaked in 6$\times$ EGR, and the results are given in the middle row of Table II. Notice that with this value of internal resistivity, the calculated diameter for the fibers in 6$\times$ EGR is the same as that for the control fibers for which R_i is assumed to be 200 Ω cm. A decrease of the internal resistivity of this order of magnitude may be expected if the intracellular water content is reduced during the immersion in the hypertonic solution.

To examine this possibility, the electrical properties were calculated using the fiber diameters measured with an accuracy of $\pm$ 12 μ in the same group of fibers as in the previous calculations, and the results are given in the lower three rows of Table II. No significant difference ($P > 0.2$) was found between the fiber diameters of the three groups, i.e. control, 6$\times$ EGR, and uncoupled. The internal resistivity was reduced to 42.5% control in the hypertonic solution; this decrease is in the same order of magnitude as that predicted in the previous calculation.

From the results given in the lower three rows of Table II, it is apparent that the only changes in the cable properties of the fibers soaked in 6$\times$ EGR are a decrease in R_i and the resultant increase in the length constant, both being statistically significant ($P < 0.05$ and $P < 0.01$, respectively). After uncoupling two major changes in the cable properties of the fibers occurred: a decrease in specific membrane capacity to 60% of the controls ($P < 0.05$) and an increase in specific membrane resistance to 168% of the controls ($P < 0.05$). As the internal resistivity returned to the normal value upon uncoupling, the length constant also returned to the normal level. No difference in the values for the membrane time constant was found among the three experimental conditions.

Intracellular Water Content and Potassium Concentration

Since the internal resistivity decreases while the muscle is soaked in 6$\times$ EGR, a reduction of the water content of the fiber and a simultaneous increase in intracellular potassium concentration are expected to occur. In order to examine such changes, one sartorius muscle was kept in normal Ringer solution

TABLE II

CABLE PROPERTIES OF MUSCLE FIBERS BEFORE AND AFTER E-C UNCOUPLING WITH ETHYLENE GLYCOL RINGER (EGR)

	Condition	Specific membrane resistance	Specific membrane capacitance	Internal resistivity	Diameter	Length constant	Time constant	Input resistance	Number of fibers
		$\Omega\ cm^2$	$\mu F/cm^2$	$\Omega\ cm$	μ	μ	*msec*	$M\Omega$	
1	Control	2076±359	4.18±0.73	(200)	53±5	1152±122	7.70±0.51	0.56±0.08	5
2	In 6× EGR	3197±204	2.31±0.18	(200)	72±4	1693±85	7.15±0.42	0.43±0.04	10
3	After 6× EGR	3079±640	2.21±0.18	(200)	50±3	1328±100	6.63±1.39	0.71±0.1	5
4	In 6× EGR	2261±640	3.18±0.26	(100)	51±3	1693±85	7.15±0.42	0.43±0.04	10
5	Control	2576±498	3.58±0.83	398±124	64±5	1152±122	7.70±0.51	0.56±0.08	5
6	In 6× EGR	2636±260	3.08±0.45	148±30	60±4	1693±85	7.15±0.42	0.43±0.04	10
7	After 6× EGR	4324±754	1.69±0.24	296±134	57±8	1330±129	7.34±1.53	0.76±0.1	4

Data of lines 1–4 were calculated assuming the internal resistivity as indicated in the parenthesis. Data of lines 5–7 were from the same sets of experiments as 1–3, but calculations were made using the measured fiber diameters. Membrane potentials were: controls (from four muscles), -81.2 ± 6.34 mv; in 6× EGR (from two muscles), -84.6 ± 1.59 mv; after uncoupling (from four muscles), -74.6 ± 4.74 mv.

as the control, while the other muscle from the same frog was treated with 6× EGR. Fig. 6 summarizes the results of measurements of potassium content. There was no significant change in potassium content during and after soaking in 6× EGR.

Changes in water content and potassium concentration are shown in Fig. 7. The water content decreased while soaking the muscle in 6× EGR and increased after uncoupling, whereas the potassium concentration changed in

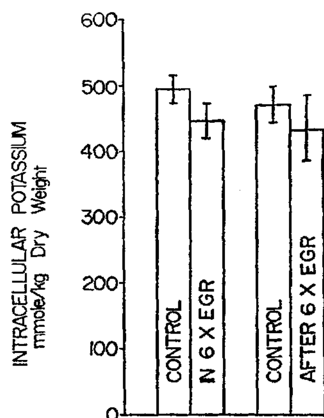

FIGURE 6

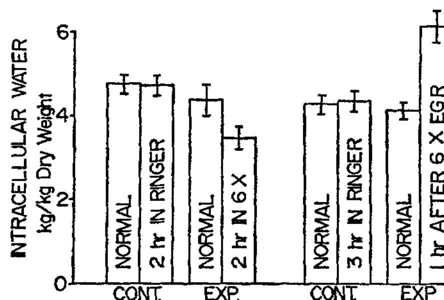

FIGURE 7

FIGURE 6. Intracellular potassium contents in the muscle soaked in 6X ethylene glycol Ringer (EGR) for 2 hr at 5°C and in the muscle uncoupled with 6X EGR. Each control measurement was made with the contralateral muscle of the same frog kept in normal Ringer for the same period of time and at the same temperature. Data are given as the mean ± standard error of mean of three muscles (control at left) or four muscles (the other three). The difference between control and test in each set is insignificant ($P > 0.05$).

