The Roles of Potassium Currents in *Drosophila* Flight Muscles

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The roles of different K^+ currents in regulating the generation and waveform of action potentials in *Drosophila* dorsal longitudinal flight muscles (DLMs) were examined in current-clamp experiments. In response to depolarizing current, DLMs displayed an initial transient rectification of the electronic potential lasting for up to hundreds of milliseconds. This delay in excitation was followed by oscillations or graded spikes that finally gave way to sharply rising spikes. Previous voltage-clamp studies of DLMs have revealed an inward Ca^{2+} current and at least three K^+ currents: I_A and I_K, which are voltage-dependent, and I_C, which is Ca^{2+} dependent. I_A and I_C are early inactivating currents, while I_K is a slow, noninactivating current. In mature adults, selective elimination of I_A either with Shaker (*Sh*) mutations or with 4-aminopyridine (4-AP), had no effect on spike duration or on the delay in excitation. In contrast, when I_C was specifically eliminated with the slowpoke (*slo*) mutation, there was no delay before excitation, the amplitude of the spikes was significantly increased, and the spike duration was increased by 10-fold. Similar results were obtained by reducing I_C in normal muscle by intracellular injections of EGTA or by use of low Ca^{2+} saline. Furthermore, DLM spikes evoked in *slo* by stimulation of the motorneuron were also broadened, suggesting that I_C functions in a similar manner during normal flight as in current-clamped muscles. Elimination of I_K along with I_A and I_C in saline containing tetraethylammonium or Ba^{2+} resulted in further prolongation of the DLM spike. In Ba^{2+} saline, there was an additional increase in spike amplitude as well. We conclude that in mature adults, I_C, rather than I_A, plays the major role in repolarization of DLM spikes and in the delay before excitation. In the absence of I_C, spike duration and amplitude is limited by I_K and Ca^{2+} inactivation. We also present evidence showing that prior to the full development of I_C in the young adult, I_A is responsible for early spike repolarization.

The diversity in electrical properties among excitable cells is determined in large part by the array of ion channel types present in the membrane of different cells. To elucidate the underlying basis of the signaling properties of particular excitable cells, it is necessary to identify the types of ion channels present and to assess the functional role(s) of each channel type (e.g., Thompson, 1977; Miller, 1987). The availability of toxins and other pharmacological agents that specifically block certain channel types have been extremely useful in studies to obtain this kind of information. In *Drosophila melanogaster* this approach can be further extended by the use of mutations that specifically disrupt or eliminate the function of particular channels (for reviews, see Ganetzky and Wu, 1986; Salkoff and Tanouye, 1986; Tanouye et al., 1986).

The flight muscles of *Drosophila* adults and the body wall muscles of larvae are favorable for studies of this kind because the various ion currents present in these cells have been studied by voltage-clamp experiments and because several of these currents can be individually blocked by different mutations (Salkoff and Wyman, 1981, 1983; Salkoff, 1983a; Wu et al., 1983; Wu and Haugland, 1985). In the dorsal longitudinal flight muscles (DLMs) of mature adult flies, a single inward current and at least 3 distinct outward currents have been described (Salkoff and Wyman, 1983). The inward current, which gives rise to the muscle action potential, is carried by Ca^{2+}. The outward currents are carried by K^+ and comprise 2 classes: voltage-dependent K^+ channels (I_A and I_K) and Ca^+-dependent K^+ channels (I_C). I_A and I_C are early inactivating currents, while I_K is a slow noninactivating current. Recently, an additional slow, Ca^{2+}-dependent K^+ current has been described in pupal DLMs (Wei and Salkoff, 1986). These K^+ currents are similar to those that have been identified and studied in other systems.

In axons of squid and other organisms, I_K has been shown to play the primary role in repolarization of action potentials (Hodgkin and Huxley, 1952). A fast, inactivating Ca^{2+}-dependent K^+ current, similar to I_C, has been shown to be involved in repolarizing action potentials in the somata of some vertebrate neurons (Adams et al., 1982; MacDermott and Weight, 1982; Rogawski et al., 1985). In various molluscan neurons, and in some vertebrate neurons as well, I_A modulates the voltage dependence of spike frequency and is also responsible for the delay of excitation in response to steady depolarization (Conner and Stevens, 1971a, b; Byrne, 1980; Getting, 1983; Shimahara, 1983; Segal et al., 1984).

