Delayed Rectification and Anomalous Rectification in Frog's Skeletal Muscle Membrane

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ABSTRACT Delayed rectification was elicited in frog's skeletal muscles bathed in choline-Ringer's solution, in normal Ringer's solution with tetrodotoxin, in 40 mM Na_2SO_4 solution with tetrodotoxin, and even in 40 mM K_2SO_4 solution when the membrane had been previously hyperpolarized. However, after a sustained depolarization current-voltage relations in 40 mM K_2SO_4 and in 40 mM Na_2SO_4 solutions revealed a rectifier property in the anomalous direction. This indicates that the increase in potassium conductance which is brought about upon depolarization is a transient phenomenon and is inactivated by a maintained depolarization, and that this potassium inactivation process converts the delayed rectification into the anomalous rectification. In normal Ringer's solution with tetrodotoxin and in the 40 mM Na_2SO_4 solution with tetrodotoxin the apparent resistance was increased when the membrane was hyperpolarized beyond about −150 mv. This is thought to be due to a decrease of K conductance caused by a strong hyperpolarizing current. In the 40 mM Na_2SO_4 solution with tetrodotoxin a de- or hyperpolarizing current pulse induced a prolonged depolarizing response. During the early phase of this response the effective resistance was lower, and during the following phase greater than that in the resting fiber. An interpretation in terms of the ionic hypothesis was made of the nature of this response.

INTRODUCTION

In 1949 Katz made an observation that muscles in 50 mM K_2SO_4 displayed rectification in the anomalous direction; *i.e.*, in the opposite direction to the delayed rectification of the squid axon. Recently Hodgkin and Horowicz (1959 *b*) studied the influence of K and Cl on the resting potential of single skeletal muscle fibers and concluded, in agreement with Katz (1949), that the rectification of K channel was in the anomalous direction. The anomalous

rectifying property of the skeletal muscle membrane was also confirmed by Hutter and Noble (1960), by Adrian (1960), and by Freygang and Adrian (1960).

However, there is a wholly different line of evidence which suggests that depolarization leads to an increase in K permeability. Fenn and Cobb (1936) on rat muscles, and Hodgkin and Horowicz (1959 *a*) on frog skeletal muscles observed the release of potassium ion with muscle activity, suggesting an increase in K permeability during the repolarization phase of the action potential. Jenerick (1953, 1959) reported the existence of delayed rectification in the skeletal muscle fiber, and Narahashi *et al.* (1960) observed the occurrence of delayed rectification in muscle rendered inexcitable with tetrodotoxin, a toxic substance obtained from the puffer.

This noticeable discrepancy led some investigators (Hodgkin and Horowicz, 1959 *a*, *b*; Adrian, 1960; Grundfest, 1960) to speculate that when the fiber was depolarized, the period of raised potassium conductance was followed by one of lowered potassium conductance.

The present paper describes experiments in which the behavior of the potassium conductance over a wide range of membrane potentials was observed in frog's skeletal muscle bathed in solutions of varying ionic composition. The results obtained, in keeping with the supposition described above, showed that increase in K permeability which was brought about upon depolarization was transient and was inactivated by a maintained depolarization, and that as a result of this inactivation the anomalous rectification became apparent. In order to be able to measure the current-voltage relation over a wide range, hypertonic bathing solutions were used (Hodgkin and Horowicz, 1957), thus abolishing the mechanical response. The use of microelectrodes filled with Na citrate (Boistel and Fatt, 1958) also facilitated the performance of the present experiments. The opportunity has also been taken to study the nature of the prolonged response which was observed in the muscle immersed in 40 mM Na_2SO_4 solution with tetrodotoxin.

Part of the results of the present investigation has been reported in a preliminary communication (Nakajima, Iwasaki, and Obata, 1961).

METHODS

The isolated sartorius of *Rana nigromaculata* was mounted with its inner surface facing upward, in a slightly stretched condition in a bath. Two microelectrodes were inserted into one fiber less than 200 μ apart from each other. The one filled with 3 M KCl was used to record potential. The other, which was filled with 2 M Na citrate and had a resistance about 10 MΩ, was used to apply polarizing current. The potential recorded was fed into a DC amplifier with an input cathode follower. Polarizing currents were monitored with a series resistor of 1 to 3 KΩ, which was inserted between the bath

and the ground, and a high gain DC amplifier. Potentials and currents were observed simultaneously on a dual beam cathode ray oscilloscope.

Nearly all the experiments were done with muscles immersed in the solutions made hypertonic (2.5 times the normal) by adding sucrose in order to avoid the movement of muscle (Hodgkin and Horowicz, 1957). In the experiments in normal Ringer's solution and 40 mM Na_2SO_4 solution, tetrodotoxin (1×10^{-7} gm/ml) was applied to render the muscle inexcitable.

Test solutions were the same as those used by Hodgkin and Horowicz (1959 *b*). The normal Ringer's solution contained (mM) NaCl 116, KCl 2.5, $CaCl_2$ 1.8, Na_2HPO_4 1.08, NaH_2PO_4 0.43. The choline-Ringer's solution was made by replacing NaCl with equimolar amounts of choline chloride. The 40 mM K_2SO_4 solution contained (mM) K_2SO_4 40, $CaSO_4$ 8, Na_2HPO_4 1.08, NaH_2PO_4 0.43, sucrose 113. The 40 mM Na_2SO_4 solution contained (mM) Na_2SO_4 38.7, K_2SO_4 1.3, $CaSO_4$ 8, Na_2HPO_4 1.08, NaH_2PO_4 0.43, sucrose 113.

