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PROPERTIES OF POTASSIUM CURRENTS AND THEIR ROLE IN MEMBRANE EXCITABILITY IN *DROSOPHILA* LARVAL MUSCLE FIBERS

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Summary

The larval muscle fibers of *Drosophila* show four outward K^+ currents in addition to the inward Ca^{2+} current in voltage-clamp recordings. The *Shaker (Sh)* and the *slowpoke (slo)* mutations, respectively, eliminate the voltage-activated fast K^+ current (I_A) and the Ca^{2+}-activated fast K^+ current (I_{CF}). Quinidine specifically blocks the voltage-activated delayed K^+ current (I_K) at micromolar concentrations. We used *Sh*, *slo* and quinidine to remove specifically one or more K^+ currents, so as to study physiological properties of these currents not previously characterized, and to examine their role in membrane excitability. A linear relationship was observed between the peak I_{CF} and the peak I_{Ca} at different membrane potentials. I_{CF} inactivated considerably during a 140 ms pulse to +20 mV. Recovery from inactivation was not complete for up to 2 s at the holding potential of −50 mV, which is much slower than the recovery of Ca^{2+} current from inactivation. In addition to I_A and I_{CF}, two delayed K^+ currents are also observed in these fibers, the voltage-activated I_K and the Ca^{2+}-activated I_{CS}. Near the end of a 500 ms depolarizing pulse, both I_A and I_{CF} are inactivated. Ca^{2+}-free and 20 mmol l^{-1} Ca^{2+} saline were used to examine the tail currents of the remaining I_K and I_{CS}. The tail currents of I_{CS} were slower than those of I_K and reversed between −30 and −50 mV in different fibers. We further studied the dose-dependence of the blockade of I_K by quinidine, which did not indicate a simple one-to-one binding mechanism. Current-clamp recordings from normal, *Sh*, *slo* and the double-mutant *Sh;slo* fibers suggested that I_{CF} plays a stronger role than I_A in repolarization of the larval muscle membrane. Elimination of I_{CF} facilitates the occurrence of action potentials. Further elimination of I_K prolonged the action potentials to several hundred milliseconds.

Introduction

The existence of mutations which affect specific ionic currents in *Drosophila* provides an opportunity to analyze the properties of individual currents and to

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study the roles of these currents in membrane excitability. The advantage of mutational analysis can be complemented with the use of pharmacological agents that specifically block certain currents. The larval body-wall muscles in *Drosophila* support four K^+ currents which can be distinguished and separated from each other with the use of mutations and drugs. These currents include two voltage-activated K^+ currents, the fast I_A and the delayed I_K, and two Ca^{2+}-activated K^+ currents, the fast I_{CF} and the delayed I_{CS} (Salkoff, 1983; Wu *et al.* 1983; Wu and Haugland, 1985; Gho and Mallart, 1986; Wei and Salkoff, 1986; Singh and Wu, 1989). Among these currents, I_A and I_{CF} are eliminated by the *Shaker (Sh)* (Salkoff and Wyman, 1981; Wu *et al.* 1983; Wu and Haugland, 1985) and the *slowpoke (slo)* (Elkins *et al.* 1986; Singh and Wu, 1989) mutations, respectively, and I_K is blocked by quinidine at micromolar concentrations (Singh and Wu, 1989). We have used *Sh*, *slo* and quinidine to study properties of the K^+ currents that have not been described previously, and to look at their role in membrane excitability in larval body-wall muscle fibers.

Different names have been used previously for the Ca^{2+}-activated K^+ currents in *Drosophila*. The fast current has been designated as I_{Acd} (Salkoff, 1983), I_{Ac} (Gho and Mallart, 1986; Wei and Salkoff, 1986) and I_C (Elkins *et al.* 1986), and the slow current as I_{Kc} (Wei and Salkoff, 1986) and I_C (Gho and Mallart, 1986). To avoid confusion and to preserve consistency with the conventional names used in other species we use the present nomenclature, i.e. I_{CF} for the fast C current and I_{CS} for the slow C current (Singh and Wu, 1989). Part of the results presented here have appeared in abstract form previously (Singh *et al.* 1986; Singh and Wu, 1987).

Materials and methods

The wild-type strain Canton-S was used as the normal control. An extreme allele of the *Shaker* (Kaplan and Trout, 1969) locus, Sh^{KS133} (Wu and Haugland, 1985), was used for eliminating I_A. Only one allele of *slo* (Elkins *et al.* 1986) is available and was used in these experiments. These two mutant alleles have been extensively characterized in larval muscle (Wu *et al.* 1983, 1989; Wu and Haugland, 1985; Singh and Wu, 1989; Haugland and Wu, 1990).

The body-wall muscle preparation of mature third-instar larvae (Jan and Jan, 1976) was used for recordings, as described previously (Wu and Haugland, 1985; Singh and Wu, 1989). Two-microelectrode voltage-clamp recordings were made with a holding potential of −50 or −80 mV, as specified in the figure legends. Both the voltage-clamp and the current-clamp recordings were made at 4 °C. Recording saline contained (in mmol l^{-1}) 128 NaCl; 35.5 sucrose; 2 KCl and 5 Hepes, and was buffered at pH 7.1. In addition, the Ca^{2+}-free saline had 0.5 mmol l^{-1} EGTA and $MgCl_2$ and was adjusted to 20 mmol l^{-1} for improving membrane stability (Wu and Haugland, 1985). Unless otherwise specified, the saline with Ca^{2+} contained 20 mmol l^{-1} $CaCl_2$, 4 mmol l^{-1} $MgCl_2$ and no EGTA. Quinidine and 4-aminopyridine (4-AP) were obtained from Sigma Chemical Co. (St Louis, MO).

The muscle fibers used in these experiments are supercontracting muscles (Osborne, 1967). The contractions induced during recordings, especially during strong depolarizations, damage the fiber quickly. Although it is possible to make reliable recordings from a fiber, it is difficult to take a single fiber through repeated experiments using different experimental paradigms or recording solutions. Therefore, all individual experiments described here were performed on separate fibers.

Voltage or current pulses were generated at intervals of 10 s by a Master-8-cp pulse generator (A.M.P.I., Jerusalem, Israel). The voltage-clamp circuit was equipped with a high-voltage head stage (model HVHD, Almost Perfect Electronics, Basel, Switzerland). The current electrode was shielded up to a few millimeters from the tip with a coaxial double shield. The inner shield was driven at the potential of the electrode and the outer shield was grounded. The voltage electrode was filled with KCl and the current electrode with a mixture of KCl and potassium citrate. The electrodes were pulled to a resistance between 5 and 15 MΩ. The current signal was recorded through the virtual ground using the amplifier M701 (WPI, New Haven, CT). The current and the voltage signals were pulse-coded using a Neurocorder unit DR-384 (Neuro Data Instruments Corp., New York, NY), and recorded using a video cassette recorder. The data were digitized and subsequently analyzed on an Apple-Macintosh II computer. Current densities in voltage-clamp recordings were obtained by normalizing the current with respect to the fiber capacitance (Haugland, 1987; Haugland and Wu, 1990). Leakage current was subtracted digitally and the initial 3 ms of recordings which contain capacitive transients have been omitted in the figures for the sake of clarity, as described previously (Singh and Wu, 1989).

