Delayed Rectification in the Transverse Tubules

Origin of the Late After-Potential in Frog Skeletal Muscle

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ABSTRACT   Tetanic stimulation of skeletal muscle fibers elicits a train of spikes followed by a long-lasting depolarization called the late after-potential (LAP). We have conducted experiments to determine the origin of the LAP. Isolated single muscle fibers were treated with a high potassium solution (5 mM or 10 mM K) followed by a sudden reduction of potassium concentration to 2.5 mM. This procedure produced a slow repolarization (K repolarization), which reflects a diffusional outflow of potassium from inside the lumen of the transverse tubular system (T system). Tetanic stimulation was then applied to the same fiber and the LAP was recorded. The time courses of K repolarization and LAP decay were compared and found to be roughly the same. This approximate equality held under various conditions that changed the time courses of both events over a wide range. Both K repolarization and the LAP became slower as fiber radius increased. These results suggest that LAP decay and K repolarization represent the same process. Thus, we conclude that the LAP is caused by potassium accumulation in the T system. A consequence of this conclusion is that delayed rectification channels exist in the T system. A rough estimation suggests that the density of delayed rectification channels is less in the T system than in the surface membrane.

INTRODUCTION

When muscle fibers are suddenly depolarized, the potassium permeability of the membrane is increased (Jenerick, 1953; Hodgkin and Horowicz, 1959a; Narahashi et al., 1960; Nakajima et al., 1962). This phenomenon is called "delayed rectification" (Hodgkin et al., 1949) or "K-activation" (Grundfest, 1961). When the depolarization is maintained for a few seconds, the increased K permeability diminishes and disappears completely (K inactivation) (Nakajima et al., 1962; Adrian et al., 1970a). In recent years knowledge about physiological properties of the transverse tubular system (T system) of muscle has greatly increased (Costantin, 1975). Yet it is not clear if delayed rectification channels are located

in the T system membrane (see Stanfield, 1973). This is the question that we have tried to answer in the experiments described here.

In 1964 Freygang et al. (1964a) studied the long-lasting depolarization which occurs after muscle fibers are stimulated tetanically. They called this depolarization the late after-potential (LAP) and gave an explanation for its origin. According to them, the LAP is a consequence of an accumulation of K ions in the T system. Thus, when the T-system membrane is depolarized repetitively by tetanic stimulation, the membrane undergoes a permeability increase to potassium ions, resulting in an accumulation of K ions in the T system. This idea of the origin of the LAP implies that delayed rectification channels exist in the T system, since otherwise an accumulation of a large amount of K ions, which could account for the magnitude of the LAP, would not be expected.

These considerations led us to undertake experiments which would test the validity of Freygang's K accumulation theory. In this way we hoped to gain more information about the membrane properties of the T system. The results of our experiments suggest that the K accumulation theory is correct and that delayed rectification channels exist in the T system. Preliminary accounts have appeared (Kirsch et al., 1975; Kirsch, 1976; Nakajima and Kirsch, 1977).

MATERIALS AND METHODS

The essential procedures of the main experiments were: (a) to isolate a single muscle fiber; (b) to record changes in membrane potential accompanying sudden changes of external potassium concentration [K]$_0$; and (c) to stimulate the fiber tetanically and record the late after-potential (LAP) from the same fiber.

Single twitch fibers isolated from the semitendinosus muscle of *Rana temporaria* were used throughout except for two experiments, in which the LAP was measured by using bundles consisting of a few fibers (see legends to Table II and Fig. 6). The condition of the frogs was critical for the success of the experiments (see below for the criteria of success). We spent considerable time and effort to acquire healthy frogs. It would have been wiser to use *Xenopus*, which can be kept in healthy condition in the laboratory. *Rana pipiens* was not suitable for the present experiments because the fiber size is rather small.

An experimental chamber similar to that described by Hodgkin and Horowicz (1959b) was used for the quick change of solutions. Solutions could be exchanged with a flush lasting about 1 s. We measured the speed of the solution exchange using an electrode potential as an indicator. At a flow rate of about 1.8 ml/s, the rate which we generally used, the exchange was complete within 0.2 s with a half-time of about 0.04 s. The isolated fiber was mounted in the chamber, and to minimize contraction the fiber was stretched to about 150% of the slack length, as was done by Freygang et al. (1964a). The membrane potential was measured as a potential difference between an intracellular microelectrode (3 M KCl, 5–30 MΩ) and an external microelectrode of low resistance (3 M KCl, 2–3 MΩ). When the LAP was being recorded, floating microelectrodes of the type described by Heistracher and Hunt (1969) were used, whereas when effects of quick solution changes were investigated, conventional rigid shank microelectrodes were used.

The fiber radius a_1, measured optically as described in Hodgkin and Nakajima (1972a), refers to the value in normal Ringer at slack length, whereas the radius a_2 is the value when the fiber is stretched by 50% in normal Ringer. Thus, $a_1 = a_2 \sqrt{1.5}$. The experiments were performed at room temperature (22–26°C).

Table I gives the composition of the main solutions containing normal K concentration (2.5 mM). High K solutions, not listed in Table I, were made by replacing Na with K on an

equimolar basis. The low chloride solutions were made by substituting methanesulfonate for chloride as in Table I, except that in a few early experiments sulfate solution (Hodgkin and Horowicz, 1959*b*) was used; data obtained in the sulfate solution (four fibers) were included only in Fig. 6*A*. The low Cl solution contained 2.7 mM calcium instead of the normal 1.8 mM (Table I). This was done in the hope of obtaining better and more stable fibers in the low chloride condition. But the normal Ca concentration would have been equally good. Dextran was obtained from Sigma Chemical Co. (St. Louis, Mo.) and had an average molecular weight of 17,700.

Criteria of Success

Only K repolarization and the LAP data which met the following criteria were included: (*a*) the fiber had an initial resting potential more negative than −80 mV; (*b*) the membrane potential did not drift more than 1 mV over the period from 15 to 30 s after the tetanic stimulation or after the sudden reduction of $[K]_0$; and (*c*) each of the tetanic stimuli (10 ms apart, 9–17 stimuli) elicited an action potential.

TABLE I
SOLUTIONS

Solution	K^+	Na^+	Ca^{2+}	Cl^-	$MeSO_3^-$	HPO_4^{2-}	$H_2PO_4^-$	Sucrose	Dextran
	mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*g/100 ml*
2.5 K, 120 Cl (normal)	2.5	120	1.8	121	0	2.15	0.85	0	
2.5 K, low Cl	2.5	120	2.7	5.4	117	2.15	0.85	0	
2.5 K, hypertonic (232)	2.5	120	1.8	121	0	2.15	0.85	232	
2.5 K, hypertonic (350)	2.5	120	1.8	121	0	2.15	0.85	350	
2.5 K, low Cl, Dextran	2.5	120	2.7	5.4	117	2.15	0.85	0	15

$MeSO_3$ = methanesulfonate.

RESULTS

Two Types of After-Potentials

Fig. 1 shows action potentials and after-potentials elicited by single or repetitive stimulation recorded from three isolated single fibers bathed in normal Ringer (A1, A2, A3), in low-Cl solution (B1, B2, B3), and in hypertonic solution (C1, C2, C3). A1, B1, and C1 show single spikes and the subsequent early after-potentials (EAP) recorded with an oscilloscope. The spike, after reaching the peak, quickly repolarizes to within 15–30 mV of the resting potential. Then the potential drifts back to the resting level with a half-time of 10–15 ms; this slow phase of repolarization is the early after-potential (EAP) (Freygang et al., 1964*a*). As mentioned by Persson (1963) and by Adrian and Peachey (1973), a hump frequently occurs at the beginning of EAP. This hump is clearly seen in B1.

In A2, B2, and C2 the fibers were stimulated repetitively. These records were obtained by using a chart recorder at low chart speed and high gain; thus, the spike potentials cannot be seen. The train of spike potentials was recorded with the oscilloscope (in parallel with the chart recorder) at lower gain and faster sweep speed, and is displayed in A3, B3, and C3. As illustrated by A2, B2, and C2, the later after-potential (LAP) is a long-lasting depolarization which persists after repetitive stimulation. In almost all the fibers we used, the initial part of the LAP was complicated by a hump of various sizes (very distinct in B2 and C2). This hump, which was also observed by Freygang et al. (1964*a*), is probably not a

movement artifact since it was present in nearly every record with a fairly con-
sistent time course and polarity. It was also present in hypertonic solutions
which largely inhibited muscle contraction.