TABLE I

INFLUENCES OF HYPERTONIC SUCROSE AND TETRODOTOXIN ON RESTING POTENTIAL AND K ACTIVATION POTENTIAL OF MUSCLE FIBER

Row	Solution	Resting potential	K activation potential	No. of observations
		mv	*mv*	
1	Normal Ringer's	−85±1		7 muscles, 107 fibers
2	Hypertonic Ringer's	−78±1		7 muscles, 105 fibers
3	Choline-Ringer's	−82±1	−49±1	5 muscles, 32 fibers
4	Choline-Ringer's with 2×10^{-7} tetrodotoxin	−83±1	−49±1	5 muscles, 33 fibers
5	Hypertonic choline-Ringer's	−72±1	−49±2	7 muscles, 16 fibers

All measurements were done 15 to 45 minutes after immersion of the muscle into the solution. ± limits show the standard error of the mean. The microelectrodes used in the experiments in the first and second rows had tip potentials less than 5 mv. Resting potentials given in the third to fifth rows were measured with two microelectrodes inserted into the same fiber; hence, the comparison with the resting potentials in the first and second rows should not be made. Temperature 21–23°C.

The experiments were performed at room temperature which ranged from 21 to 27°C.

The membrane potential will be given as inside potential minus outside potential.

RESULTS

A. Influences of Hypertonic Sucrose and Tetrodotoxin on Membrane Properties

Table I summarizes the results of the experiments in which the effects of hypertonic sucrose and tetrodotoxin on the permeability to potassium were observed. As is shown in the first and second rows of Table I the hypertonic

solution decreases the resting potential: the difference is 7 ± 2 mv (t test, 5 per cent confidence limits).

Fig. 1 shows the current-voltage relation of the muscle membrane in the choline-Ringer's solution. It will be seen that the rectification over the range between −100 and −55 mv is in the anomalous direction but at −55 mv there is a remarkable deviation toward the direction of the abscissa, indicating the existence of delayed rectification. This result partly coincides with Jenerick's observation (1959). The potential indicated by the arrow *a* in Fig. 1 is the potassium activation potential defined by Jenerick (1959). This is the potential at which delayed rectification becomes first noticeable.

Narahashi *et al.* (1960) showed that tetrodotoxin at a concentration of 1×10^{-7} gm/ml abolished the action potential, whereas the resting potential was unchanged and delayed rectification became apparent. In the experiments

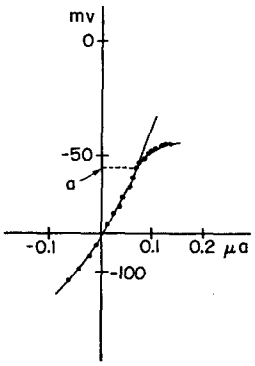

FIGURE 1. Current-voltage relation of muscle membrane bathed in the choline-Ringer's solution. Ordinate, membrane potential at the end of the rectangular current pulse of about 100 msec. duration. Abscissa, intensity of the current pulse. Outward current positive. The arrow *a* indicates the K activation potential. Temperature 21°C.

given in the third and fourth rows of Table I effects of tetrodotoxin (2×10^{-7} gm/ml) on the resting potential and on the potassium activation potential were examined. It can be seen that tetrodotoxin does not alter the potassium activation potential, suggesting that the drug at this concentration may not alter the increase of K permeability which occurs upon depolarization.

The fifth row of Table I shows that the potassium activation potential is also unchanged in muscle treated with the hypertonic sucrose solution, although the resting membrane potential decreases by about 10 mv.

These results indicate that the application of hypertonic sucrose solution or 1×10^{-7} gm/ml tetrodotoxin does not profoundly interfere with the increase in K permeability which occurs upon depolarization.

B. Current-Voltage Relationship in Muscles in the Hypertonic 40 mM K_2SO_4 Solution

In Fig. 2 the current-voltage relation is plotted for a muscle in the hypertonic 40 mM K_2SO_4 solution. Fig. 3 represents some of the actual records. An

inward conditioning direct current was first applied bringing the potential from the original value of −21 mv to −83 mv. After a few minutes, rectangular current pulses of about 100 msec. duration and of different intensity and direction were superimposed on it, and the current-voltage curve was plotted from measurements of the potential at the end of the current pulse (Fig. 2 I, Fig. 3 i–o). In Fig. 2 the intensity of the conditioning current is not shown. Hence, the point at which the curve (I) of Fig. 2 crosses the ordinate (the

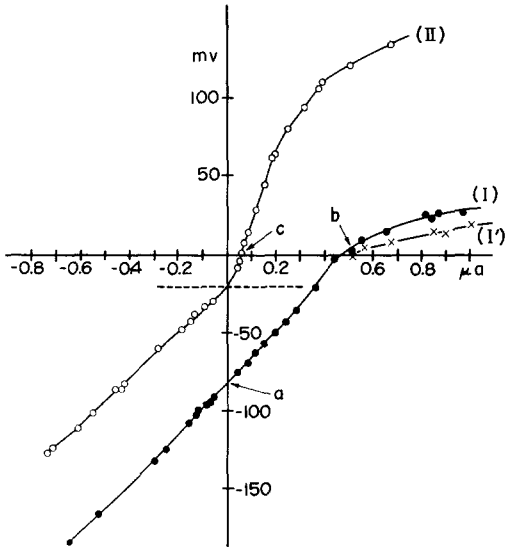

FIGURE 2. Current-voltage relation of muscle membrane bathed in the hypertonic 40 mM K_2SO_4 solution. Ordinate, membrane potential at the end of the rectangular current pulse of about 100 msec. duration. The dashed line represents the resting membrane potential. Abscissa, intensity of the current pulse. Outward current positive. (I), the membrane has previously been hyperpolarized to −83 mv by a conditioning direct current. The arrow *a* indicates the potential level with the direct current flowing. (I'), same as (I), except that the current and potential were measured at about 20 msec. after the onset of current pulses. (II), no conditioning current applied. Temperature 27°C.

arrow *a*) does not indicate the potential at zero current intensity, but one with the conditioning current flowing. It will be seen from the curve (I) of Fig. 2 that when the potential is inside-negative the current-voltage relation reveals a slight anomalous rectification. However, marked deviation is seen to occur near 0 mv, indicating the existence of delayed rectification. The occurrence of delayed rectification is also shown in the records k and l of Fig. 3. The potassium activation potential was on the average of −2 ± 3 mv (SE of the mean, fourteen fibers in six muscles), when the potential had been previously hyperpolarized to −84 ± 2 mv level at 24–27°C.