In this paper, we describe the respective roles of the different K^+ currents in regulating the generation and waveform of action potentials in the *Drosophila* DLMs. In particular, we examined the consequences of selectively eliminating various K^+ currents on action potential repolarization and delayed excitation. These experiments were initiated with the isolation of a mutation, named slowpoke (*slo*), that specifically eliminates I_C (Elkins et al., 1984, 1986). We also took advantage of Shaker (*Sh*) mutations that specifically eliminate I_A (Salkoff and Wyman, 1981; Salkoff 1983a; Wu and Haugland, 1985). Conclusions about the
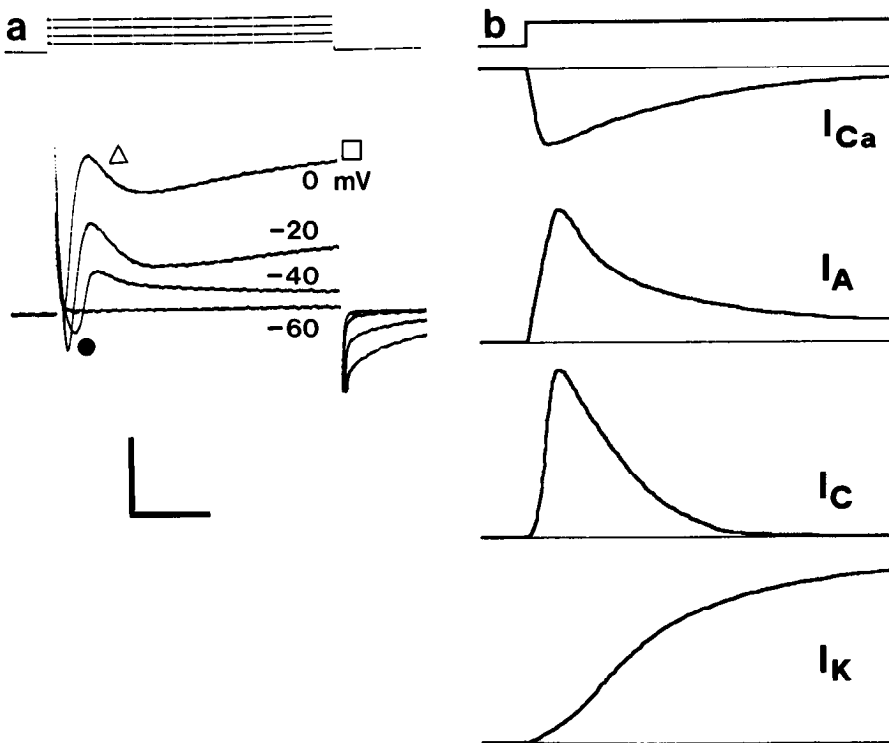
Figure 1. *a*, Membrane currents in dorsal longitudinal flight muscles (DLMs). Current responses (*lower traces*) to voltage-clamp steps (*upper traces*) in a normal DLM fiber. The membrane was held at -80 mV and stepped to -60, -40, -20, and 0 mV for 140 msec, as indicated. The pulse to -60 mV activates only passive leakage current. At -40 mV an inward (downward) Ca^{2+} current (*closed circle*) is followed by an inactivating outward current (*triangle*). This outward current is separable into 2 components, the voltage-dependent I_A and the Ca^{2+}-dependent I_C (see text for details). At -20 mV, a delayed outward current, I_K, is evident (*square*). *b*, Idealized diagram of the individual ion currents, showing their approximate amplitudes and time courses in response to the voltage step to 0 mV above. Calibration: 0.2 V, 400 nA, 40 msec.

roles of these currents based on the analysis of mutants were confirmed and extended by ion-substitution experiments and by the use of specific blocking agents. From these experiments, it appears that I_C plays the major role both in action potential repolarization and delayed excitation in these cells. The possible functions of the other K^+ currents are also discussed.

Materials and Methods

Mutants used in these studies include Sh^{KS133} (Jan et al., 1977) and *slo* (Elkins et al., 1986). Previous voltage-clamp experiments have demonstrated that Sh^{KS133} causes the specific elimination of I_A (Salkoff and Wyman, 1981; Salkoff, 1983a; Wu and Haugland, 1985), while *slo* specifically abolishes I_C (Elkins et al., 1986). Neither mutation causes a detectable alteration in any other current in the DLMs. Control flies were from the Canton-S wild-type strain.