Table II summarizes the characteristics of the EAP and LAP recorded from
isolated single fibers (see the legend for exception) in different solutions. The
average half-time of LAP decay in normal Ringer was 0.7 s. This value is about
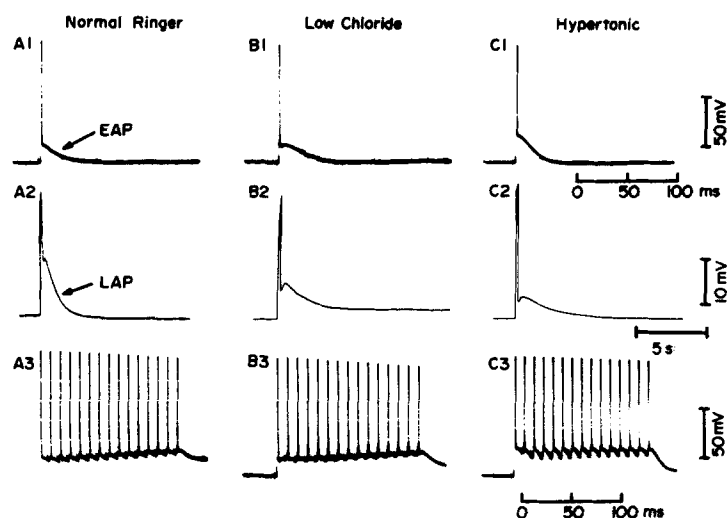twice that obtained by Freygang et al. (1964a), probably reflecting a difference in

FIGURE 1. Two types of after-potentials. A1, A2, A3: membrane potential in an
isolated single fiber (radius, a_1 = 59.5 μm) immersed in normal Ringer solution.
A1: oscilloscope record of a single action potential followed by an early after-
potential (EAP). A2: strip-chart record of action potentials elicited by tetanic
stimulation of 15 pulses at 10 ms intervals, followed by a late after-potential (LAP).
The spike potentials themselves are not seen. Note the difference in calibration
between A1 and A2. A3: same train of action potentials as in A2 recorded on the
oscilloscope to show individual spike potentials at fast sweep. B1, B2, B3: mem-
brane potential in another single fiber (radius, a_1 = 37 μm) immersed in low-
chloride 2.5 mM K solution. C1, C2, C3: membrane potential in another single fiber
(radius, a_1 = 61 μm) immersed in Ringer's solution made hypertonic by the
addition of 232 mM sucrose. Resting potential (average): A, −91 mV; B, −93 mV;
C, −91 mV. Room temperature was 23–24°C.

the fiber size. It will be shown presently that the time course of the LAP is
strongly influenced by fiber size. The average radius of our sample (Table II)
was fairly large (60.4 μm) because we used Rana temporaria and tended to isolate
large fibers. Freygang et al. (1964a) used surface fibers of the whole sartorius of
Rana pipiens; thus the size of their fibers could have been much smaller than
ours. (From our experience in isolating single muscle fibers, we know that fibers
from Rana pipiens are substantially smaller than those from Rana temporaria of
comparable body size. Costantin and Taylor [1973] made a similar observation.
Eisenberg and Gage [1969] gave 25–30 μm as the average radius of surface fibers
of the sartorius of Rana pipiens.)

In agreement with Freygang et al. (1964*b*), the results in Table II show that the half-time of LAP decay in low-Cl solution was about 50% larger than that in normal Ringer. Freygang et al. (1964*b*) also reported that hypertonic Ringer prolonged the decay of LAP about fivefold. Our results show that hypertonic solution produced much less effect on the time course of the LAP. Since we treated the fiber with this solution for only a short period of time (~5 min) a quantitative comparison is difficult. All in all, the results in Table II, which were obtained by using isolated single fibers, are in fair agreement with those previously obtained on surface fibers of the whole sartorius (Persson, 1963; Freygang et al., 1964*a, b*).

Comparison of LAP with K-Repolarization

The main purpose of the present experiments was to test the validity of Freygang et al.'s idea that the LAP is caused by potassium accumulation inside the

TABLE II

TWO TYPES OF AFTER-POTENTIALS IN MUSCLE FIBERS

Solution	EAP			LAP			No. of fibers
	Radius (a_1) mean ± SD	Decay half time mean ± SD	No. of fibers	Radius (a_1) mean ± SD	Decay half time mean ± SD	Peak amplitude mean ± SD	
	μm	ms		μm	s	mV	
Normal	60.4±4.8	10.4±2.0	6	60.4±10.3	0.7±0.3	11.8±8.0	11
Hypertonic (232 mM)	58.8±11.1	8.1±1.4	9	51.3±9.0	1.2±0.3	5.8±0.6	3
Hypertonic (350 mM)	51.9±20.2	9.0±1.5	5	47.7±19.9	1.2±1.1	6.1±2.8	3
Low chloride	55.5±17.0	14.7±4.9	21	57.2±15.9	1.1±0.5	14.7±6.0	33
Dextran (15%)	—	—		71.5±3.2	1.7±0.3	20.6±9.1	4

All data were obtained from isolated single fibers, except for two fibers in low-chloride solution: in the latter two cases we used small bundles of fibers, and isolated the fiber after the experiment to determine its radius. The peak amplitude of the LAP is the amplitude at $t = 0$, determined by extrapolation, as described in the text.

tubules. For this purpose we utilized the phenomenon of K-repolarization (Hodgkin and Horowicz, 1960; Nakajima et al., 1973). An example of K repolarization is seen in Fig. 2 A, in which the external potassium concentration $[K]_0$ was changed quickly from 2.5 mM to 5 mM and back to 2.5 mM, all the solutions having a low chloride concentration (5.4 mM). The speed of repolarization upon decreasing $[K]_0$ was slow with a half-time of about 1 s. Hodgkin and Horowicz (1960) gave an explanation, which was substantiated by Nakajima et al., (1973), for this slow phase of K repolarization. According to this explanation, when $[K]_0$ is suddenly reduced, it will take some time for the potassium ions, which are retained in the tubules, to diffuse out, and this diffusional outflow manifests itself as the slow repolarization. Thus, this phenomenon gives us a good way to test Freygang et al.'s idea about the origin of the LAP. Assuming that their idea is correct, the time course of LAP decay in any given fiber should be identical with the time course of K-repolarization in the same fiber.

Now returning to the explanation of Fig. 2, after record A was taken, the

microelectrode (a conventional type) was withdrawn, the fiber was again impaled with a floating electrode, and tetanic stimuli were delivered. This resulted in repetitive spike potentials shown in B2 (recorded with the oscilloscope), which were followed by an LAP illustrated in B1 (recorded with the chart recorder). As shown in records A and B1, there seems to be good agreement between the decay time course of the LAP and the time course of K repolarization.

Another example of this kind of experiment is illustrated in Fig. 3. In this case all the solutions contained 120 mM chloride. First, K repolarization was recorded by using a solution change from 2.5 mM K to 5 mM K and back to 2.5 mM K (A). Then the LAP was recorded together with the spike train (B1 and B2), and finally the K repolarization was again recorded (C), this time with the sequence: 2.5 mM K to 10 mM K and back to 2.5 mM K.
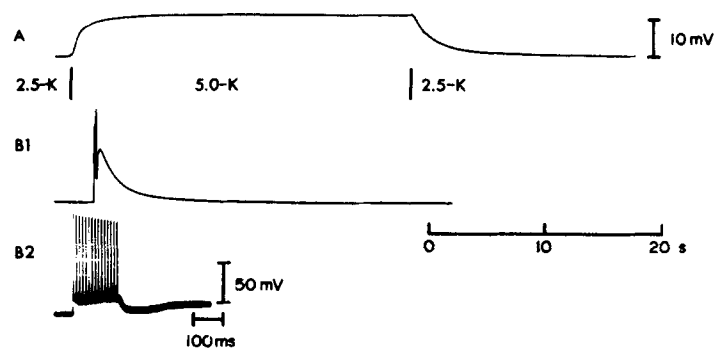

FIGURE 2.   K repolarization and the LAP recorded from an isolated single fiber in low chloride solution. A: strip chart record of the changes in membrane potential in response to changing $[K]_0$ from 2.5 mM to 5.0 mM and back to 2.5 mM, all in low chloride solution (5.4 mM). B1: strip-chart record of membrane potential in response to tetanic stimulation (15 pulses at 10-ms intervals). Spike potentials are obscured, but the LAP is clearly seen. B2: oscilloscope record of the same action potential train as in B1 showing the spike potentials. Resting potential was −80 to −91 mV. Radius (a_1) = 54.5 μm. Room temperature, 24°C.