When the current-voltage relation was measured after terminating the conditioning current (Fig. 2 II, Fig. 3 a-h), the outward current evoked a much greater electrotonic potential than did the inward one. This is the indication of the anomalous rectification first described by Katz (1949). It

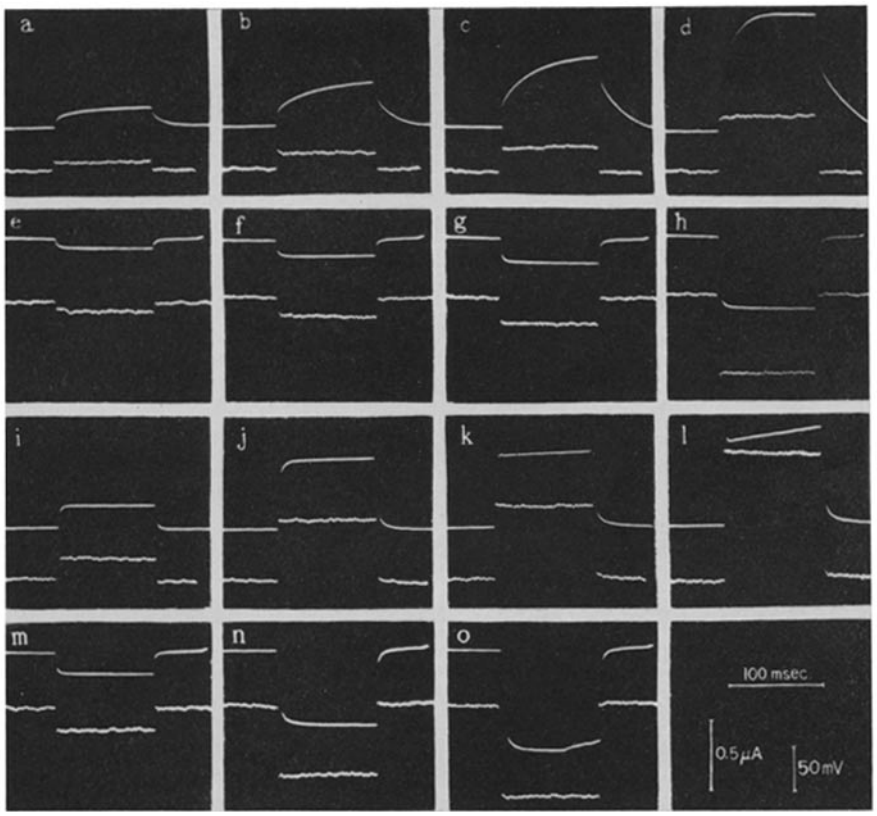

FIGURE 3. Electrotonic potentials (upper traces) evoked by rectangular current pulses (lower traces) in the hypertonic 40 mM K_2SO_4 solution. Selected from the data illustrated in Fig. 2. Inside positive direction of the potential and outward direction of current upward. Records *a–h* correspond to the curve (II) and *i–o* to the curve (I) of Fig. 2. The base lines of the potential records of *a–h* represent the resting membrane potential, while those of *i–o* −83 mv level. Temperature 27°C.

can be seen from Fig. 2 that the slope resistance in curve (II) near 0 mv (the arrow *c*) is much greater than that at the corresponding potential in the curve (I) (the arrow *b*). This indicates that the increased K permeability induced by a depolarization is again eliminated by a long lasting depolarization. Or, put another way, delayed rectification is converted into anomalous rectification by a maintained depolarization.

The elimination of delayed rectification can also be inferred from records

k and l of Fig. 3, which show that electrotonic potentials increase with small slopes after having passed through slight minima. The curve (I′) of Fig. 2 was plotted at about 20 msec. after the onset of the current pulse. The difference between slopes of curve (I) and (I′) illustrates the onset of elimination of delayed rectification by the outward currents of 100 msec. duration.