Adult female flies 1–2 d posteclosion were used for all experiments except where noted otherwise. To observe the responses of DLMs to motorneuron activity, a 20 mV, 200 μsec pulse was applied to the cervical connective with a pair of insulated tungsten electrodes that were electrically isolated from ground, thus stimulating the giant fiber pathway that includes the DLM motorneurons (King and Wyman, 1980). A tungsten electrode inserted through the abdomen measured a reference potential, while the fly was immobilized in air by gluing it to a pin with cyanoacrylate adhesive.

For current- and voltage-clamp experiments, the dorsal thoracic cuticle was removed with tungsten scalpels and the DLMs were exposed to saline. The thoracic and abdominal spiracles were aerated as reported by Ikeda and Kaplan (1974). The preparation was cooled on a Peltier plate to 18°C for current-clamp and to 4°C for voltage-clamp experiments. The saline was composed of 0.05 (low) or 1.8 mM (normal) $CaCl_2$, 8 mM $MgCl_2$, 123 mM NaCl, 2 mM KCl, 35.5 mM sucrose and buffered at pH 7.1 with 5 mM HEPES.

All experiments were performed on DLM 45a or b according to the nomenclature of Miller (1965). Only cells with a resting potential of -70 mV or greater were used. Glass microelectrodes were filled with 2.5 M KCl and beveled using a silicon carbide grit (Kripke and Ogden, 1974) to 5–10 MΩ for easier penetration and drug injection. Each experiment was repeated on 3–15 individuals.

Two-microelectrode current-clamp and voltage-clamp experiments were performed according to standard methods with a Dagan 5700

current/voltage clamp. Signals were photographed from a Tektronix storage oscilloscope.

For EGTA injection, a current-passing electrode was filled with 100 mM EGTA. Long DC pulses of 10 nA current were applied for up to 2 min, producing an estimated EGTA transfer of 6×10^{-12} moles. Active responses were then evoked by applying 120 msec depolarizing pulses.

Results

Ion currents in the DLM membrane

In addition to the inward Ca^{2+} current, at least 3 outward K^+ currents are present in the DLMs of normal flies (Salkoff and Wyman, 1983; Salkoff, 1983a). Voltage-clamp traces from a normal DLM, in which these currents are superimposed, are shown in Figure 1. During a voltage step to -40 mV, the inward (downward) current is followed by 2 early, inactivating outward currents that superimpose, the Ca^{2+}-dependent I_C and the voltage-dependent I_A. At steps to -20 and 0 mV, these 2 outward currents are followed by I_K, which does not inactivate during the current pulse.

Delayed excitation and graded spikes

When a normal DLM was depolarized with a steady current, it did not immediately respond with an all-or-none action potential (Salkoff and Wyman, 1980). Instead, voltage became partially rectified, reaching a plateau at about -50 mV. After charging the membrane for tens to hundreds of milliseconds, oscillations or graded spikes of increasing amplitude were often seen that gave way to sharply rising spikes (Fig. 2*a*). As the magnitude and rate of depolarization were increased, the extent of the delay before spiking decreased (Salkoff and Wyman, 1980). At peak amplitude, the spikes did not overshoot 0 mV but reached membrane potentials of about -10 mV.

To investigate the roles of I_A and I_C in the delay of excitation, we examined the response to steady depolarization under conditions where each of these currents was selectively blocked.