One of the most obvious differences between the results obtained in low-Cl solution (Fig. 2) and those in 120 mM Cl solution (Fig. 3) is that the time courses of the LAP and K repolarization were faster in 120 mM Cl than in low-Cl solution (also see Table II). This is in agreement with the results of Hodgkin and Horowicz (1960) on K repolarization and with those of Freygang et al. (1964 b) on the LAP. According to Hodgkin and Horowicz (1960), KCl influx will occur from the tubular lumen into the myoplasm in the presence of chloride, and this influx will accelerate the disappearance of K during K repolarization. The same explanation could be applied equally well to the LAP if we assume that both the LAP and K repolarization represent the K accumulation.

Another point in Fig. 3 is that upon reduction of $[K]_0$ the potential did not return quite to the original level even 20 s after the solution had been changed. Part of this could be due to a KCl influx into the myoplasm during the treatment with the high K solution, thus disturbing the Donnan equilibrium (Hodgkin and

Horowicz, 1959*b*). But part is due to irreversible damage to the fiber. (We had the impression that the fibers tended to deteriorate more quickly in the present experiments than in the earlier ones of Nakajima et al. [1973]. The condition of the frogs might have been different, or the discrepancy could also be due to the fact that the present study was done in low K solutions, whereas the earlier one [Nakajima et al., 1973] was done in high K solution [40 mM and 165 mM]. In high K solutions the fiber would be less sensitive to leakage caused by damage.)

In Fig. 4 the time courses of K repolarization (circles) and LAP decay (crosses) are plotted on semilogarithmic coordinates for two fibers (A and B). Time zero
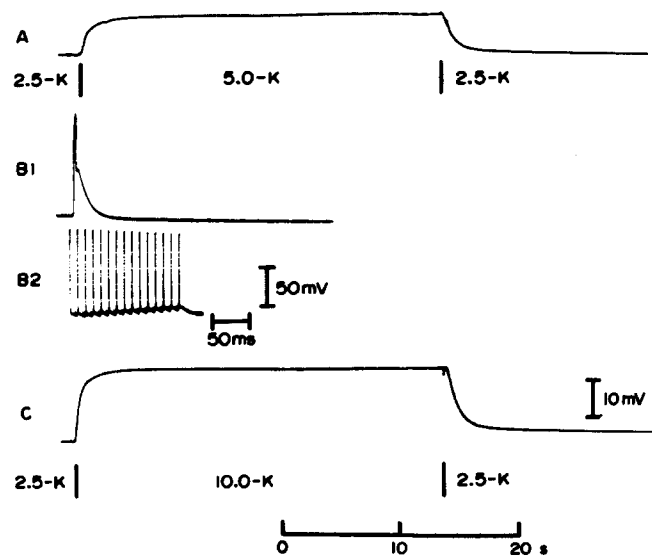

FIGURE 3.   K repolarization and the LAP recorded from a single fiber in normal chloride solution. A and C: strip-chart records of the changes in membrane potential in response to changing $[K]_0$ in the sequences: 2.5 mM–5.0 mM–2.5 mM (A) and 2.5 mM–10.0 mM–2.5 mM (C). B1: strip-chart record of membrane potential in response to tetanic stimulation (15 pulses at 10-ms intervals). Spike potentials are obscured but the LAP is clearly seen. B2: oscilloscope record of the same action potential train as in B1 showing the spike potentials. Resting potential was −80 to −91 mV. Radius (a_1) = 59.5 μm. Room temperature, 24°C.

was defined as the point at which the solution exchange started (i.e. the abrupt inflexion point of the membrane potential) or as the point where the tetanic stimulation was completed. In about two-thirds of the fibers data points could be fitted fairly well by a single straight line (Fig. 4 A), while in the remaining one-third more than one straight line seemed necessary (either downward or upward convex, Fig. 4 B). In these latter cases, since the inflection point usually became obvious at a very small depolarization (2 mV or less), little significance can be attached. For each record, the best straight line was fitted by eye using the following considerations: the initial part (time 0 to about 200–400 ms) was disregarded because in the case of K repolarization the solution exchange was not instantaneous and in the case of the LAP the initial part was obscured by the

hump (Fig. 1); the points below 1 mV depolarization were also ignored. From these straight lines the half-time of LAP and K repolarization was determined.

In Fig. 5 the half-time of K repolarization is plotted against the half-time of the LAP. For a given fiber the experiment was done either in 120 mM Cl or in low Cl, or in hypertonic solutions. In 43 isolated single fibers both K repolarization and the LAP were recorded from the same fiber, and the data satisfied the criteria decribed in Materials and Methods. In some fibers we used either 5 mM
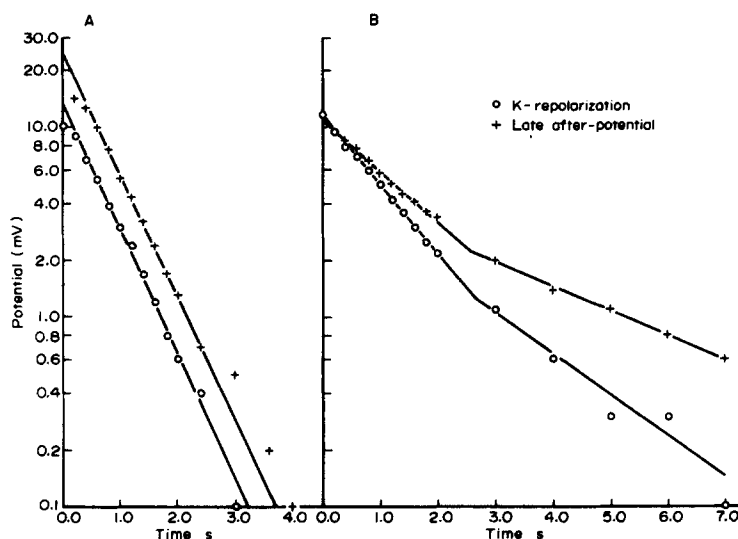

FIGURE 4. The time course of K repolarization and LAP decay. The abscissa is time in seconds. The ordinate is membrane depolarization measured from the final resting level (average membrane potential 15–30 s after stimulation) in millivolts (log scale). A: data obtained from a single fiber (radius, a_1 = 36 μm) in low-chloride solution. The half-time of K repolarization elicited by a change from 5.0 to 2.5 mM K, and the half-time of the decay of an LAP elicited by 14 spikes at 10-ms intervals were 0.42 s and 0.48 s, respectively. B: data obtained from another fiber (a_1 = 40.5 μm) also in low-chloride solution. K repolarization was recorded by changing from 5.0 to 2.5 mM K. The LAP was elicited by 14 spikes, 10-ms intervals. Room temperature was 25°C in both A and B.