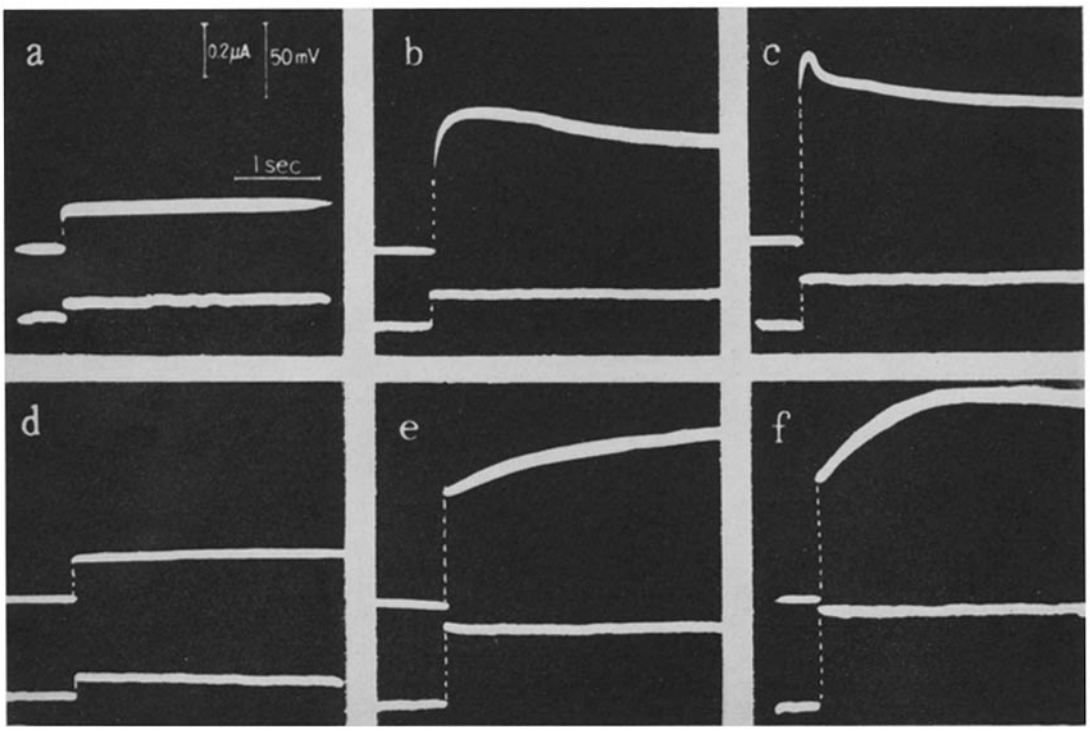

FIGURE 4. Records on a slow time base of electrotonic potentials (upper traces) produced by current steps (lower traces) in the hypertonic 40 mM K_2SO_4 solution. Inside positive direction of the potential and outward direction of the current upwards. In records *a–c*, no conditioning current has been applied, so that the base lines correspond to the resting membrane potential of −25 mv. In records *d–f*, the membrane had been hyperpolarized to −76 mv by a conditioning direct current. Records *b* and *c* show the breakdown of the membrane, while *e* and *f* show the conversion of the delayed rectification into the anomalous. Temperature 27°C.

When increasing intensities of outwardly directed current pulses carried the potential beyond +70 mv, the effective resistance was reduced again (Fig. 2 II). However, this lowering of resistance is thought to be different from delayed rectification; since in the former case the lowered resistance was not raised again by a maintained outward current, but it continued to decline as long as outward current was being applied. This is clearly demonstrated in Fig. 4, in which the electrotonic potentials are displayed at slow sweep

speed. A conditioning inward current was applied bringing the potential to −76 mv, and outward current steps were superimposed on it (d-f). In e and f we can see the slowly developing electrotonic potentials which indicate the elimination of delayed rectification by maintained outward currents. However, when the outward currents were passed without prior hyperpolarization (a-c), there appeared an early maximum in the electrotonic potential (b and

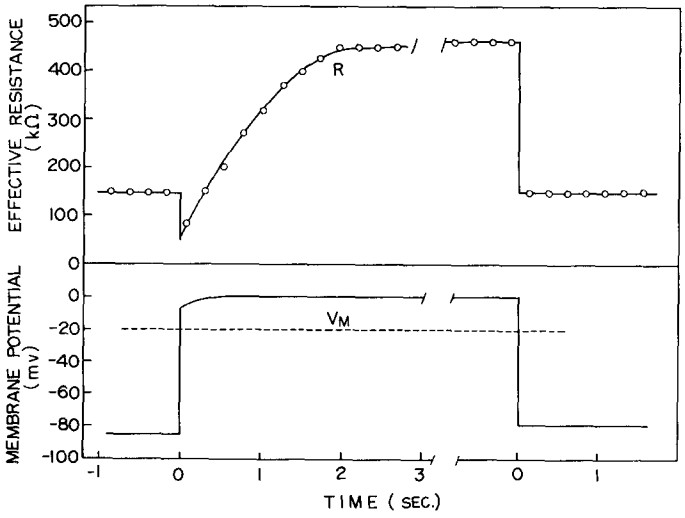

FIGURE 5. Time course of the change in the effective resistance ($R = dV_M/dI_o$) induced by depolarization (V_M) in the muscle bathed in the hypertonic 40 mM K_2SO_4 solution. The effective resistance was measured with small current pulses of about 80 msec. duration at a frequency of 4.2/sec. The membrane had been previously hyperpolarized from −20 mv to −85 mv by a conditioning current, and at zero time (left-hand side), outward direct current was passed bringing the potential to about 0 mv; after a few minutes, the outward direct current was turned off, the potential falling to −80 mv (right-hand side). The dashed line represents the resting potential. Temperature 27°C.

c): this is supposed to indicate a breakdown of the membrane. Similarly, the lowering of resistance at membrane potentials beyond +70 mv (Fig. 2 II) is thought to represent the breakdown rather than delayed rectification.

C. Time Course of the Shift of Delayed into Anomalous Rectification in Muscles in the Hypertonic 40 mM K_2SO_4 Solution

The time course of the conversion of the delayed rectification into the anomalous was followed in muscles in the hypertonic 40 mM K_2SO_4 (Fig. 5). The membrane was hyperpolarized by a conditioning current to −85 mv; and at zero time (the left-hand side of Fig. 5) an outward direct current was superimposed bringing the potential to 0 mv level. Since the constant current

technique was used instead of the voltage-clamp technique, a small shift in potential was inevitable owing to the change in the resistance. But this was frequently of small magnitude because the resting potential was only about −20 mv. The effective resistance was measured by superimposing depolarizing pulses of about 80 msec. duration at a frequency of 4.2/sec.