FIGURE 7. Intracellular water content. "Normal" represents the muscles immediately after dissection, and others the same muscles after treatment as indicated. The water content decreased significantly after soaking in 6X ethylene glycol Ringer (EGR) for 2 hr, and increased after uncoupling. Same muscles as those used for the experiments presented in Fig. 6. All solutions were kept at 5°C. Data are given as the mean ± standard error of mean.

the opposite direction during these treatments. Hyperpolarization observed in 6× EGR and depolarization after uncoupling may be at least partly related to these changes in potassium concentration. However, it should be noted that the measurement of intracellular potassium and water content were made on large muscles. Therefore, uncoupling may not have been completed in deep fibers causing some underestimate in the magnitude of the changes.

DISCUSSION

The coupling between electrical activity and concentration was completely blocked after treatment of the muscle with a Ringer solution made hypertonic

with ethylene glycol. The muscle was first soaked in 6× or 5× EGR for 2 or 4 hr and brought back to normal Ringer solution. Electrical stimulation failed to produce contraction some 15–30 min after return to Ringer. The resting membrane potential declined by about 10 mv, but the depolarization was much less than that observed after treatment with glycerol (14–35 mv depolarization) (Howell, 1969; Henderson, 1970; Eisenberg et al., 1971). However, Eisenberg et al. (1971) have recently reported that the depolarization is slowed by an addition of magnesium at a concentration of 5 mM and an increase in calcium concentration to 5 mM. One of the possible mechanisms involved in the depolarization after ethylene glycol treatment is a decrease in internal potassium concentration as a result of water movement.

The active membrane potential underwent no change after uncoupling with ethylene glycol. The mean value of 32 mv is almost exactly the same as that obtained with normal muscle fibers by Nastuk and Hodgkin (1950). In the muscle fibers uncoupled with glycerol, the active membrane potential also remained essentially unchanged (Gage and Eisenberg, 1969 *a*).

However, a difference is noted between glycerol and ethylene glycol treatments in their effects on early afterpotential. Although EAP was modified in amplitude and duration in the muscle fibers uncoupled with glycerol (Gage and Eisenberg, 1967, 1969 *a*), it was present in normal amplitude and duration in the EGR-treated fibers. This difference appears to be due at least in part to different resting membrane potentials. When the membrane was depolarized, the amplitude of EAP was decreased and eventually reversed its polarity, the reversal potential being estimated to be −64 mv, or 27 mv less negative than the normal resting potential. This reversal potential is the same as that obtained with normal muscle fibers by Persson (1960, 1963). In addition, Henderson (1970) did observe the EAP in the muscle fibers in which the membrane potential was restored to near normal value some 4 hr after uncoupling with glycerol.

The specific membrane capacity and membrane time constant obtained in the present study with normal muscle fibers are smaller than those reported previously (Fatt and Katz, 1951; Ishiko and Sato, 1960; Falk and Fatt, 1964), the capacity being about one-half and the time constant about one-third. However, the differences virtually disappear when the data by Ishiko and Sato (1960) are recalculated using the internal resistivity of 200 Ω cm. In addition, Hodgkin and Nakajima (1972) have recently found that in frog skeletal muscle fibers the specific membrane capacity is a function of the fiber diameter and reported a value of $4.6 \pm 0.17\ \mu F/cm^2$ ($n = 9$) for 50-μ fibers. This value is not significantly different from $3.58 \pm 0.83\ \mu F/cm^2$ ($n = 5$) described in the present paper ($P > 0.10$).

Drastic changes were observed in the specific membrane capacity and the specific membrane resistance after uncoupling with ethylene glycol. The ca-

pacity decreased from the normal control value of 3.58 to 1.69 $\mu F/cm^2$, while the membrane resistance increased from the control value of 2576 to 4324 Ω cm^2. Because of these opposite changes, the membrane time constant remained essentially unchanged. In addition, the late afterpotential and the creep of anelectrotonic potential also decreased after uncoupling with ethylene glycol. The LAP and creep are interpreted as being due to changes in potassium concentration in the T-system lumen (Adrian and Freygang, 1962). Therefore, these changes in membrane capacity, membrane resistance, LAP, and creep are compatible with the notion that the T system is functionally eliminated in the muscle uncoupled with ethylene glycol. It should be noted that the uncoupling using glycerol results in similar changes in those electrical parameters except for the membrane time constant which decreases.

It has been shown that after treatment with glycerol the T system forms swollen vesicles (Howell and Jenden, 1967; Eisenberg and Eisenberg, 1968; Krolenko, 1969; Howell, 1969). Furthermore, electron microscope marker molecules such as ferritin or horseradish peroxidase can no longer enter the T system (Eisenberg and Eisenberg, 1968). It should be noted that such structural changes in T system produced by 200 mM glycerol are reversible (Krolenko, 1969). This suggests that a narrowing of the lumen or a coalescence of the sarcotubular membranes rather than a complete disruption of the T system is more likely to occur. The fact that the resting membrane potential after uncoupling with ethylene glycol is relatively stable is in favor of this view. However, we cannot exclude the possibility that the T system is actually disrupted after ethylene glycol treatment but the resultant holes in the muscle membrane seal by themselves quickly.

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