K or 10 mM K for determining the time course of K repolarization. Each of these gave one datum point in Fig. 5. In other fibers we could record K repolarization twice using 5 mM K and using 10 mM K (Fig. 3 is an example). Each of these experiments gave two data points. We tried to isolate single fibers over a wide size range in order to obtain a broad range of K repolarization and LAP half-times. Fig. 5 shows that although there is some scatter of the data points, there is a fairly good correlation between the two quantities (the correlation coefficient r was 0.72). (The scatter probably comes from base-line drift. For example, in the data of Fig. 4 A a 1 mV error in determining the base line will produce an error of 30% in determining the half-time.) Not only is there a positive correlation, but the absolute values of these two half-times are roughly the same for each fiber

over this large range. However, closer inspection will reveal that when the K repolarization was determined with 10 mM K (open symbols of Fig. 5), the half-times of K repolarization were somewhat larger than the half-times of the LAP. Thus, most of the open symbols are located above the identity line in Fig. 5. The
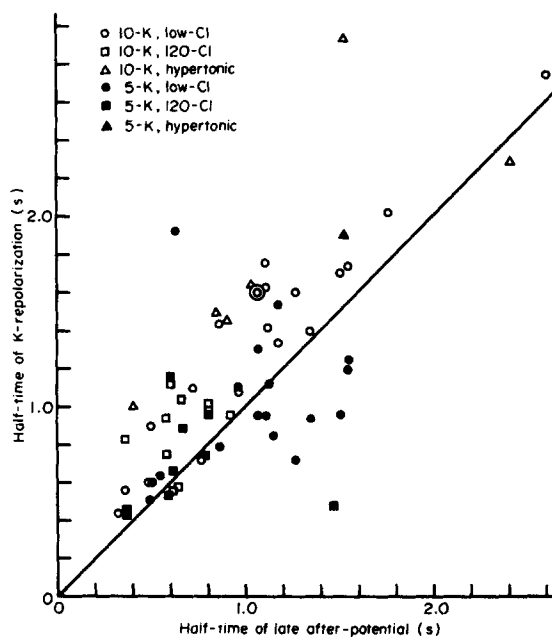

FIGURE 5.   Comparison of the half-time of LAP decay (abscissa) and the half-time of K repolarization (ordinate) in isolated fibers. In 23 fibers, either 10 mM K to 2.5 mM K, or 5 mM K to 2.5 mM K solution change was performed in addition to recording LAP. Each of these fibers gave one datum point. In 20 fibers, both 10 mM K to 2.5 mM K and 5 mM K to 2.5 mM K solution changes as well as recording LAP were performed. Each of these gave two data points. The line drawn through the data is the identity line. The double symbol indicates the coincidence of the data. *10-K, low-Cl* means, for example, that in these fibers K repolarization was recorded by 10 mM K to 2.5 mM K solution change, all under the low-Cl condition, and the LAP was recorded in the 2.5 mM K, low Cl solution. Among the experiments in hypertonic solutions, three data points (from three fibers) were obtained in 350 mM sucrose, and four data points (from three fibers) were obtained in 232 mM sucrose. All data are from experiments on isolated single fibers. In two experiments using 120 mM Cl solutions the radius of the isolated fiber was not measured. Room temperature was 23-25°C.

half-time of K repolarization using the 5 mM K to 2.5 mM K sequence (filled symbols) does not seem to be larger than that of the LAP. This is probably caused by the logarithmic relation between $[K]_0$ and the membrane potential. Because of this relationship, as the K concentration in the T system becomes higher and higher, the time course of K repolarization becomes slower and slower, even if the diffusion rate of K is the same. The fact that the open symbols

are located mainly above the identity line may indicate that the tetanic stimuli elevated K concentration in the tubules to some degree but not as much as 10 mM. (This point will be discussed in more detail later.) Thus, although there is some scatter in the data and although the time course of K repolarization is somewhat slower than that of the LAP, we regard the result of Fig. 5 as fairly good evidence for the K accumulation theory of LAP. Further support for the theory will be presented next.
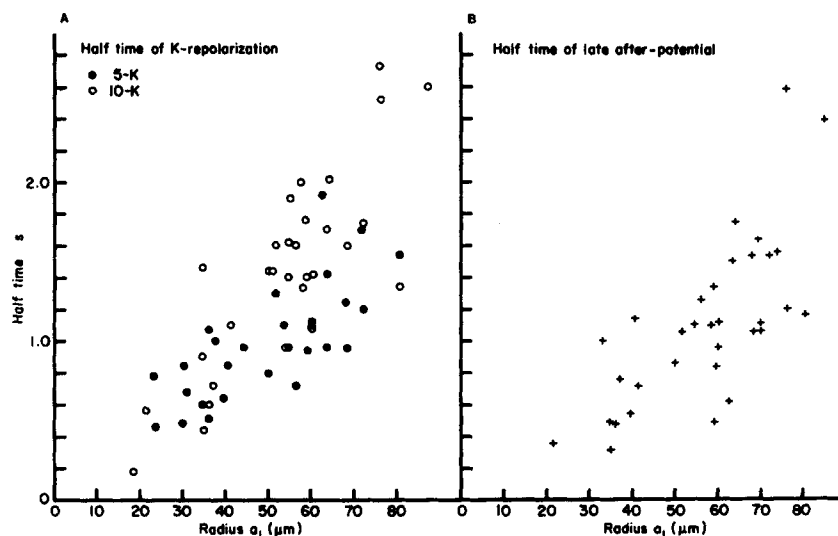

FIGURE 6. Variation of repolarization time course with fiber radius. A: half-time of K repolarization elicited by a change from 5.0 to 2.5 mM K (filled circles) or 10.0 to 2.5 mM K (open circles) plotted as a function of fiber radius (a_1). B: half-time of LAP decay produced by 12-17 spikes, plotted as a function of fiber radius (a_1). Data in both A and B were obtained from fibers immersed in low-chloride solution. Low-chloride solution was methanesulfonate substituted (Table I) except for four experiments in A, in which sulfate substitution was used. All data are from isolated single fibers except for two data points in B $(a_1 = 33.1$ and 59.6 μm): in the latter two cases bundles of a few fibers were used, and after the experiment the fiber in question was isolated and the radius was determined. Room temperature was 23-26°C.

Effects of Fiber Size

If both K repolarization and the LAP represent the diffusional outflow of tubular potassium, the time courses should be slower in larger fibers. We observed this to be the case. In Fig. 6 the half-time of K repolarization (A) and the half-time of the LAP (B) are plotted against fiber radius. All the data are from experiments performed in low chloride solutions. Part of the data in Fig. 6 is from the data included in Fig. 5, and part is from experiments in which only K repolarization or only the LAP was recorded. In A K repolarization was recorded by using the sequence either of 5 mM K to 2.5 mM K (filled circles) or of 10 mM K to 2.5 mM K (open circles). Obviously a positive correlation exists not only in the relationship between K repolarization and radius (in confirmation

with Hodgkin and Horowicz, 1960), but also in the relationship between the LAP and radius. These results support the idea of Freygang et al. (1964a) about the origin of the LAP. It is also obvious that, as was seen in Fig. 5, the time course of K repolarization using the 10 mM K to 2.5 mM K sequence (open circles in Fig. 6 A) is slower than that using the 5 mM K to 2.5 mM K sequence (filled circles in Fig. 6 A).

As discussed by Endo (1966), if the hindrance to the diffusion is located evenly along the T system, the half-time will be proportional to the second power of the radius (this comes from the diffusion equation), whereas if a limiting resistance is located only near the mouths of the T system, the half-time will be proportional to the radius (this comes from the fact that the volume of the T system is proportional to the cross-sectional area of the fiber, while the number of T-system mouths would increase in proportion to radius). The scatter of the data precludes us from deciding which relationship holds for the graphs in Fig. 6.

The Effect of High Viscosity

Caputo and DiPolo (1973) observed that the time course of K repolarization became slower in the presence of 15% Dextran. They thought that the high-viscosity medium retarded ionic movement, resulting in the slower K repolarization. Whatever the explanation of this finding is, since we are testing the identity of the origin of the LAP and of K repolarization, it was necessary to see whether Dextran produces the same effect on the LAP.

Fig. 7 illustrates the effects of the addition of Dextran on the LAP recorded from an isolated fiber. In low-Cl solution the half-time of LAP decay was 1.1 s. After changing to high viscosity solution and allowing 12 min for equilibration, the half-time became 1.3 s. The average increase in the half-time of the LAP was 41% (four fibers, ranging from 12% to 85%).