From Fig. 5, it is apparent that when the membrane is depolarized to 0 mv, the effective resistance is first decreased (delayed rectification), followed by a gradual increase, reaching eventually the value much higher than that

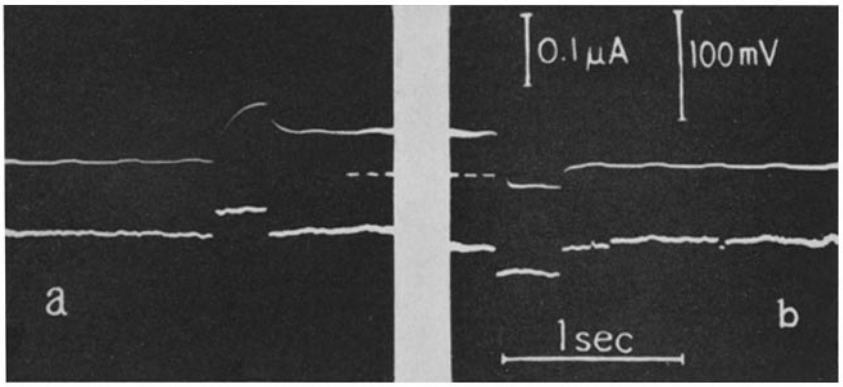

FIGURE 6. Records suggesting the presence of a negative conductance region in the muscle membrane immersed in the hypertonic 40 mM K_2SO_4 solution. Upper traces represent potential changes, the lower ones currents applied. Inside positive direction of the potential and outward direction of the current upwards. Dashed line represents the outside zero potential. A conditioning outward current had been previously applied. The potential level was switched back and forth between the two stable levels of +10 and +35 mv by delivering outward (*a*) and inward (*b*) current pulses alternately. The potential could never be brought to the level of about +20 mv. Temperature 27°C.

at −85 mv (anomalous rectification). The half-time of this increase in the effective resistance ranged from 0.3 to 1.1 sec. with an average of 0.7 ± 0.2 sec. (SE of the mean, eleven fibers in five muscles) when the potential was brought from −85 ± 2 mv to +4 ± 1 mv at 27°C. When the potential was brought back almost to the original level, the resistance reverted at once to the original value (right-hand side of Fig. 5).

D. Discontinuity in the Potential in Muscles in the Hypertonic 40 mM K_2SO_4 Solution

Occasionally we encountered a discontinuous phenomenon in fibers in the K_2SO_4 solution. This region was observed only if the inside potential was more positive than about −20 mv. Fig. 6 shows an experiment in which a conditioning outward current was being passed, and the test current pulses were superimposed. We tried to bring the potential to about the +20 mv level,

which was never attained. When an outward current pulse of a certain intensity was applied (Fig. 6 *a*), the potential was raised regeneratively, and even after the pulse was turned off the potential stayed at about +35 mv. An inward current applied resulted in a wholly reversed phenomenon (Fig. 6 *b*). The potential could be switched back and forth between the two stable

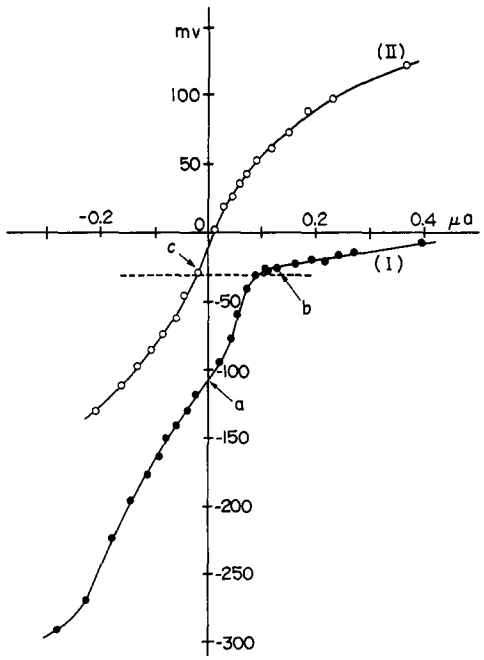

FIGURE 7. Current-voltage relation of muscle membrane immersed in the hypertonic 40 mM Na_2SO_4 solution with 1×10^{-7} gm/ml tetrodotoxin. Ordinate, membrane potential at the end of the rectangular current pulse of about 100 msec. duration. The dashed line represents the resting membrane potential. Abscissa, intensity of current pulse. Outward current positive. (I), the membrane has been hyperpolarized to −107 mv by a conditioning current. (II), conditioning outward current is applied bringing the potential to −11 mv level. Temperature 26°C.

levels of +10 and +35 mv by delivering inward and outward current pulses alternately.

E. Current-Voltage Relation in the Hypertonic 40 mM Na_2SO_4 Solution

In Fig. 7 the current-voltage relation was plotted for a muscle fiber bathed in the hypertonic 40 mM Na_2SO_4 solution with 1×10^{-7} gm/ml tetrodotoxin. Even after allowing more than 30 min. for equilibration in this solution, the resting potential was found to show great variability between the values of −20 and −70 mv. It was often unstable, and exhibited a long or indefinitely lasting response when a short current pulse was applied. The nature of this

response will be discussed later. This unstable character of the resting potential made it impossible to measure the current-voltage relationship. But it was found that when a conditioning current was applied hyper- or depolarizing the membrane by a few tens of millivolts, the membrane became stable; thus, enabling us to measure the current-voltage relation.