Results

Early membrane currents

Voltage-clamp recordings made in 1.8 mmol l^{-1} Ca^{2+}, the concentration generally used in *Drosophila* saline, showed considerable variation in the Ca^{2+}-dependent currents. This problem was overcome by increasing the external Ca^{2+} concentration. Saline with 20 mmol l^{-1} Ca^{2+} was therefore used (Singh and Wu, 1989) for voltage-clamp measurements of Ca^{2+}-dependent currents in this paper.

Early membrane currents recorded from different genotypes are shown in Fig. 1. The fast current peak seen in the normal fibers (Fig. 1A) consists of two outward currents, the voltage-activated (I_A) and the Ca^{2+}-activated (I_{CF}) K^+ currents, and an inward Ca^{2+}-current, I_{Ca}. As shown previously (Gho and Mallart, 1986; Singh and Wu, 1989), the outward currents mask the inward current which is therefore not apparent in the figure. Lack of I_{CF} in the *slo* mutation (Elkins *et al.* 1986; Singh and Wu, 1989) revealed the sum of I_A and I_{Ca}, in which the outward peak was reduced but the inward current was still masked (Fig. 1B). In a similar way, the *Sh* mutant that lacks I_A (Salkoff and Wyman, 1981; Wu *et al.* 1983; Wu and Haugland, 1985) showed the sum of I_{CF} and I_{Ca} (Fig. 1C). The

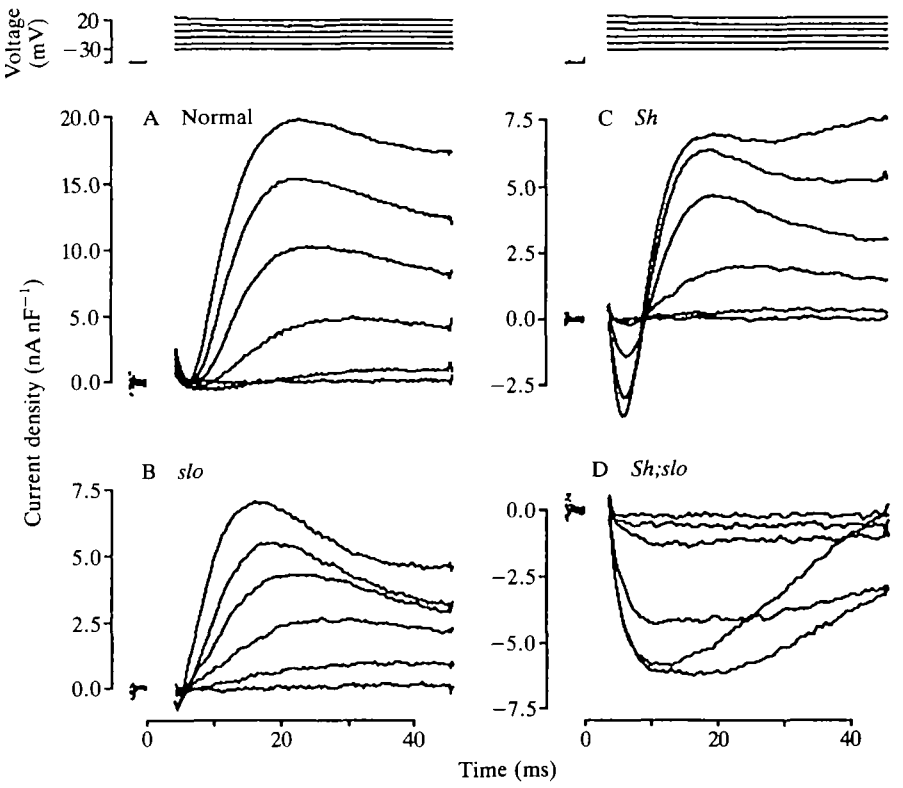

Fig. 1. Voltage-clamp recordings of the early currents from the larval muscle fibers in *Drosophila*. Superimposed traces show currents elicited by voltage steps to −30, −20, −10, 0, 10 and 20 mV from a holding potential of −50 mV in saline containing 20 mmol l^{-1} Ca^{2+}. The early current in the normal fibers (A) consists of I_A, I_{CF} and I_{Ca}. Among these, the inward I_{Ca} is not apparent as it has been masked by the two outward currents I_A and I_{CF}. The *Sh* mutation eliminates I_A and shows the sum of I_{CF} and I_{Ca} (C). Similarly, the sum of I_A and I_{Ca} is seen in *slo* which lacks I_{CF} (B). The *Sh;slo* double mutant lacks both I_A and I_{CF} and thus unmasks the inward Ca^{2+} current I_{Ca} (D), which is otherwise obscured by the two outward currents. The current is shown as normalized to the membrane capacitance. Approximately the first 3 ms of recordings show capacitive transients and have been omitted from the figure for the sake of clarity. Note the difference in the current scale for the normal fibers and the rest of the fibers. For this and the following figures, all experiments were conducted at 4°C.

inward Ca^{2+} current can be seen during the short interval before it is masked by the outward I_{CF}. Since these fibers have both I_A and I_{CF}, the total outward current is stronger than in *Sh* or in *slo*. Elimination of both the outward currents (I_A and I_{CF}) in the *Sh;slo* double mutant (Fig. 1D) unmasks the inward I_{Ca}.

I_{CF} inactivation

Both I_A and I_{CF} in larvae (Wu and Haugland, 1985; Gho and Mallart, 1986) and

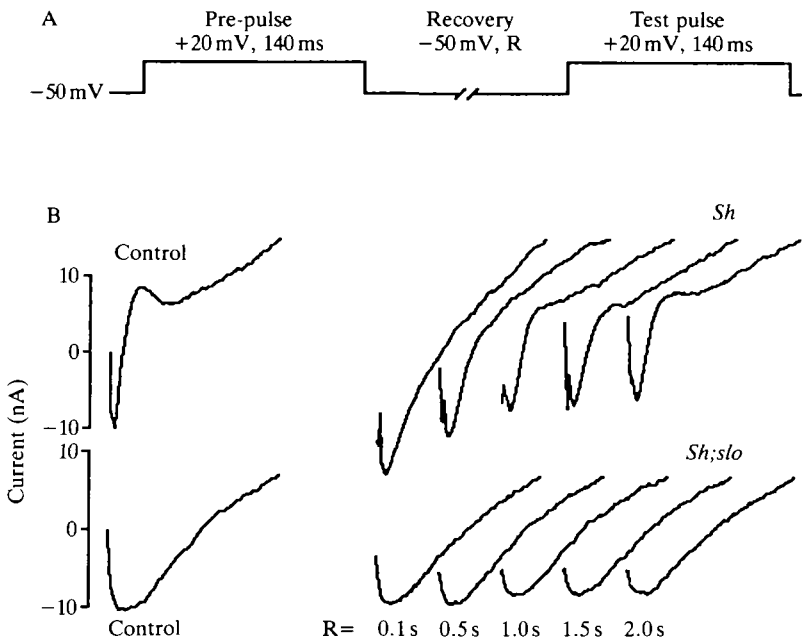

Fig. 2. Inactivation and recovery of I_{CF}. (A) Pulse paradigm. R indicates recovery time between the pre-pulse and the test pulse, each of 140 ms duration, to +20 mV from the holding and the recovery potential of −50 mV. (B) Sequential recordings were obtained from the same fibers. Recovery times of 0.1, 0.5, 1.0, 1.5 and 2.0 s were given, as shown beneath the different traces. Recordings from *Sh;slo* show I_{Ca} and those from *Sh* show the contribution of both I_{Ca} and I_{CF}. The shortest recovery time given in the experiment, 100 ms, was enough for the recovery of I_{Ca} from whatever, if any, inactivation took place. In contrast, recovery of I_{CF} was not complete even after 2 s at the holding potential. In this figure, the current is shown in nA and not as current density and the time scale is indicated by the pulse paradigm.