The addition of large molecules is expected to result in a reduction of mobilities of small ions by lengthening the effective paths of the moving ions, although the extent of the reduction would not be as much as Stokes's law predicts (p. 307, Robinson and Stokes, 1965). We measured the effect of Dextran on viscosity (with an Ostwald viscometer) and on conductivity of the solution, and found that the addition of 15% Dextran to the low-Cl solution increased viscosity 4.3-fold and decreased conductivity by 35% at 24°C (a small increase in conductance produced by Dextran itself was subtracted). Thus, the observed prolongation of the LAP could be explained by a retardation of K diffusion as proposed by Caputo and DiPolo (1973) in their K repolarization experiments. However, since 15% Dextran could substantially increase the osmotic pressure (Tanford, 1966), we are not sure whether diffusion retardation is a sufficient explanation. Although the effect is too small to merit emphasis, the results are not in disagreement with the notion that the origin of LAP and the origin of K repolarization are the same.

Analysis of the Time Course of K-Repolarization and LAP

In Fig. 4 we have shown that the time course of K repolarization and decay of the LAP are approximated by a single exponential until the magnitude becomes

very small. An analysis of the time courses was made to see whether this single exponential curve could be expected from a diffusional process.

To simplify the problem a single muscle fiber is treated as a circular cylinder. If the tubular lumen is preloaded with potassium at a concentration C_1, and the external concentration is changed suddenly to C_0, the average degree of saturation (y) will be (Hill, 1928):

$$y = \frac{\bar{C} - C_0}{C_1 - C_0} = 4 \sum_{n=1}^{\infty} \left\{ \frac{\exp(-T\alpha_n^2)}{\alpha_n^2} \right\}, \tag{1}$$
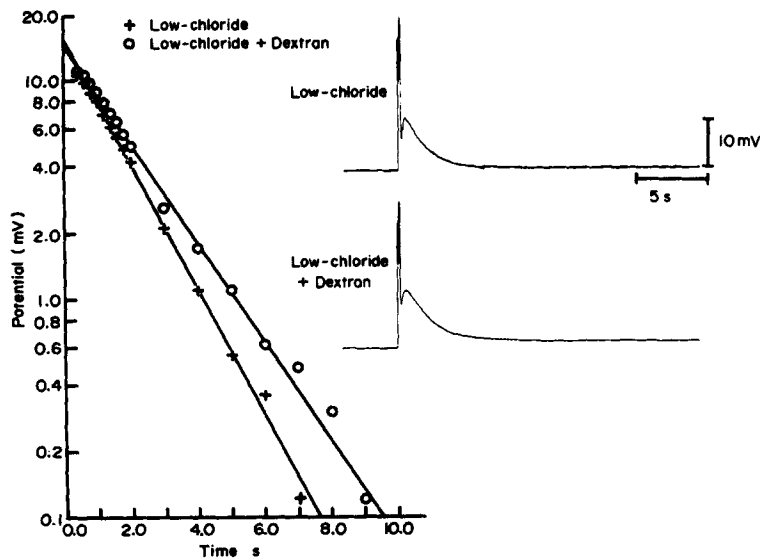

FIGURE 7.   The effect of Dextran on LAP time course. Right-hand traces are strip-chart records of LAPs before (upper) and after (lower) addition of 15% Dextran to the external solution. The fiber was treated in the Dextran solution for about 12 min. LAP decay half-times determined from the semilogarithmic plot on the left hand side were 1.1 s in low-chloride solution and 1.3 s in low-chloride + Dextran. a_1 = 70 μm. Room temperature, 23°C.

where T is normalized time and

$$T = \frac{D't}{a^2}. \tag{2}$$

Also, $\bar{C}$ = average concentration of K in the T system, D' = apparent diffusion constant of K, a = fiber radius, t = time, and α_n = nth root of the Bessel function of the first kind and zero order. Ignoring for the moment the presence of the surface membrane, the membrane potential (V) of the fiber is approximated by the average Nernst potential of the T system (in mV), namely:

$$V = 58 \log \frac{\bar{C}}{[K]_i}, \tag{3}$$

where $[K]_i$ is the internal K concentration. If $[K]_0$ is suddenly decreased from 5 mM to 2.5 mM, then $C_1 = 5$ and $C_0 = 2.5$, and the time course of K repolarization (ΔV) will be given by:

$$\Delta V = 58 \left(\log \frac{\bar{C}}{[K]_i} - \log \frac{2.5}{[K]_i} \right) = 58 \log \frac{\bar{C}}{2.5}. \tag{4}$$

From Eq. (1) and (4)

$$\Delta V = 58 \log (y + 1). \tag{5}$$

In Fig. 8 the time course of y (curve A) and the time course of $\log (y + 1)$ (curve
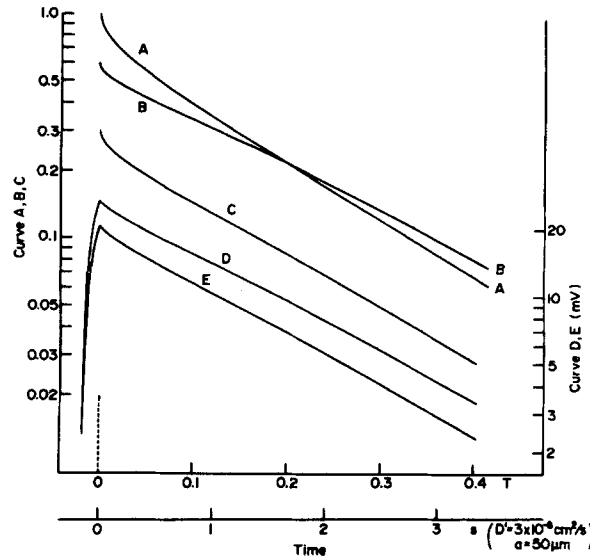

FIGURE 8. Semilogarithmic plot of the following functions. Curve A $= y$. Curve B $= \log (3y + 1)$. Curve C $= \log (y + 1)$. Curve D $=$ model of the LAP assuming each impulse produces an increment of potassium concentration $\Delta[K]$ of 0.366 mM, and 15 impulses 10 ms apart were applied. Curve E $=$ same as curve D, except that $\Delta[K]$ was 0.248 mM. See text for details.

C) were plotted semilogarithmically. In the latter case it is equivalent to plot log {log $(y + 1)$} on linear coordinates. The abscissa includes normalized time T as well as the scale for real time (in seconds), assuming that the apparent diffusion constant $D' = 3 \times 10^{-6}$ cm^2/s and $a = 50$ μm. It is obvious from curve C that ΔV $(= 58 \log [y + 1])$, when plotted semilogarithmically, becomes approximately linear a few hundred milliseconds after the solution change. This is the justification for determining half-times from semilogarithmic plots as in Fig. 4.

We have mentioned that the time course of K repolarization is slower after a 10 mM K to 2.5 mM K exchange than after a 5 mM K to 2.5 mM K exchange. The following is the quantitative explanation for this. If the solution change is from 10 mM K to 2.5 mM K, then $C_1 = 10$ mM and $C_0 = 2.5$ mM. Thus, from Eq. (1) and (4),

$$\Delta V = 58 \log (3y + 1). \tag{6}$$

The semilogarithmic plot of log $(3y + 1)$ against T is curve B of Fig. 8. It is apparent that curve B is less steep than curve C $(= \log [y + 1])$. Although this is certainly one important factor responsible for the slowness of K repolarization when 10 mM K is used, we are not sure that it is a sufficient explanation. It is possible that anomalous rectification in the tubular membrane also plays a role.

To calculate the zero-current potential of the T system by Eq. (3) is an approximation. The tubular potential would be nearly uniform during the LAP since the length constant of the T system is larger than the radius (Adrian et al., 1969). However, this potential is not given by Eq. (3). A better way is to calculate the average of the Nernst potentials for all locations of T system with a weight factor proportional to the local membrane conductance, which varies with the local potassium concentration. A similar problem was discussed in a previous paper dealing with the diffusion process during K repolarization (Nakajima et al., 1975). A more general theory describing a nonhomogeneous tubular model such as that developed by Barry and Adrian (1973) would give a more precise time course of the K repolarization and the LAP.