Figure 2. Membrane potential changes produced by constant depolarizing current pulses in normal and mutant DLMs. In this figure and those that follow, the traces shown are representative of all individuals examined for a given genotype and experimental condition. The number of cells examined in each case is shown. *a*, Normal (Canton S), $n = 50$. *b*, Normal in saline containing 5 mM 4-AP, $n = 5$. *c*, Shaker mutant (Sh^{KS133}), $n = 10$. *d*, Slowpoke mutant (slo), $n = 70$. *e*, slo in saline containing 5 mM 4-AP, $n = 5$. *f*, Sh^{KS133}; slo double mutant, $n = 10$. Temperature for all current-clamp experiments: 18°C. Calibration: 20 mV, 100 nA, 20 msec.
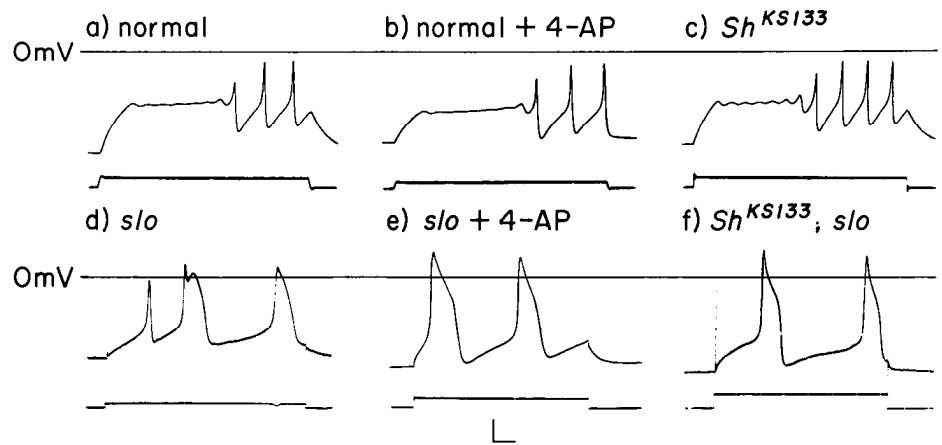
Although I_A is completely absent in the DLMs of Sh^{KS133} (Salkoff and Wyman, 1981), delayed excitation occurs as in normal flies (Fig. 2c). Similarly, when normal flies are treated with 4-AP, which selectively eliminates I_A (Salkoff, 1983a), there is no effect on delayed excitation (Fig. 2b). These results indicate that the DLMs, unlike molluscan neurons, do not require I_A for delayed excitation.

The result was very different when similar experiments were performed on slo, a mutation that has been shown to eliminate I_C completely and specifically (Elkins et al., 1986). In the DLMs of this mutant, a depolarizing pulse caused the cell to reach firing threshold without any delay (Fig. 2d). Since a specific block in I_C results in the loss of delayed excitation, it is evident that this current normally plays the major role in producing delayed excitation.

The response to steady depolarization in slo DLMs is unusual in another respect. The first spike evoked by depolarization rapidly repolarizes and is followed by spikes whose duration is markedly prolonged (Fig. 2d). This raises the question of how the spike waveform is normally shaped by the various K⁺ currents.

Roles of I_A and I_C in spike repolarization

Under current-clamp conditions, a train of action potentials is evoked in DLMs of normal flies that repolarize after 1.9 ± 0.2 msec (measured at ½ maximal amplitude) (Fig. 2a). These spikes do not overshoot. The action potentials evoked in Sh^{KS133} DLMs or in normal DLMs treated with 4-AP did not differ from normal in amplitude or duration (Fig. 2, b, c; cf. Salkoff, 1985). In contrast, following the first spike of a train, the action potentials evoked in DLMs of slo flies remained depolarized for 19 ± 1 msec and were invariably overshooting, reaching membrane potentials around +10 mV (Fig. 2d). These results suggest that during a train of action potentials evoked under current-clamp conditions, I_C but not I_A is normally required for early spike repolarization and for limiting the spike amplitude (Elkins et al., 1984; Salkoff, 1985).

However, even in slo DLMs, the first spike in a train usually repolarized with approximately normal kinetics and did not overshoot, suggesting that repolarization of the first spike in a train occurs by a different mechanism from that of subsequent spikes. To investigate further the mechanism involved in repolarization of the first spike in a train, we assayed the response in flies lacking both I_A and I_C. Flies lacking both currents were

produced either by treating slo flies with 5 mM 4-AP or by constructing Sh^{KS133}; slo double mutants. In both of these experiments, the first spike evoked under current-clamp conditions became as prolonged as the following spikes and were overshooting as well (Fig. 2, e, f). We conclude that the prompt repolarization of the first spike in a series is normally accomplished by I_A. Elimination of both I_A and I_C then results in uniformly prolonged spikes of increased amplitude.

If I_A is sufficient to bring about the early repolarization of the first spike in a series, why is it apparently unable to do the same thing for subsequent spikes? One possible explanation is the inactivation of I_A during prolonged depolarization (Salkoff, 1985). Measurements of the exponential time constant for recovery from inactivation of I_A in voltage-clamp experiments (Salkoff and Wyman, 1983) suggest that, at the membrane potentials reached between spikes in our experiments, I_A would not appreciably recover from inactivation.