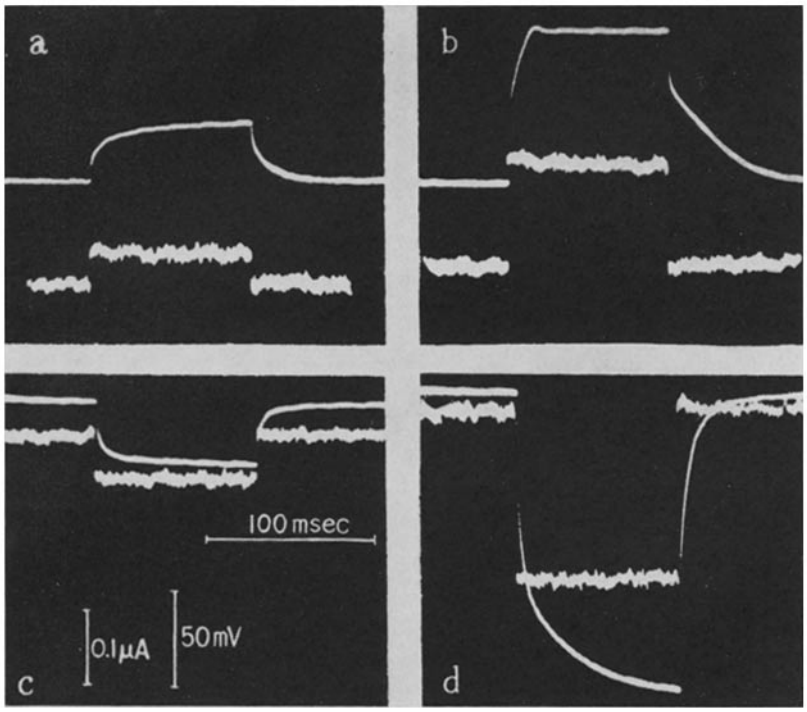

FIGURE 8. Potential changes (thinner traces) produced by rectangular current pulses (thicker traces) in muscle bathed in the hypertonic 40 mM Na_2SO_4 solution with 1×10^{-7} gm/ml tetrodotoxin. Selected from the data illustrated in Fig. 7 (I). Inside positive direction of the potential and outward direction of the current upwards. The potential at the base lines is −107 mv. Temperature 26°C.

Curve (I) of Fig. 7 shows an experiment in which a conditioning current was applied hyerpolarizing the membrane from the original potential of −31 to −107 mv, and rectangular current pulses of 100 msec. duration were superimposed on it. The curve shows that at about −30 mv, the delayed rectification appears. The potassium activation potential was −39 ± 2 mv (SE of the mean, eight fibers in four muscles) when the membrane had been previously hyperpolarized to −94 ± 8 mv at 26°C. The rectification between −120 and −40 mv is of the anomalous type; this is in keeping with the result of Hutter and Noble (1960). But as the intensity of the hyperpolarizing current pulse is further increased taking the potential below −150 mv, the

slope of the curve is increased again. Finally at about −300 mv, the apparent resistance is lowered greatly, suggesting the occurrence of a breakdown of the membrane. In Fig. 8 some of the actual records are displayed. Record b illustrates the occurrence of delayed rectification. Record d shows that when a strong inward current pulse is applied a slowly developing electrotonus of

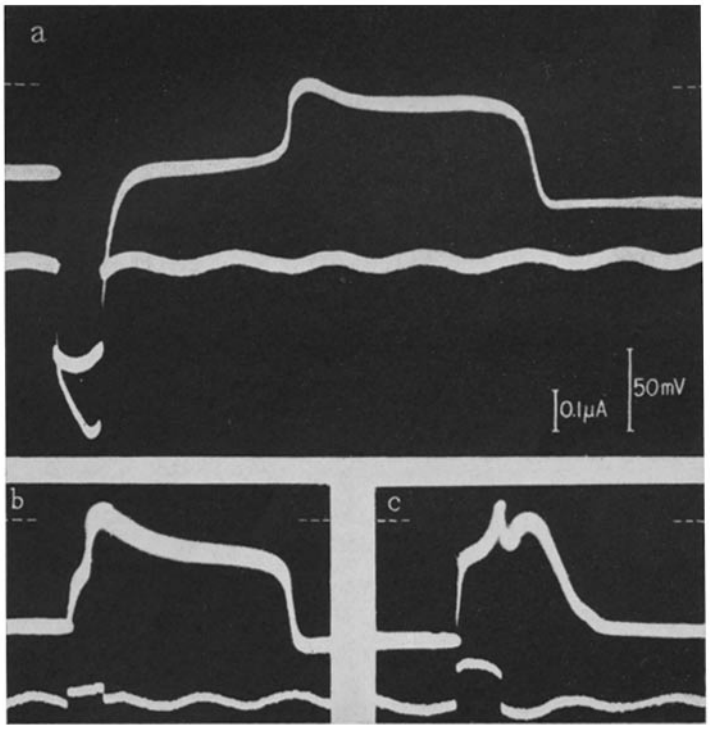

FIGURE 9. Prolonged responses (upper traces) evoked by hyperpolarizing (*a*), or depolarizing (*b*, *c*) current pulses (lower traces) in the hypertonic 40 mM Na_2SO_4 solution with 1×10^{-7} gm/ml tetrodotoxin. Action potential was totally abolished by the drug. No conditioning direct current applied. Dashed lines represent the outside zero potential. Time marker 1 cycle/sec. superimposed on the current trace. Temperature 24°C.

great amplitude appears; this corresponds to the increase in the apparent resistance between −150 and −250 mv in Fig. 7 (I).

Fig. 7 (II) shows the current-voltage relation in which a conditioning outward current was applied instead of the inward one. It will be seen that as in the case in the K_2SO_4 solution delayed rectification is eliminated by a maintained depolarization. The slope resistance at *c* is also greater than that at *a* or *b*: this indicates that the delayed rectification is converted into the anomalous, though the anomalous rectifier property is not so marked as in the case in the K_2SO_4 solution. Qualitatively the same result was obtained for

the muscle fiber which had a stable resting potential of about −60 mv, except that in that case the anomalous rectifying property was less apparent than in the case with unstable resting potential.

F. Current-Voltage Relation in the Hypertonic Ringer's Solution

In the hypertonic Ringer's solution with 1×10^{-7} gm/ml tetrodotoxin qualitatively the same result as that in the Na_2SO_4 solution was obtained; *i.e.*, the occurrence of the delayed rectification as well as the slowly developing hyperpolarizing electrotonus was observed.