The Amount of K Released into the Tubules during Action Potential

If we accept the idea that the LAP represents K accumulation in the tubules, we can estimate the amount of net exit of K ions during the action potential. From all the measurements of the LAP in low chloride solution shown in Fig. 6 B, we have chosen only the fibers having a resting potential more negative than −87 mV (Table III). The average resting potential of this sample (just before and after measurement of the LAP) was −92.5 mV. This arbitrary selection was necessary to convert the membrane potential V into $[K]_0$ using the relationship:

$$V = 58 \log \left(\frac{[K]_0 + 0.01 [Na]_0}{140} \right). \tag{7}$$

This equation was shown by Hodgkin and Horowicz (1959b) to hold for fibers whose average resting potential was about −92 mV (Fig. 5 of their paper).

The time course of the LAP was plotted semilogarithmically and the magnitude of the LAP at time zero was determined by the linear extrapolation as explained in relation to Fig. 4. These values (column IV, Table III) were converted into potassium concentration [K] in the T system by the use of Eq. (7), assuming that the recorded potentials were the average potential of the T system and disregarding the contribution from the surface membrane. The increment of potassium concentration $\Delta[K]$ was calculated by $\Delta[K] = [K] -2.5$ (column V). For each fiber we calculated the volume of the T system from morphometric data (namely, 0.32% of fiber volume; Peachey 1965; Mobley and Eisenberg, 1975) and from the fact that the T system swells by 70% in low chloride solutions (Freygang et al., 1964b). The volume of the T system was multiplied by $\Delta[K]$ to give the net release of K ions during the action potential (listed in column VII). The average was 3.5 pmol/spike·cm² (referred to the surface area). This tenta-

TABLE III

POTASSIUM ACCUMULATION IN THE T SYSTEM

I Fiber	II a_2	III Resting potential	IV LAP at $t = 0$	V Δ[K] (tentative)	VI No. of spikes	VII K release* (tentative)	VIII K release* (corrected)	IX Δ[K] (corrected)
	μm	*mV*	*mV*	*mM/tetanus*		*pmol/spike·cm²*	*pmol/spike·cm²*	*mM/spike*
S-60	42.0	−87	4.9	0.7	13	0.61		
S-70	45.7	−95	20.6	4.2	15	3.4		
S-81	58.8	−92	15.5	2.8	11	4.0		
S-91	55.9	−88	21.3	4.4	15	4.4		
S-94	32.2	−90	11.0	1.8	15	1.0		
S-112	49.0	−94	20.2	4.1	15	3.6		
S-117	28.6	−93	17.7	3.4	15	1.7		
S-182	69.4	−89	12.6	2.2	16	2.6		
S-202	60.4	−89	20.2	4.1	15	4.5		
S-210	57.1	−99	19.9	4.0	15	4.1		
S-211	57.1	−98	18.4	3.6	13	4.3		
S-215	62.4	−96	30.6	7.9	16	8.3		
Mean	51.6	−92.5	17.7	3.6	14.5	3.5	5.2	0.366

Fibers that had a resting potential more negative than −87 mV at the time of recording LAP were selected from the sample of Fig. 6 B.
* Referred to surface area.
Room temperature was 22-25°C.

tive value of K release was corrected for the sources of errors described below.[1] The corrected value of K release became 5.2 pmol/spike·cm² (referred to surface membrane area), and Δ[K] became 0.366 mM/spike (columns VIII, IX).

[1] First, the tentative value of potassium release was corrected for the fact that the linear extrapolation of the semilogarithmic plot of LAP would not give a correct value of the true magnitude of depolarization at $t = 0$, since the time course predicted by the diffusion process (curves B and C of Fig. 8) has a rapidly declining initial part. Also, some potassium ions would already have escaped from the T system during the period of tetanic stimulation lasting about 150 ms. In order to estimate the errors due to these sources we have made a model LAP. As shown in Table III, the average of the tentative values of Δ[K] is 3.6 mM (column V) per 14.5 impulses (column VI), namely 0.248 mM/impulse. We assumed that there was an instantaneous [K] increment of 0.248 mM at the moment of each action potential, and that this increment was dissipated according to the diffusion equation (Eq. [1]). The increment was superimposed 15 times at 10-ms intervals. The time course of Δ[K] thus derived was converted into potential changes by the Nernst equation, and is shown by curve E of Fig. 8. From curve E the error that would be produced in calculating Δ[K] by the extrapolation procedure was estimated. The real value of Δ[K] as well as the amount of K release was found to be 32% larger than the tentative figure.

Another source of error is that the recorded LAP does not represent the average potential of the T system since it is short-circuited by the conductance of the surface membrane. This short-circuit factor was calculated by assuming that conductance is proportional to membrane area, giving due regard to the presence of anomalous rectification. Our calculation showed that neglecting this short-circuiting factor would result in an underestimate of Δ[K] by 12%.

Thus the corrected amount of K exit is 3.5 × 1.32 × 1.12 = 5.2 pmol/spike·cm² (referred to surface membrane area), and Δ[K] becomes 0.366 mM/spike. We have computed the corrected time course of the LAP using Δ[K] per impulse = 0.366 mM rather than 0.248 mM. The resulting potential change during and after the tetanus (15 pulses at 10-ms intervals) is illustrated by curve D of Fig. 8. If we short-circuit the potential of curve D by the surface conductance, it will represent the time course of the model LAP.

Density of the Delayed Rectification Channels in the T System

The isotope tracer experiments by Hodgkin and Horowicz (1959a) indicated that the net K release during an action potential in single muscle fibers (radius = 50 μm) was 9.6 pmol/spike·cm² (referred to surface area) at 20.6°C. This figure represents the sum of the net K exit from both the outer surface and tubular membranes. Since our figure of 5.2 pmol/spike·cm² (referred to surface area, average temperature was 23°C) represents the net K exit in the T system, the outer surface component will be 9.6 − 5.2 = 4.4 pmol/spike·cm² (referred to surface area). Because the area of the T system is about six times larger than the surface area in a fiber of this size, the net K exit in the T system is 5.2/6 = 0.87 pmol/spike·cm² (referred to the area of T system membrane). This means that the K-current density through the T-system membrane is much less than that through the surface, the ratio of the densities being 0.87: 4.4 ≑ 1:5. If we assume that the tubular action potential has roughly the same shape as the surface action potential, we can conclude that the density of delayed rectification channels in the T system is less than that in the surface, the ratio being roughly 1:5. It is interesting to note that measurements of tetrodotoxin binding suggest that the density of the Na channels in the T system is ~25% of that in the surface membrane (Jaimovich et al., 1976). However, since our estimation is based on data which are subject to large errors,[2] we do not strongly hold the conclusion of this section.

Since the density of the delayed rectification channels seem to be sparse in the T system, it is pertinent to inquire whether the LAP would still occur if delayed

[2] The sources of the errors and uncertainties are the following. (a) The above estimation was based on the pure diffusional model without extra-resistance (access resistance) at the mouths of the T system. If there were a large access resistance, the time course of K concentration change in the T system would follow a simple exponential, and the amount of K exit into the T system is estimated to be 4.1 pmol/spike·cm² (referred to surface area). If one uses this value, the ratio of the density of the K current (or the density of delayed rectification channels) in the T system to that in the surface will become 1:8. However, this would be unlikely in view of the results of Endo (1966), Schneider (1970), and Valdiosera et al. (1974). (b) The conclusion about the density of the delayed rectification channels (not the conclusion about the K-current densities) was based on the unproven assumption that the wave-form of the tubular action potential is roughly the same as that in the surface. Relevant to this point is the computation by Adrian and Peachey (1973) which showed that the shape of the tubular action potential is not very different from the surface action potential when no access resistance is assigned, whereas it is somewhat distorted when there is a large access resistance. According to Dr. John A. Connor (personal communication), a computation using the squid axon model (Connor et al., 1977) indicated that if $\bar{g}_{Na}$ and $\bar{g}_K$ (sodium and potassium limiting conductances) were reduced to 20% of the normal values, the total potassium exit during the action potential would be reduced to 28%. (c) A large error would come from the uncertainty in the value of the T-system volume: we assumed that the T system swells by 70% (Freygang et al., 1964b), but the composition of our low-chloride solution was different from theirs (our low-chloride solution had 5.4 mM Cl and was methanesulfonate substituted; that of Freygang et al. had 30 mM Cl, sulfate substituted). If the T-system volume were actually 30% less than the value we used, the ratio of delayed rectification channel densities would become about 1:8, and if the T-system volume were 30% more, the density ratio would become 1:2.5. (d) Another source of uncertainty is that the estimate of the K current through the surface membrane is based on the difference of two independent sets of data: one, that of Hodgkin and Horowicz (1959a) and the other, our present data. If both values are in error by 20%, the ratio of the densities will range from 1:11 to 1:1.4. And if they both are in error by 30%, then the surface component of K current could become zero.