in adults (Salkoff, 1983; Salkoff and Wyman, 1983) have been shown to inactivate with membrane depolarization. The I_{CF} inactivation in larvae is almost complete at a membrane potential of −20 mV (Gho and Mallart, 1986), a potential at which I_{Ca} or I_{CF} have still not been activated. This implies that the I_{CF} inactivation is voltage-dependent and not Ca^{2+}-dependent. However, there is no study on the recovery of I_{CF} from inactivation in larvae. This was studied by using different experimental protocols and recovery times in recordings from *Sh* (44 fibers) and *Sh;slo* (36 fibers). Fig. 2 shows the effect of a 140 ms pre-pulse to +20 mV on I_{CF} and I_{Ca}. Their recovery, after different times at the holding potential of −50 mV, was tested by an identical test pulse. The pulse paradigm is shown in Fig. 2A. The first traces in both the *Sh* and the *Sh;slo* recordings in Fig. 2B show the membrane current during the pre-pulse, which serves as a control. Since I_K and I_{CS} rise slowly with a delay, the early phase (0–40 ms) of this current mainly consists of the inward I_{Ca} in *Sh;slo* and the sum of I_{Ca} and I_{CF} in *Sh*. The traces on the right show

membrane currents in response to the test pulse, after the indicated recovery period at −50 mV. I_{CF} was considerably inactivated by the pre-pulse. Note that the first test pulse (after 0.1 s recovery time) shows stronger inward current than the control current seen with the pre-pulse in *Sh* (Fig. 2B). Since the *Sh* fibers lack I_A, the inactivation of I_{CF} unmasks the inward current to a greater degree. Even if inactivation did occur in I_{Ca} during the pre-pulse, it recovered during the first 100 ms, as indicated by recordings in *Sh;slo*. In contrast, I_{CF} showed clear inactivation which did not recover completely within 2 s (compare *Sh* and *Sh;slo* in Fig. 2B). The recovery from inactivation of I_{CF} was slower than that of I_A, which shows substantial recovery within 1 s at a comparable temperature (Wu and Haugland, 1985). The main result is that the recovery of I_{CF} from inactivation may not be linked to that of I_{Ca}.

Relationship between peak I_{CF} and I_{Ca}

The possibility of extracting I_{CF} with the use of mutations enabled us to look at the relationship between this current and the inward Ca^{2+} current that is responsible for its activation. The current–voltage relationship for the extracted I_{CF} is given in Fig. 3A. The values for I_{CF} were obtained by subtracting the averaged current densities recorded in *Sh;slo* from those recorded in *Sh*. The

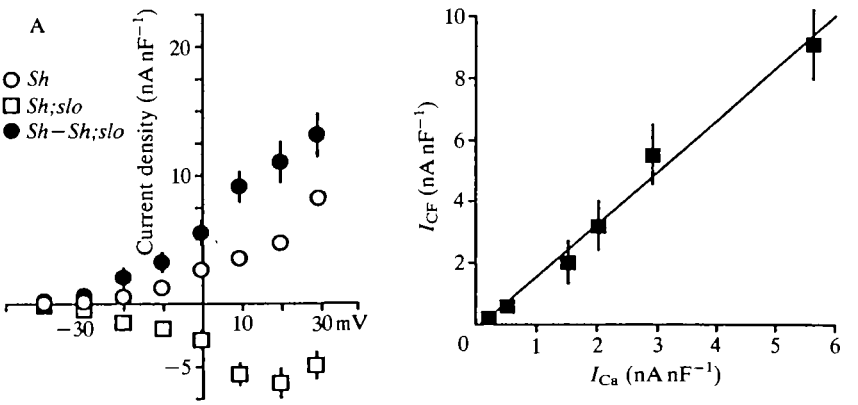

Fig. 3. (A) Current–voltage relationship for I_{CF}. Currents were normalized to fiber capacitance. Peak values for the fast current plotted here were calculated as described in the text. Current from *Sh;slo* fibers (□) lacks both I_A and I_{CF}. Current from *Sh* fibers (○) lacks I_A but not I_{CF}. Digital subtraction of the *Sh;slo* current from the *Sh* current thus gives I_{CF} (●). Recordings represent averages from a number of fibers (*F*) taken from a number of larvae (*L*). *Sh;slo*: *F*=7, *L*=3; *Sh*: *F*=10, *L*=4. Bars represent standard error of the mean. (B) Relationship between the Ca^{2+}-activated fast K^+ current I_{CF} and the inward current I_{Ca}. Same data as shown in A. Mean±S.E.M. for I_{CF} is plotted against mean I_{Ca} at the membrane potentials of −40, −30, −20, −10, 0 and +10 mV. Measurements of I_{Ca} become less reliable at more positive potentials because the inward peak becomes masked by the more rapid rise of the delayed outward currents. The continuous line represents linear regression.

current for *Sh* is shown as the early peak of the outward current at different membrane potentials. For the purpose of subtraction, the inward I_{Ca} in *Sh;slo* was measured at the time when a peak occurred for the outward current in *Sh* at the corresponding voltage. This time was a few milliseconds after the actual inward peak in *Sh;slo* (see Fig. 1C and D), which gives values very similar to the ones shown in Fig. 3A.

Fig. 3B shows the relationship between I_{CF} and I_{Ca}, determined as described for Fig. 3A. It shows a linear dependence of I_{CF} on I_{Ca}. This is consistent with a one-to-one correspondence between the Ca^{2+} entering the fiber and the opening of individual I_{CF} channels. A linear relationship between the amount of Ca^{2+} injected directly into the cell and the value of the Ca^{2+}-activated K^+ current has also been reported in the dorsal unpaired median neurons in the cockroach (Thomas, 1984). However, the data cannot be taken as conclusive evidence for a one-to-one correspondence, because the amount of free Ca^{2+} in the cytosol depends on the influx of Ca^{2+}, on the nature of the Ca^{2+} sequestering process and on a number of other factors.

The Ca^{2+}-activated slow I_{CS}

In addition to the fast Ca^{2+}-activated I_{CF}, the larval muscle fibers in *Drosophila* also show a slow Ca^{2+}-activated outward I_{CS} (Gho and Mallart, 1986; Singh and Wu, 1989). We performed experiments with 0 mmol l^{-1} Ca^{2+} (20 fibers for *Sh* and 10 fibers for *Sh;slo*) or 20 mmol l^{-1} Ca^{2+} (19 fibers for *Sh* and 13 fibers for *Sh;slo*) in the saline. I_{CS} was observed in recordings made in saline with 20 mmol l^{-1} Ca^{2+}. *Sh* and *Sh;slo* gave similar results because I_{CF} is almost completely inactivated during the 500 ms pulse used in these experiments (Fig. 2 shows inactivation produced by a 140 ms pulse) and the contamination from residual I_{CF} is expected to be minor. Fig. 4A shows the voltage-activated I_K in Ca^{2+}-free saline, which reaches a steady state before the end of the 500 ms pulse. In Fig. 4B,C, the delayed outward current recorded in saline with Ca^{2+} consisted of I_K and I_{CS}. I_{CS} can be seen as changes in the overall amplitude of the slow outward current and the tail currents after the voltage pulse has ended. Unlike I_{CF}, I_{CS} did not inactivate, and in fact continued to rise for at least 500 ms. This contrast between the fast and the slow Ca^{2+}-activated outward currents, I_{CF} and I_{CS}, parallels the difference between the two voltage-activated outward currents, the fast transient I_A and the delayed sustained I_K.