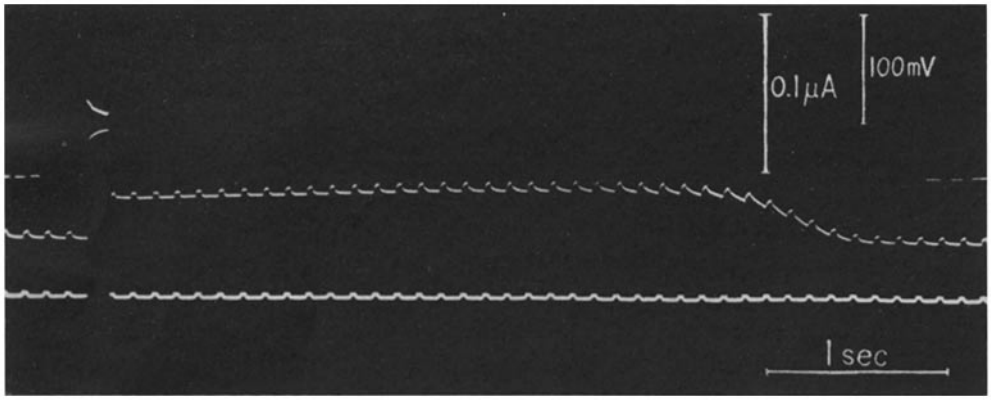

FIGURE 10. Change in the slope resistance during a prolonged response in the hypertonic 40 mM Na_2SO_4 solution with 1×10^{-7} gm/ml tetrodotoxin. Upper trace, potential change. Lower trace, current applied. Dashed line represents the outside zero potential. No conditioning current applied. Temperature 21°C.

G. Prolonged Response in the Hypertonic 40 mM Na_2SO_4 Solution

As was described above, the membrane potential in the hypertonic 40 mM Na_2SO_4 solution was often unstable. Even though the action potential was abolished by tetrodotoxin (1×10^{-7} gm/ml), a depolarizing (Fig. 9 *b*, *c*) or even a hyperpolarizing current pulse (Fig. 9 *a*) often induced a long lasting response. The duration of the response was variable, and occasionally steady depolarization lasted indefinitely. Falk and Landa (1960) reported that replacement of the chloride of Ringer's solution with impermeant anions resulted in a prolonged response or plateau following the spike potential in frog's muscle. The similarity of the present observation to that of Falk and Landa is apparent.

The effective resistance during the response was measured using recurring depolarizing current pulses (Fig. 10). It can be seen that at the early phase of the response the effective resistance is smaller than the resting value, but this initial decrease gives way to an increase in the resistance, and the resis-

tance gradually becomes greater than the resting value. The relatively rapid repolarization phase is accompanied by an abrupt return of the effective resistance to the resting value.

DISCUSSION

Cole (1961) and Cole and Curtis (1941) showed that for a non-linear uniform cable the relation among total current (I_o), membrane potential (V_M), and membrane current density (I_M) is

$$I_M \propto I_o \frac{dI_o}{dV_M}. \quad (1)$$

This equation is applicable for the case illustrated in Fig. 2 (II) and Fig. 7 (II), but not applicable for the case in Fig. 2 (I) and Fig. 7 (I), because in the latter two cases only the region near the microelectrodes was sufficiently hyperpolarized to become capable of giving rise to the delayed rectification upon depolarization.

In Fig. 11 the relationship between the membrane current density and membrane potential is shown for the fiber in the 40 mM K_2SO_4 solution calculated from the data in Fig. 2 (II). Hodgkin and Horowicz (1959 *b*) showed that for a muscle fiber in 95 mM K, the chord conductance for inward K current (g_k) at -32 mv was 100 times greater than that for outward K current at $+70$ mv. A similar comparison was made with the data in Fig. 11: the ratio of the chord conductance at -32 mv and that at $+70$ mv was 6:1. A possible explanation for the discrepancy in the value of this ratio would be that (*a*) the great electrotonic potential evoked by an outward current did not sufficiently reach a plateau value with current pulses of 100 msec. duration, and (*b*) the membrane conductance began to increase at $+70$ mv probably owing to the breakdown of the membrane in our experiment, whereas this did not seem to occur at this potential level in the experiment of Hodgkin and Horowicz (1959 *b*).

It was shown, confirming the observations made by Jenerick (1953, 1959) and by Narashashi *et al.* (1960), that delayed rectification occurred also in the skeletal muscle membrane. Since it was elicited for the muscle bathed in the Na_2SO_4 and K_2SO_4 solution as well as in choline-Ringer's solution and normal Ringer's solution with tetrodotoxin, it seems reasonable to assume that, as in the squid giant axon (Hodgkin, Huxley, and Katz, 1949), the delayed rectification is indicative of an increase in the potassium conductance which occurs upon depolarization. The magnitudes of potassium activation potential in the K_2SO_4 and in the Na_2SO_4 solution were definitely larger than those in choline-Ringer's solution. This may indicate some deleterious action of SO_4 upon the K activation mechanism, probably owing to the reduced

level of ionized calcium. But, strictly speaking, a quantitative comparison seems to be difficult owing to the cable property of muscle fibers. In the K_2SO_4 solution only the region near the microelectrodes was sufficiently hyperpolarized by the conditioning current, but the rest of the membrane stayed at the depolarized level; whereas in the choline-Ringer's solution the whole length of the fiber had the resting potential of about −80 mv.

In the 40 mM Na_2SO_4 as well as in the normal Ringer's solution a strong hyperpolarizing current induced a slowly developing electrotonus (Fig. 8 *d*).

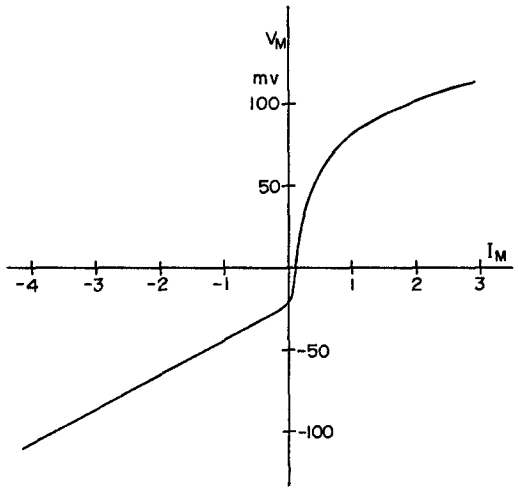

FIGURE 11. Relation between membrane current density (I_M) and membrane potential (V_M) for the fiber in the 40 mM K_2SO_4 solution. Calculated from the data of Fig. 2 (II) by equation (1). Abscissa, membrane current density in arbitrary units.