rectification channels were absent in the T system. Under this condition the charge would be dissipated mainly through the K channels in the surface membrane during the repolarizing phase of action potential, and thus we can assume that the action potential in the T system would not be as prolonged as one would expect. Thus the following calculation assumes that the T-system action potential has a wave shape similar to the normal action potential. From the data of Hodgkin and Nakajima (1972b), the resting conductance of the T-system is considered to be ~0.03 mmho/cm² (referred to the T-system area), which would be mostly due to K conductance (Eisenberg and Gage's [1969] value is 0.01 mmho/cm², referred to the T-system area). The ionic current flowing through this resting conductance during a single action potential and part of the early after-potential was estimated by measuring the area of the action potential multiplied by the resting conductance. The value obtained was equivalent to 0.3 pmol/spike·cm² (referred to surface area). With this small amount of K efflux, the magnitude of the LAP caused by the tetanus (15 spikes) would be only about 1.5 mV. This may be an underestimate since the action potential may be prolonged in the T system, whereas it may be an overestimate since we did not take into account the presence of anomalous rectification in the T system.

Apparent Diffusion Constant of K in the T System

The curves in Fig. 8 provide a simple way to calculate the approximate value of the apparent diffusion constant D' of K ions in the T system bathed in low chloride solution. Curve C, which roughly represents the time course of K repolarization after a solution change from 5 mM K to 2.5 mM K, indicates that the half-time of the late linear part is $T = 0.13$. If, for example, the half-time of the linear part of K repolarization of a fiber is 1.25 s and a_2 is 55.4 μm, we find from Eq. (2) that $D' = 0.13 \times (55.4)^2/1.25 = 3.2 \times 10^{-6}$ cm²/s. Our model of the LAP (curve D) shows that the late linear part has a half-time of $T = 0.135$, and thus D' can be similarly calculated from a semilogarithmic plot of the LAP. (For similar treatments see Keynes, 1954.)

Table IV summarizes the values of D' calculated from several groups of data in the present experiments. The values range from 2.1×10^{-6} to 2.9×10^{-6} cm²/s. This simple method of estimating D' using Eq. (1) and (2) has several sources of errors, and the values obtained should be regarded as first-order approximations (for a discussion of the errors see Nakajima et al., 1975). Nevertheless, the values obtained are in good agreement with those calculated by Almers (1972) for recovery from "creep" induced by hyperpolarizing current. (His value of diffusion constant, when converted into our nomenclature, $D' = 2.85$ cm²/s.) But they are larger than D' calculated from the time course of twitch recovery (1 $\times 10^{-6}$ cm²/s) or from the time course of K repolarization upon 165 mM K to 40 mM K solution exchange ($0.9 \sim 1.5 \times 10^{-6}$ cm²/s) (Nakajima et al., 1975). In the former case the discrepancy may be explained by the fact that D' was calculated from the diffusion of Na ions into a Tris-loaded fiber (diffusion of Tris should be slower). In the latter case the discrepancy cannot be explained by the differences in the method of calculating the diffusion constant. The difference

may suggest that the structure of the T system changes in high-K solutions.[3]

The values of D' obtained in the present study are about sixfold less than the diffusion constant of K in aqueous solution. Thus, the tortuosity factor of the T system involving K diffusion in low-chloride solution is about 1/6. For further discussion of the tortuosity factor (electrical and diffusional) see Adrian et al. (1969), Barry and Adrian (1973), Costantin (1975), Nakajima et al. (1975), and Mathias et al. (1977).

TABLE IV

APPARENT DIFFUSION CONSTANT OF POTASSIUM IN THE T SYSTEM IN LOW CHLORIDE

Radius a_2	Sample no.	D'	Method	Data from
μm	n	$10^{-6}\ cm^2/s$		
46.7±2.3	33	2.9±0.21	LAP	Fig. 6 B
40.8±2.5	28	2.3±0.19	K repolarization (5 mM)	Filled symbols in Fig. 6 A
44.3±2.5	30	2.1±0.14	K repolarization (10 mM)	Open symbols in Fig. 6 A
45.9±2.6	17	2.9±0.26	LAP	Filled circles in Fig. 5
45.9±2.6	17	2.8±0.20	K repolarization (5 mM)	Filled circles in Fig. 5

Values are mean ±SEM. K-repolarization (5 mM) means that the analysis was done using the time course of the K repolarization upon changing from 5 mM K to 2.5 mM K. See text for the method of calculating the apparent diffusion constant D'.

DISCUSSION

To recapitulate the main results: (a) the LAP and K repolarization were recorded from the same single muscle fiber. The speeds of the decay of both events were approximately equal. This rough equality held under various conditions which changed the time courses of both events over a wide range. (b) Both the time courses of LAP decay and the K repolarization became slower as fiber radius increased. Taken together the results support the K-accumulation theory of LAP origin, which was proposed by Freygang et al. (1964a) on the basis of the Adrian-Freygang model (1962).

Our results, which are essentially an observation of parallelism, do not constitute a proof of the theory, and other explanations cannot be eliminated altogether. For example, it is still possible that the LAP is caused mainly by a permeability change of the membrane. But the likelihood of this alternative explanation seems to be low, because it would lead to the peculiar conclusion that the time course of the permeability change roughly coincides with the time course of K diffusion from the tubules for each fiber and that both events change almost concomitantly as fiber size or external solution is altered. This

[3] The half-time of K repolarization upon exchange of 165 mM K to 40 mM K was reported to be 2.3 s (a_2 = 45.2 μm) by Nakajima et al. (1973). On the other hand, the half-time of K repolarization upon 10 mM K to 2.5 mM K exchange was 1.4 s (a_2 = 44.3 μm) in the present experiments (Fig. 6 A, open symbols). This large discrepancy cannot be easily explained. The time course of K repolarization upon exchange from 165 mM K to 40 mM K should follow the equation, $\Delta V = 58 \log (3.13y + 1)$, which is hardly different from the case of 10 mM K to 2.5 mM K, i.e. $\Delta V = 58 \log (3y + 1)$. Thus, the apparently slower diffusion of K in the T system when the fiber is treated with a high-K solution seems to be genuine.

conclusion is somewhat difficult to accept now, since this kind of relation has never been shown in any excitable tissue.

A corollary of the K accumulation theory for the LAP is that delayed rectification channels exist in the T system. However, our estimate suggests that the density of the K current and delayed rectification channels in the tubules may be less than of those in the surface, the ratio of the densities being roughly one to five. The error associated with this estimation is large and the extent of the error has already been described.

Another point to be discussed is the fact that Adrian et al. (1970b), in their voltage clamp experiments using long-lasting polarizations, described a small, very slowly changing component of K conductance which could account for the LAP without the need for postulating potassium accumulation. Thus, one may wonder how this experimental fact can be accommodated into the present picture of the LAP. The time constant of the K conductance change that Adrian et al. (1970b) described is about 0.5 s at 3°C. Although the rate of this K conductance change at higher temperatures was not analyzed fully, it is quite possible that the time constant becomes on the order of 100 ms at room temperature. The present results have shown that the time constant (not half-time) of the LAP is on the order of 1 s, and thus the two kinds of after-potentials could be completely different phenomena. As already described, the initial several hundred milliseconds of our LAP is obscured by a hump. This hump could be somehow connected with the slow conductance changes of Adrian et al. (1970b).

Thus, our present view is that the LAP at room temperature is primarily determined by K accumulation, possibly modified by slow permeability changes. As the temperature drops, since conductance changes usually have a larger Q_{10} than diffusional processes, the time course of the LAP would be more and more influenced by events related to permeability changes. And at 3°C the phenomenon due to the K conductance changes may overshadow the K accumulation, as was observed by Adrian et al. (1970b). By the same token, even at room temperature the LAP of a small fiber could be more strongly influenced by slow permeability changes.