The tail currents were recorded by stepping the membrane potential to +20 mV and then repolarizing it to different voltages (Fig. 4). The tail currents immediately after the end of the voltage pulse are either inward or outward, depending on the membrane potential reached. Inward tail currents are expected if the membrane potential is repolarized below the reversal potential for the current, and outward tails are expected if it is above the reversal potential. Only outward tail currents were seen in recordings made in Ca^{2+}-free saline (Fig. 4A). This is because I_K does not show inward tail currents as there is strong rectification (Wu and Haugland, 1985). Inward tails were seen when recordings were made in the

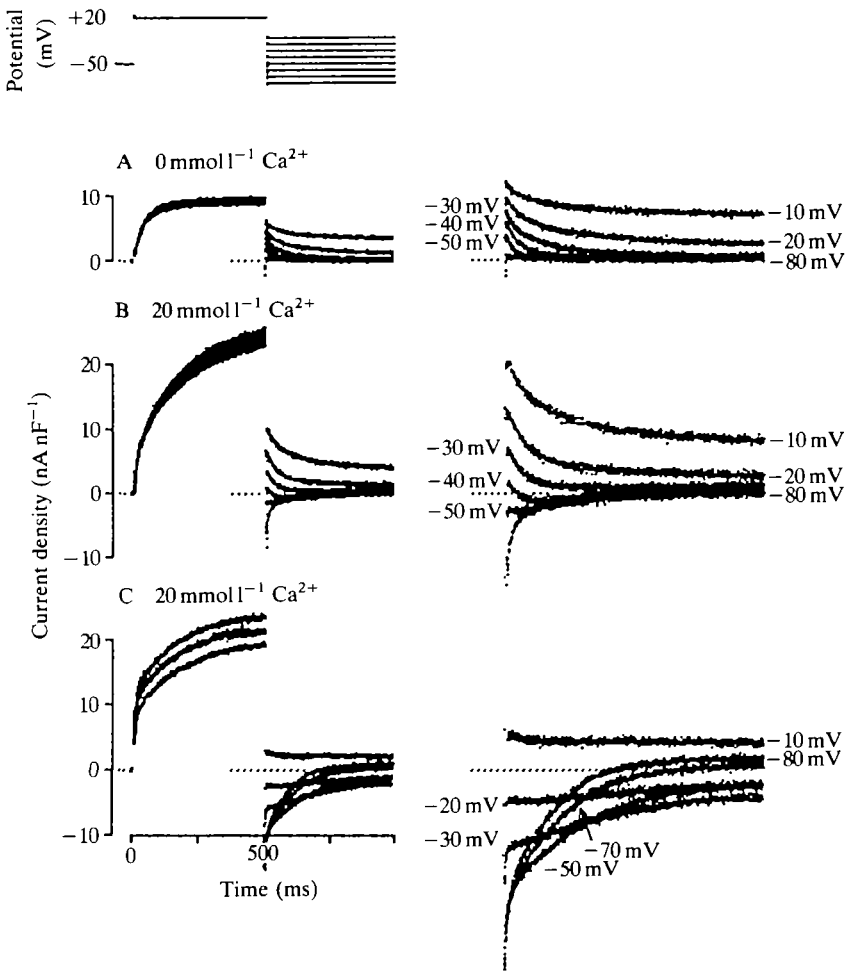

Fig. 4

presence of Ca^{2+}. Fig. 4B shows inward tail currents for I_{CS} and combined outward tail currents for I_K and I_{CS}. Inward tails are seen for potentials up to −40 mV. At −30 mV, the tails were completely outward. At this potential, I_{CS} makes only a marginal contribution to the outward tail in Fig. 4B, as can be seen by comparing with the tail at −30 mV in Fig. 4A. The reversal potential for I_{CS} in Fig. 4B thus lies between −40 and −30 mV, near to −30 mV. In different fibers, the reversal potential ranged between −30 and −50 mV.

In some fibers, depolarization by repeated test pulses showed a cumulative effect on the outward current and gave rise to more complex tail currents. Fig. 4C shows an example where inward tail current is seen up to −20 mV. The outward tail for −10 mV (Fig. 4C) is also not as strong as the I_K tail at the same voltage (Fig. 4A). The reversal potential for the current in addition to I_K in this case seems to be more positive than −10 mV. A possible interpretation of this is suggested in the Discussion.

Fig. 4. Measurement of tail currents from *Sh* fibers in Ca^{2+}-free saline and in saline containing 20 mmol l^{-1} Ca^{2+}. The voltage signal is shown at the top to indicate the pulse paradigm. The left part of the figure shows the membrane currents during the voltage step as well as the tail currents. The right side of the figure shows the tail currents magnified twofold on the time scale as well as the current scale. Membrane potential was stepped from the holding value of −50 mV to +20 mV for 500 ms. The membrane was then repolarized to different levels as specified along the magnified tail currents. Tail currents at selected voltages are shown for the sake of clarity. The horizontal dotted lines represent the zero level of the current when no active current is going across the membrane. (A) Recordings in Ca^{2+}-free saline show only the voltage-activated current I_K, as I_A is missing because these are *Sh* fibres. Only outward tail currents are seen, as I_K channels show strong rectification. (B,C) Recordings with 20 mmol l^{-1} Ca^{2+} in the saline. Activation of I_{CS} is indicated by the continuing rise of outward currents during the pulse and the changes in the tail currents, which now include an additional component due to I_{CS}, as indicated by the appearance of the inward tails. This additional component in most fibers showed a reversal potential between −30 and −40 mV (B). In some fibers, the outward current showed cumulative effects during repeated pulses (only the first three traces are shown in C to avoid cluttering) and the tail currents showed a stronger negative component which reversed at more positive potentials. In the case shown here, the additional tail component reversed near −10 mV (C).

The relative role of I_A and I_{CF} in membrane excitability

The ability to eliminate individual K^+ currents by means of mutations and pharmacological agents allows an analysis of the role of different currents in membrane excitability. Mutational elimination of I_A and I_{CF} has been used in adult dorsal longitudinal flight muscles (DLM) of *Drosophila* for this purpose (Elkins and Ganetzky, 1988). We used the lack of I_A in *Sh* and the lack of I_{CF} in *slo* to study the role of these currents in the excitability of larval muscle fibers. This was done by looking at the membrane potential in response to constant current injection.