This peculiar behavior of the electrotonus is similar to that observed by Lorente de Nó (1947) in frog nerve bundles, and seems to be closely related to the hyperpolarizing responses in lobster muscle fibers recently reported by Reuben, Werman, and Grundfest (1961). As in the case of lobster muscle fibers (Reuben, Werman, and Grundfest, 1961), this slow electrotonus can be explained by assuming a decrease in the potassium conductance due to hyperpolarizing current. The cause of the decrease in potassium conductance may be explained by assuming that the strong inward current results in a reduction of the potassium concentration in the space just outside the membrane. This is compatible with the fact that the similar decrease in resistance was not observed in the 40 mM K_2SO_4 solution, where the inward current would not cause the drop in the potassium concentration in this space. In this connection it is noteworthy that Takeuchi and Takeuchi (1961) obtained evidence suggesting a decrease in K concentration near the end-plate region during the passage of an inward current.

The ionic theory of Hodgkin and Huxley (1952) quantitatively applicable to the squid axon did not include a term for K inactivation, because without the latter it was possible to describe accurately the behavior of the action potential which lasted only a few milliseconds. But the existence of a slowly developing potassium inactivation process in the squid giant axon was first inferred by Moore in 1959, when he tried to interpret the recovery from the regenerative process in K-rich media. In Ranvier nodes Frankenhaeuser and Waltman (1959) reported the existence of a potassium inactivation process, and Lüttgau (1960) observed in the same material that K permeability was inactivated with a half-time of about 15 sec. The peculiar behavior of K current recently observed by Hagiwara, Kusano, and Saito (1961) on the *Onchidium* nerve cell can also be interpreted by assuming a relatively rapid developing K inactivation process.

In eel electroplaques a potassium inactivation process initiated by outward current was early suggested by Grundfest (1957). Recently Werman, McCann, and Grundfest (1961) and Werman and Grundfest (1961) showed that inactivation of the potassium conductance by alkali earth or onium ions played an important part in converting graded activity into an all-or-none type in arthropod muscle. Thus, Grundfest (1960, 1961) proposed a four factor ionic hypothesis to interpret the various phenomena which appeared not to be interpretable solely in terms of the original Hodgkin-Huxley theory.

The present experiments provided convincing evidence that also in frog's skeletal muscles a potassium inactivation process occurs during depolarization, and that this process converts the delayed rectification into the anomalous. Hence, the conclusions obviously support the applicability of the four factor ionic hypothesis to the frog's skeletal muscle, and are also in keeping with the presumption of Hodgkin and Horowicz (1959 *a*, *b*) and Adrian (1960) that the behavior of the K channel at short times is different from that in the steady state.

The time course of the conversion of delayed rectification into anomalous rectification was observed in 40 mM K_2SO_4 solution: the value for the half-time was estimated as about 0.7 sec. when the potential was brought from −85 mv to +4 mv at 27°C. But again this value must not be taken to represent the true time course of the K inactivation process which occurred in the "membrane," because the non-linear cable property of the muscle membrane complicates the results. The non-linear cable analysis presented by Cole and Curtis (1941) and Cole (1961) does not seem to be applicable to the present case; the analysis of the transient properties of a non-linear cable is required. However, it might be safe to conclude that the onset of potassium inactivation in the frog's skeletal muscle is much faster than that in Ranvier's nodes (Lüttgau, 1960).

The membrane potential of the muscle in the 40 mM K_2SO_4 solution

occasionally showed a discontinuous phenomenon at a positive inside potential region. It is apparent that if the variational membrane conductance (dI_M/dV_M) has no region of negativity, the experimentally measured variational conductance (dI_O/dV_M) is always positive, since neither the axoplasm resistance nor the external fluid resistance is thought to have a negative resistance. Although a precise quantitative analysis of a cable whose component has a negative conductance region would be a rather complicated problem, we may suppose that if a negative conductance characteristic in the membrane exists, there is a possibility of the occurrence of a negative region in the experimentally measured variational conductance, and this would give rise to a discontinuous manifestation in the membrane potential under the constant current condition. It is noteworthy that Adrian (1960) has found a similar discontinuity in the membrane potential for a KCl-loaded muscle fiber which was losing Cl and K into the chloride-free solution. Although the condition involved in our experiment is not analogous to Adrian's, the same mechanism might underlie both observations; and we may suppose, as Adrian, that the variational K conductance has a region of negativity at inside positive potentials.

Obviously, as Falk and Landa (1960) maintained, the primary cause for the production of the prolonged response in skeletal muscles is the elimination of the shunting effect of chloride ion. The present data showed that in the early phase of the response the effective resistance was small but gradually increased. One possible way of interpreting this response is to assume the presence of a negative conductance region in K channel. Thus, depolarization resulted in a decrease in K permeability which in turn leads to further depolarization, and these processes proceed in a regenerative manner. However, this interpretation seems to be incompatible with the facts that (*a*) at the early phase of the response the resistance was smaller than the resting value, and (*b*) in the K_2SO_4 solution a negative conductance region was not found at inside potentials more negative than -20 mv. The most plausible explanation would be to assume that depolarization leads to a small elevation of Na permeability even after application of tetrodotoxin. This small increase in Na permeability is thought to be insufficient for producing the spike potential and to be inactivated very slowly. The diminished resistance during the early phase is readily explained by assuming that at this phase the increased Na inward current is balanced by an increase of K outward current. During the maintained depolarization both factors are slowly inactivated, resulting in a gradual augmentation of the resistance. Finally, the inward Na current becomes insufficient for counteracting the potassium outward current, producing the relatively abrupt repolarization phase.

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