REFERENCES

ADRIAN, R. H., W. K. CHANDLER, and A. L. HODGKIN. 1969. The kinetics of mechanical activation in frog muscle. *J. Physiol. (Lond.).* **204:**207-230.

ADRIAN, R. H., W. K. CHANDLER, and A. L. HODGKIN. 1970a. Voltage clamp experiments in striated muscle fibres. *J. Physiol. (Lond.).* **208:**607-644.

ADRIAN, R. H., W. K. CHANDLER, and A. L. HODGKIN. 1970b. Slow changes in potassium permeability in skeletal muscle. *J. Physiol. (Lond.).* **208:**645-668.

ADRIAN, R. H., and W. H. FREYGANG, 1962. The potassium and chloride conductance of frog muscle membrane. *J. Physiol. (Lond.).* **163:**61-103.

ADRIAN, R. H., and L. D. PEACHEY. 1973. Reconstruction of the action potential of frog sartorius muscle. *J. Physiol. (Lond.).* **235:**103-131.

ALMERS, W. 1972. The decline of potassium permeability during extreme hyperpolarization in frog skeletal muscle. *J. Physiol. (Lond.).* **225:**57-83.

BARRY, P. H., and R. H. ADRIAN. 1973. Slow conductance changes due to potassium depletion in the transverse tubules of frog muscle fibers during hyperpolarizing pulses. *J. Membr. Biol.* **14:**243-292.

CAPUTO, C., and R. DiPOLO. 1973. Ionic diffusion delays in the transverse tubules of frog twitch muscle fibres. *J. Physiol. (Lond.).* **229:**547-557.

CONNOR, J. A., D. WALTER, and R. McKOWN. 1977. Neural repetitive firing: modifications of the Hodgkin-Huxley axon suggested by experimental results from crustacean axons. *Biophys. J.* **18:**81-102.

COSTANTIN, L. L. 1975. Contractile activation in skeletal muscle. *Prog. Biophys. Mol. Biol.* **29:**197-224.

COSTANTIN, L. L., and S. R. TAYLOR. 1973. Graded activation in frog muscle fibers. *J. Gen. Physiol.* **61:**424-443.

EISENBERG, R. S., and P. W. GAGE. 1969. Ionic conductances of the surface and transverse tubular membranes of frog sartorius fibers. *J. Gen. Physiol.* **53:**279-297.

ENDO, M. 1966. Entry of fluorescent dyes into the sarcotubular system of the frog muscle. *J. Physiol. (Lond.).* **185:**224-238.

FREYGANG, W. H., D. A. GOLDSTEIN, and D. C. HELLAM. 1964a. The after-potential that follows trains of impulses in frog muscle fibers. *J. Gen. Physiol.* **47:**929-952.

FREYGANG, W. H., D. A. GOLDSTEIN, D. C. HELLAM, and L. D. PEACHEY. 1964b. The relation between the late after-potential and the size of the transverse tubular system of frog muscle. *J. Gen. Physiol.* **48:**235-263.

GRUNDFEST, H. 1961. Ionic mechanisms in electrogenesis. *Ann. N. Y. Acad. Sci.* **94:**405-457.

HEISTRACHER, P., and C. C. HUNT. 1969. The relation of membrane changes to contraction in twitch muscle fibres. *J. Physiol. (Lond.).* **201:**589-611.

HILL, A. V. 1928. The diffusion of oxygen and lactic acid through tissues. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **104:**39-96.

HODGKIN, A. L., and P. HOROWICZ. 1959a. Movements of Na and K in single muscle fibres. *J. Physiol. (Lond.).* **145:**405-432.

HODGKIN, A. L., and P. HOROWICZ. 1959b. The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol. (Lond.).* **148:**127-160.

HODGKIN, A. L., and P. HOROWICZ. 1960. The effects of sudden changes in ionic concentration on the membrane potential of single muscle fibres. *J. Physiol. (Lond.).* **153:**370-385.

HODGKIN, A. L., A. F. HUXLEY, and B. KATZ. 1949. Ionic currents underlying activity in the giant axon of the squid. *Arch Sci. Physiol.* **3:**129-150.

HODGKIN, A. L., and S. NAKAJIMA. 1972a. The effect of diameter on the electrical constants of frog skeletal muscle fibres. *J. Physiol. (Lond.).* **221:**105-120.

HODGKIN, A. L., and S. NAKAJIMA. 1972b. Analysis of the membrane capacity in frog muscle. *J. Physiol. (Lond.).* **221:**121-136.

JAIMOVICH, E., R. A. VENOSA, P. SHRAGER, and P. HOROWICZ. 1976. Density and

distribution of tetrodotoxin receptors in normal and detubulated frog sartorius muscle. *J. Gen. Physiol.* **67**:399–416.

JENERICK, H. P. 1953. Muscle membrane potential, resistance and external potassium chloride. *J. Cell Comp. Physiol.* **42**:427–448.

KEYNES, R. D. 1954. The ionic fluxes in frog muscle. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **142**:359–382.

KIRSCH, G. E. 1976. Potassium permeability in the transverse tubule system and latency relaxation: a study of excitation-contraction coupling. Ph.D. thesis. Purdue University, West Lafayette, Ind.

KIRSCH, G. E., R. A. NICHOLS, and S. NAKAJIMA. 1975. The origin of the late after-potential in frog skeletal muscle. *Fed. Proc.* **34**:467.

MATHIAS, R. T., R. S. EISENBERG, and R. VALDIOSERA. 1977. Electrical properties of frog skeletal fibers interpreted with a mesh model of the tubular system. *Biophys. J.* **17**:57–93.

MOBLEY, R. A., and B. R. EISENBERG. 1975. Size of components in frog skeletal muscle measured by methods of stereology. *J. Gen. Physiol.* **66**:31–45.

NAKAJIMA, S., S. IWASAKI, and K. OBATA. 1962. Delayed rectification and anomalous rectification in frog's skeletal muscle membrane. *J. Gen. Physiol.* **46**:97–115.

NAKAJIMA, S., and G. E. KIRSCH. 1977. Delayed rectification in the transverse tubules of skeletal muscle. Abstracts of the 27th International Congress of Physiological Sciences, Paris. In press.

NAKAJIMA, S., Y. NAKAJIMA, and J. BASTIAN. 1975. Effects of sudden changes in external sodium concentration on twitch tension in isolated muscle fibers. *J. Gen. Physiol.* **65**:459–482.

NAKAJIMA, S., Y. NAKAJIMA, and L. D. PEACHEY. 1973. Speed of repolarization and morphology of glycerol-treated frog muscle fibres. *J. Physiol. (Lond.).* **234**:465–480.

NARAHASHI, T., T. DEGUCHI, N. URAKAWA, and Y. OHKUBO. 1960. Stabilization and rectification of muscle fiber membrane by tetrodotoxin. *Am. J. Physiol.* **198**:934–938.

PEACHEY, L. D. 1965. The sarcoplasmic reticulum and transverse tubules of the frog's sartorius. *J. Cell Biol.* **25**:209–231.

PERSSON, A. 1963. The negative after-potential in frog skeletal muscle fibres. *Acta Physiol. Scand.* **58** (Suppl. 205): 2-32.

ROBINSON, R. A., and R. H. STOKES. 1965. Electrolyte Solutions. Butterworth & Co., (Publishers) Ltd., London.

SCHNEIDER, M. F. 1970. Linear electrical properties of the transverse tubules and surface membrane of skeletal muscle fibers. *J. Gen. Physiol.* **56**:640–671.

STANFIELD, P. R. 1973. The onset of the effects of zinc and tetraethylammonium ions on action potential duration and twitch amplitude of single fibres. *J. Physiol. (Lond.).* **235**: 639–654.

TANFORD, C. 1966. Physical Chemistry of Macromolecules. John Wiley & Sons, Inc., New York.

VALDIOSERA, R., C. CLAUSEN, and R. S. EISENBERG. 1974. Impedance of frog skeletal muscle fibers in various solutions. *J. Gen. Physiol.* **63**:460–491.