Regenerative potentials have recently been demonstrated in larval preparations in which air was supplied through tracheoles to maintain the resting potentials up to −80 mV (Yamaoka and Ikeda, 1988). Most previous reports, which used unaerated preparations, described lower resting potentials and only graded potentials in response to current injection in normal physiological saline, which contains 1.8 mmol l^{-1} Ca^{2+} (Suzuki and Kano, 1977; Wu and Ganetzky, 1988). We found that regenerative responses were easier to elicit when the resting potential was sufficiently negative, either in a freshly dissected preparation or because of injection of a hyperpolarizing current even in the absence of aeration. Recordings were made from the normal (4 fibers), *Sh* (9 fibers) and *slo* (13 fibers). Fig. 5 shows recordings made in fibers with the resting potential kept near −60 to −70 mV by injecting hyperpolarizing current. The regenerative peak in these recordings is more pronounced in *slo* and in *Sh*. A smaller amount of current was required to elicit regenerative potentials in *slo* as compared to *Sh*. This indicates that I_{CF} is more effective than I_A in repolarizing the regenerative potential supported by I_{Ca}.

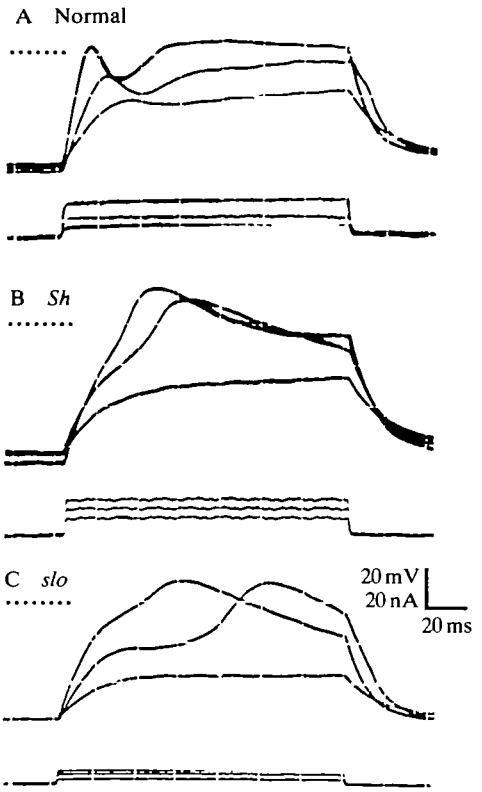

Fig. 5. Membrane potential (upper traces) in response to constant current injection (lower traces). The saline contained 1.8 mmol l^{-1} Ca^{2+}. The resting membrane potential in these recordings was maintained around −60 to −70 mV by a hyperpolarizing current. The zero potential level is represented by the horizontal dotted lines. The *Sh* (B) and the *slo* (C) fibers show more clear regenerative potentials than the normal ones (A). The amount of current needed for eliciting regenerative potentials is less for *slo* than for *Sh*. The fiber sizes used in these three experiments are different. For example, the size of the particular *Sh* fiber shown in B is larger than that of the particular normal fiber shown in A. Therefore, a comparable amount of current charges the membrane faster in the normal fiber than in the *Sh* fiber shown here.

The relative role of different outward currents can be seen more clearly by using saline containing 20 mmol l^{-1} Ca^{2+}. With increased Ca^{2+} concentration, pronounced regenerative potentials could be elicited when one or more outward currents were removed by mutations or drugs. This could be achieved without aeration in preparations with resting potentials as low as −20 mV. Fig. 6 shows recordings made in saline containing 20 mmol l^{-1} Ca^{2+} without injection of hyperpolarizing current. The normal fibers showed graded potentials in response to increasing amounts of current injection (Fig. 6A) and did not show action potentials. Elimination of I_A by *Sh* led to increased depolarization, which was still graded in nature (Fig. 6B). However, when I_{CF} was eliminated by the *slo* mutation, the fibers underwent all-or-none action potentials (Fig. 6C). This is

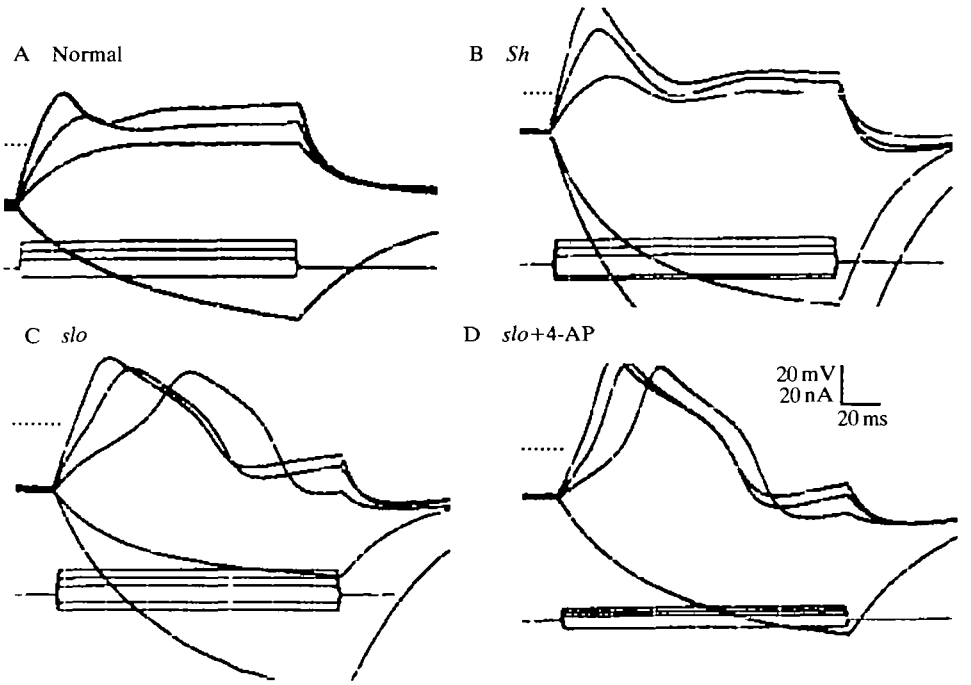

Fig. 6. Membrane potentials in response to current injection with 20 mmol l^{-1} Ca^{2+} in the saline. No hyperpolarizing holding current was applied. The horizontal dotted line represents the zero potential level. The normal (A) and the *Sh* (B) fibers show graded responses to current injection, the latter showing a stronger initial depolarization. Elimination of I_{CF} in *slo* fibers gives rise to action potentials (C). If 4-aminopyridine (4-AP, 100 μmol l^{-1}), which blocks I_A, is added to the saline, *slo* fibers require less current injection to give rise to action potentials (D).

consistent with the observation shown in Fig. 5 that I_{CF} plays a stronger role than I_A in repolarizing the membrane. As expected, *slo* fibers treated with 4-AP (100 μmol l^{-1}), which preferentially blocks I_A at this concentration, also exhibit all-or-none action potentials, but requiring even less injection of current (Fig. 6D) than *slo* (Fig. 6C). The number of fibers tested was 11 for the normal, 10 for *Sh*, 16 for *slo* and 22 for *slo* with 4-AP.

Blockade of I_K by quinidine

Quinidine, which blocks different K^+ currents in several systems (Fishman and Spector, 1981; Hermann and Gorman, 1984; Imaizumi and Giles, 1987), blocks I_K in *Drosophila* (Singh and Wu, 1989). The inset in Fig. 7 shows membrane currents in response to voltage steps from the holding potential of −80 mV to −10, +10 and +30 mV in Ca^{2+}-free saline with and without quinidine. I_A was removed by the use of *Sh*, and 0.1 mmol l^{-1} quinidine substantially blocked the only remaining current I_K. This concentration of quinidine has earlier been shown not to block I_A,

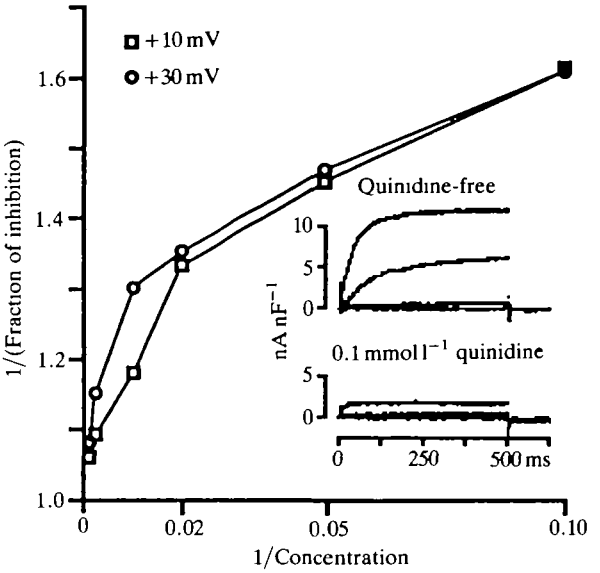

Fig. 7. Blockade of I_K by quinidine represented in a double-reciprocal plot between the fraction of inhibition and the quinidine concentration (in μmol l^{-1}). The inset shows the reduction of I_K due to 0.1 mmol l^{-1} quinidine, as recorded from *Sh* fibers in Ca^{2+}-free saline. Membrane currents are shown in response to voltage steps to −10, +10 and +30 mV from the holding potential of −80 mV. This part of the figure is adopted from Singh and Wu (1989). The values of I_K in the plot were determined from the amplitude at the end of a 500 ms pulse to +10 or +30 mV, as specified in the figure. The mean values of I_K were derived from a number of fibers (at different concentrations in μmol l^{-1}): 7 (0); 3 (10); 6 (20); 2 (50); 12 (100); 3 (500) and 3 (1000).

I_{CF} or I_{CS} (Singh and Wu, 1989). The double-reciprocal plot in Fig. 7 shows the effect of different concentrations of quinidine on the amplitude of I_K. Quinidine at 10 μmol l^{-1} reduced I_K by more than half. In this plot, the reciprocal of the fraction of inhibition is plotted against the reciprocal of quinidine concentration in micromoles per liter. A linear relationship would signify a simple saturation with one-to-one binding of quinidine molecules to the I_K channels. Fig. 7 shows a deviation from a linear relationship. Although I_K is blocked more than 50 % by 10 μmol l^{-1} quinidine (as indicated by the upper-most point in the plot), even 1.0 mmol l^{-1} quinidine leaves some current intact (the lower-most point in the plot). It is not clear at this stage if this is a manifestation of sub-populations of I_K with different sensitivity to quinidine.

Some K^+ channel blockers are known to show voltage-dependent action. The extent of inhibition by quinidine was examined at +10 and +30 mV in *Drosophila*. The plot shows small differences at the two potentials (Fig. 7). These differences could not be substantiated statistically, owing to the small sample sizes, but were far less pronounced than the voltage-dependence reported for 4-AP (Yeh *et al.* 1976) and tetraethylammonium (Armstrong, 1971).

The role of I_K in membrane excitability

Selective blockade of I_K by quinidine provided an opportunity to examine the role of this current in determining the membrane potential. Fig. 8 shows current-clamp recordings in saline containing 20 mmol l^{-1} Ca^{2+} and 100 μmol l^{-1} quinidine. In the absence of quinidine, normal and *Sh* fibers did not show action potentials, as discussed above. Removal of I_K by quinidine did not show a strong effect except for a slight regenerative potential, as indicated by a shoulder in *Sh* after the end of the current injection (Fig. 8B). In the case of *slo* and *Sh;slo*, regenerative potentials were initiated even during weak current pulses. After the end of the pulse, the regenerative potential was sustained for hundreds of milliseconds (Fig. 8C,D). The duration of the prolonged depolarization was highly

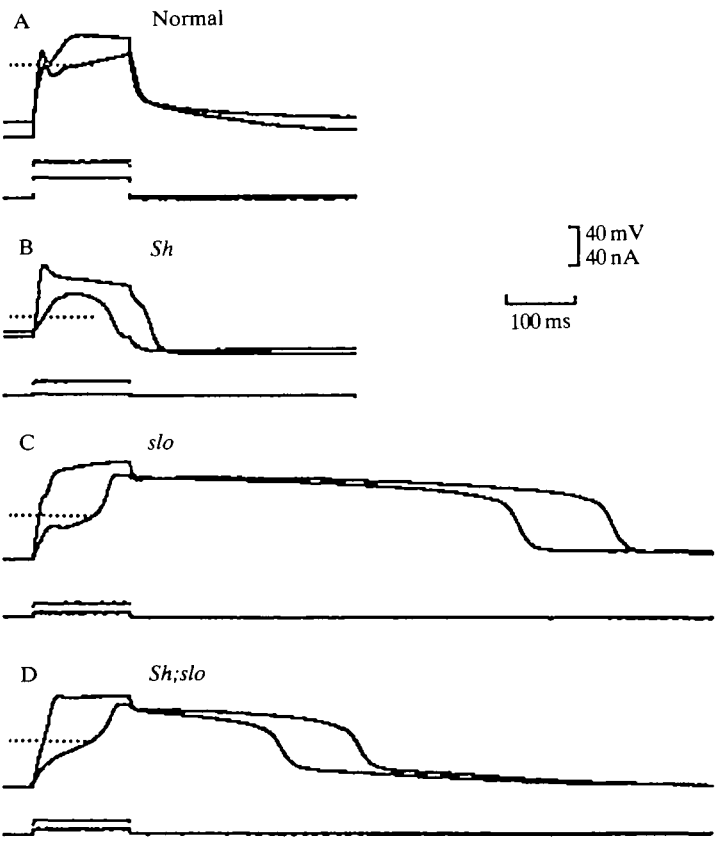

Fig. 8. Effect of quinidine on the membrane potential. The saline contained 20 mmol l^{-1} Ca^{2+}. Responses to 140 ms current pulses in the presence of 100 μmol l^{-1} quinidine are shown. The dotted horizontal lines indicate the zero potential level. The normal (A) and the *Sh* (B) fibers repolarized soon after the current pulse was over. In contrast, the regenerative potentials initiated during the current pulse in *slo* (C) and *Sh;slo* (D) fibers were sustained for several hundred milliseconds after the end of the current pulse.

variable even between fibers of the same genotype and no consistent differences were found between *slo* and *Sh;slo*. Elimination of I_K thus prevents repolarization of Ca^{2+} regenerative potentials initiated either by the lack of I_{CF} alone or by the elimination of both I_{CF} and I_A (compare Fig. 8 with Fig. 6). The number of fibers tested was 9 for the normal, 8 for *Sh*, 10 for *slo* and 7 for *Sh;slo*.

Discussion

Properties of K^+ currents

The availability of the two mutations, *Sh* and *slo*, allows a comparison between I_A and I_{CF} underlying the early phase of the membrane current. Fig. 1B,C shows that the generation of the Ca^{2+}-activated I_{CF} is preceded by the voltage-activated I_A. Presumably, I_{CF} requires Ca^{2+} influx following I_{Ca} activation, whereas I_A is activated by membrane depolarization without a lag following I_{Ca} activation. As previously reported (Gho and Mallart, 1986), I_{CF}, like I_A, was inactivated by membrane depolarization. I_{CF} recovery from inactivation followed a time course much slower than that of I_{Ca} recovery (Fig. 2), suggesting a process independent of the availability of cytosolic free Ca^{2+}.

The late outward currents consist of I_K and I_{CS}. I_K can be isolated by using *Sh* in Ca^{2+}-free saline. This allows an analysis of the effect of quinidine on I_K. A double-reciprocal plot between the fractional inhibition and the quinidine concentration did not indicate a linear relationship (Fig. 7). This observation suggests that there are different sub-populations of I_K channels with different sensitivities to quinidine, a possibility that requires further investigation.

As mentioned above, I_K does not show inward tail currents because of rectification (Fig. 4A). Outward currents are seen at potentials more positive than -60 mV but not at a potential of -70 mV. The reversal potential for this current is therefore between -60 and -70 mV, consistent with the earlier reported reversal potential for I_K (Wu and Haugland, 1985). Fig. 4B shows both outward and inward tail currents, recorded in saline containing Ca^{2+}. In different fibers, the component contributed by I_{CS} reversed at a potential between -50 and -30 mV (see Results). This is consistent with I_{CS} being a K^+ current in larvae (Gho and Mallart, 1986) as well as in adults (Wei and Salkoff, 1986). The currents observed in the presence of Ca^{2+} show a greater degree of variation in some fibers. Fig. 4C shows such a fiber with a reversal potential that is more positive than -10 mV. Such a reversal potential was generally associated with stronger tail currents as well as a cumulative effect on the variable outward currents during repeated voltage pulses (Fig. 4C). This type of behavior was generally elicited in fibers that were relatively leaky, which may be an indication of deterioration or damage. A more positive reversal potential may be accounted for by the presence of yet another set of channels or a non-selective conductance in addition to the I_{CS} channels.

The roles of K^+ currents in membrane excitability

Yamaoka and Ikeda (1988) have recently shown that the tracheole aeration of

the larval preparation is essential in maintaining the resting potential of the muscle fibers and in initiating a regenerative response. Lack of aeration results in a drop in the resting potential from about −80 mV to about −30 mV within 1 h at room temperature. It is known that the resting potentials in many insect muscles are maintained below the K^+ equilibrium potential by electrogenic pumps (Huddart and Wood, 1966; Rheuben, 1972; Henon and Ikeda, 1981). The metabolic state, which would depend upon effective aeration, is thus expected to influence the resting potential. However, it is not clear if the effect of aeration on the regenerative response is due to the membrane potential, which would determine the state of channel inactivation, or to a direct deteriorative effect on channel machinery or to some other cellular factors. Recordings in this study and in other reports (Jan and Jan, 1976; Suzuki and Kano, 1977; Wu and Ganetzky, 1988) have been made in the larval preparation without tracheole aeration. Our recordings from muscle fibers with resting potentials at different levels indicated that the metabolic condition was probably not a prerequisite for regenerative responses. Regenerative responses were easier to elicit in fibers with resting potentials between −60 and −70 mV, maintained by injection of hyperpolarizing currents or by low temperature (4°C) in freshly dissected preparations. For example, regenerative potentials could be seen in *Sh* fibers only if the resting potential was more negative than −60 mV (compare Figs 5B and 6B; note the inflexion points in Fig. 5B). An effect of resting potential on the regenerative events is expected since the Ca^{2+} channels mediating inward current inactivate to different degrees depending upon the extent of membrane depolarization. The main aim of the present study was to compare the relative role of different K^+ currents in membrane excitability. We found clear differences between the consequences of eliminating different currents on excitability, even without tracheole aeration. However, it is important to investigate these differences under normal physiological conditions with effective aeration.

An important consideration is the free Ca^{2+} concentration around the fibers as well as inside the fibers. This can be a source of variation in the Ca^{2+}-activated K^+ currents as it can affect the amount of Ca^{2+} influx and the Ca^{2+} available to trigger these currents. From electron microscopic observations (Jan and Jan, 1976; C.-F. Wu, unpublished data), the larval muscle fibers are enveloped by relatively little connective tissue. In addition, ion replacement and drug additions to the recording saline show immediate effects, indicating a lack of effective diffusion barriers (Wu and Haugland, 1985; Haugland, 1987; Singh and Wu, 1989; Wu *et al.* 1989). The level of intracellular free Ca^{2+} can conceivably vary in relation to metabolic state, e.g. a weakened sequestering mechanism, and affect the Ca^{2+} concentration gradient across the plasma membrane as well as the available free Ca^{2+} for activating I_{CF} and I_{CS}. It will be important to determine the free Ca^{2+} level directly, employing other available techniques in future investigations.

Interactions between the outward and the inward currents determine the membrane potential in response to a current stimulus. The inward current depolarizes the membrane and the outward current counteracts this by repolariz-

ing the membrane. As in other arthropod species, the inward current in *Drosophila* muscles is carried by Ca^{2+} (Suzuki and Kano, 1977; Salkoff and Wyman, 1983; Elkins *et al.* 1986; Gho and Mallart, 1986). A tetrodotoxin-sensitive inward current was recently suggested by current-clamp experiments (Yamaoka and Ikeda, 1988). It remains to be determined by voltage-clamp experiments whether it is a Na^+ inward current. The outward currents in *Drosophila* muscles consist of two voltage-activated (I_A and I_K) and two Ca^{2+}-activated (I_{CF} and I_{CS}) K^+ currents. The effectiveness of different K^+ currents in repolarizing the membrane depends on their channel density (current amplitude) and their kinetic characteristics. Because of these differences, they give rise to a variety of excitability patterns.

An ability to manipulate different currents, by the use of mutations and drugs, provides an opportunity to examine the role of individual currents in excitability. The two fast outward currents (I_A and I_{CF}) in the normal fibers limit the membrane depolarization immediately after the initiation of the current injection. As compared to the normal muscles, therefore, a lack of I_A in the *Sh* muscles allowed a larger initial depolarization with the same amount of current injection (compare Fig. 6A and 6B). However, removal of I_{CF} alone, in *slo*, had a greater effect, resulting in action potentials, even at a low resting membrane potential (see Fig. 6C). This implies that I_{CF} plays a more effective role in membrane repolarization than does I_A during prolonged current injections. It is known that I_A inactivates rapidly (Salkoff and Wyman, 1983; Wu and Haugland, 1985) and is effective in delaying membrane excitation (Salkoff and Wyman, 1983; Elkins and Ganetzky, 1988) but would not have a prolonged repolarizing effect. Although I_{CF} is similar in magnitude to I_A, its activation is coupled to the activation of I_{Ca}. In addition, it inactivates at more depolarized potentials (Gho and Mallart, 1986) and with a slower time course (S. Singh and C.-F. Wu, unpublished observations) than I_A. The effect of I_A removal became more apparent only when the resting potential was more negative (Fig. 5B) or when I_{CF} was also removed (action potentials in Fig. 6D require less current injection compared to Fig. 6C). These observations on the relative role of I_A and I_{CF} in the excitability of larval muscles are consistent with the earlier conclusions on their role in the adult flight muscles (Elkins and Ganetzky, 1988).

In contrast to the early currents, the slow outward currents activate with delay but show little inactivation. The role of I_K in muscle repolarization is revealed by the use of quinidine. In normal and *Sh* fibers, blockade of I_K by quinidine led to only marginal differences. However, when the membrane undergoes an action potential, as when I_{CF} is removed in *slo* or *Sh;slo*, I_K assumes an important role in repolarization. The action potentials lasted only a few tens of milliseconds when I_K was present (Fig. 6C,D) but lasted for several hundred milliseconds when I_K was blocked by quinidine (Fig. 8C,D). In the experiments shown in Figs 6 and 8, I_A and I_{CF} are expected to have inactivated to a great extent by the end of the current pulse. The repolarization of the action potential, once initiated, thus depends mainly on I_K and I_{CS}. With the further removal of I_K, the termination of the

prolonged action potential after the end of the current pulse will depend primarily on the amplitudes of I_{Ca} and I_{CS}, and how they change with time. The membrane during this prolonged depolarization is presumably in an unstable equilibrium and the time taken by the membrane to repolarize varied considerably, regardless of the genotype (data not shown). The sustained action potential is similar to, but less prolonged than that seen in the larval muscle fibers treated with tetraethylammonium (Suzuki and Kano, 1977; S. Singh and C.-F. Wu, unpublished observations), which blocks all four K^+ currents in this system to different degrees (Wu and Haugland, 1985; Gho and Mallart, 1986). So far no mutations or drugs remove I_{CS} specifically. Such a possibility would be very useful for determining the role of I_{CS} in membrane excitability.

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References

ARMSTRONG, C. M. (1971). Interaction of tetraethylammonium ion derivatives with the potassium channels of giant axons. *J. gen. Physiol.* **58**, 413–437.

ELKINS, T. AND GANETZKY, B. (1988). The roles of potassium currents in *Drosophila* flight muscles. *J. Neurosci.* **8**, 428–434.

ELKINS, T., GANETZKY, B. AND WU, C.-F. (1986). A *Drosophila* mutation that eliminates a calcium-dependent potassium current. *Proc. natn. Acad. Sci. U.S.A.* **83**, 8415–8419.

FISHMAN, M. C. AND SPECTOR, I. (1981). Potassium current suppression by quinidine reveals additional calcium currents in neuroblastoma cells. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5245–5249.

GHO, M. AND MALLART, A. (1986). Two distinct calcium-activated potassium currents in larval muscle fibers of *Drosophila melanogaster*. *Pflügers Arch. ges. Physiol.* **407**, 526–533.

HAUGLAND, F. N. (1987). A voltage clamp analysis of membrane potassium currents in larval muscle fibers of the *Shaker* mutants of *Drosophila*. PhD thesis, University of Iowa.

HAUGLAND, F. N. AND WU, C. F. (1990). A voltage-clamp analysis of gene-dosage effects of the *Shaker* locus on larval muscle potassium currents in *Drosophila*. *J. Neurosci.* (in press).

HENON, B. K. AND IKEDA, K. (1981). Changes in membrane properties of the *Drosophila* dorsal longitudinal flight muscles induced by sodium pump inhibitors. *J. exp. Biol.* **90**, 175–183.

HERMANN, A. AND GORMAN, A. L. F. (1984). Action of quinidine on ionic currents of molluscan pacemaker neurons. *J. gen. Physiol.* **83**, 919–940.

HUDDART, H. AND WOOD, D. W. (1966). The effect of DNP on the resting potential and ionic content of some insect skeletal muscle fibers. *Comp. Biochem. Physiol.* **18**, 681–688.

IMAIZUMI, Y. AND GILES, W. R. (1987). Quinidine-induced inhibition of transient outward current in cardiac muscle. *Am. J. Physiol.* **253**, H704-H708.

JAN, L. Y. AND JAN, Y. N. (1976). Properties of the larval neuromuscular junction in *Drosophila melanogaster*. *J. Physiol., Lond.* **262**, 189–214.

KAPLAN, W. D. AND TROUT III, W. E. (1969). The behavior of four neurological mutants of *Drosophila*. *Genetics* **61**, 399–409.

OSBORNE, M. P. (1967). Supercontraction in the muscles of the blowfly larva: an ultrastructural study. *J. Insect Physiol.* **13**, 1471–1482.

RHEUBEN, M. (1972). The resting potential of moth muscle fiber. *J. Physiol. Lond.* **225**, 529–554.

SALKOFF, L. (1983). *Drosophila* mutants reveal two components of fast outward current. *Nature* **302**, 249–251.

SALKOFF, L. AND WYMAN, R. (1981). Genetic modification of potassium channels in *Drosophila Shaker* mutants. *Nature* **293**, 228–230.

SALKOFF, L. AND WYMAN, R. (1983). Ionic currents in *Drosophila*. *J. Physiol., Lond.* **337**, 687–709.

SINGH, S. AND WU, C.-F. (1987). Genetic and pharmacological separation of four potassium currents in *Drosophila* larvae. *Soc. Neurosci. Abstr.* **13**, 579.

SINGH, S. AND WU, C.-F. (1989). Complete separation of four potassium currents in *Drosophila*. *Neuron* **2**, 1325–1329.

SINGH, S., WU, C.-F. AND GANETZKY, B. (1986). Interactions among different K^+ and Ca^{2+} currents in normal and mutant *Drosophila* larval muscles. *Soc. Neurosci. Abstr.* **12**, 559.

SUZUKI, N. AND KANO, M. (1977). Development of action potential in larval muscle fibers in *Drosophila melanogaster*. *J. cell. Physiol.* **93**, 383–388.

THOMAS, M. V. (1984). Voltage-clamp analysis of a calcium-mediated potassium conductance in cockroach (*Periplaneta americana*) central neurons. *J. Physiol., Lond.* **350**, 159–178.

WEI, A. AND SALKOFF, L. (1986). Occult *Drosophila* calcium channels and twinning of calcium and voltage-activated potassium channels. *Science* **233**, 780–782.

WU, C.-F. AND GANETZKY, B. (1988). Genetic and pharmacological analyses of potassium channels in *Drosophila*. In *Neurotox '88: Molecular Basis of Drug and Pesticide Action* (ed. G. G. Lunt), pp. 311–323. Amsterdam: Elsevier.

WU, C.-F., GANETZKY, B., HAUGLAND, F. N. AND LIU, A.-X. (1983). Potassium currents in *Drosophila*: Different components affected by mutations of two genes. *Science* **220**, 1076–1078.

WU, C.-F. AND HAUGLAND, F. N. (1985). Voltage clamp analysis of membrane currents in larval muscle fibers of *Drosophila*. Alteration of potassium currents in *Shaker* mutants. *J. Neurosci.* **5**, 2626–2640.

WU, C.-F., TSAI, M.-C., CHEN, M.-L., ZHONG, Y., SINGH, S. AND LEE, C. Y. (1989). Actions of dendrotoxin on K^+ channels and its synergistic effect on neuromuscular transmission with K^+ channel-specific drugs and mutations in *Drosophila melanogaster*. *J. exp. Biol.* **147**, 21–41.

YAMAOKA, K. AND IKEDA, K. (1988). Electrogenic responses elicited by transmembrane depolarizing current in aerated body wall muscles of *Drosophila melanogaster* larvae. *J. comp. Physiol.* **163**, 705–714.

YEH, J. Z., OXFORD, G. S., WU, C. H. AND NARAHASHI, T. (1976). Interactions of aminopyridines with potassium channels of squid axon membranes. *Biophys. J.* **16**, 77–81.