To establish that the effect of slo on repolarization of the DLM spike could be attributed solely to the elimination of I_C rather than to some additional undetected defect in the mutant, we reduced I_C experimentally in normal flies and examined the consequences on DLM spikes. In one series of experiments, DLMs were current-clamped after the extracellular Ca^{2+} was lowered from 1.8 to 0.05 mM. In voltage-clamp experiments it was found that this lowering of Ca^{2+} resulted in a 4-fold reduction in the amplitude of I_C (not shown). Under these conditions, long-duration spikes, albeit of reduced amplitude, were readily evoked by a 120 msec depolarizing pulse and the delay before excitation was shortened relative to normal (Fig. 3a).

A second series of experiments involved the injection of EGTA into normal DLMs to chelate the intracellular free Ca^{2+} that is required to activate I_C. After injection, depolarizing pulses evoked one to several spikes of short duration followed by a series of long-lasting spikes (Fig. 3b). The effect of EGTA on spikes was temporary. Within 10 sec after injection, the spikes returned to their normal duration of 2 msec. During the time that the spikes were prolonged, there was also a reduction in the delay before excitation (Fig. 3b). In contrast, in slo, injection of EGTA into the DLMs did not result in any additional increase in spike duration (Fig. 3c). That the slo phenotype can be mimicked in normal DLMs by reducing I_C in these cells whereas similar treatment has no effect on slo supports the conclusion that the altered DLM spikes in slo results specifically from the loss of I_C.
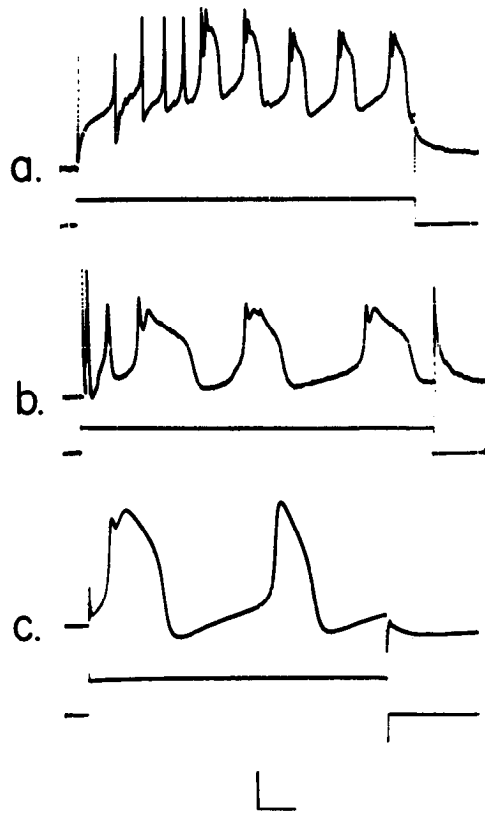
Figure 3. Membrane potential changes in DLMs produced by constant depolarizing current pulses in low Ca^{2+} or after injection of EGTA. *a*, Normal DLM in saline containing low (0.05 mM) Ca^{2+} ($n = 5$). *b*, Normal DLM following intracellular injection of EGTA. The voltage spikes at the start and end of the pulse probably result from clogging of the electrode tip by EGTA ($n = 5$). *c*, *slo* DLM following intracellular injection of EGTA ($n = 7$). The failure of the first spike to repolarize quickly in this particular cell is attributed to slight damage to the cell during injection leading to partial loss of resting potential and inactivation of I_A. Calibration: *a*, 20 mV, 100 nA, 50 msec. *b* and *c*, 20 mV, 100 nA, 20 msec.

Role of I_A and I_C in young adults

The control of DLM spike repolarization appears to differ in mature adults compared with flies newly eclosed from the pupal case. It was observed that whereas DLM spikes in newly eclosed normal flies repolarize as quickly as they do in mature adults, the spikes in newly eclosed Sh^{KS133} adults are of longer duration (Salkoff, 1985 and Fig. 4). Furthermore, the DLM spikes in *slo* adults aged 1–2 hr posteclosion are not prolonged but repolarize as quickly as in normal flies of the same age (Fig. 4). Spike repolarization in newly eclosed Sh^{KS133}; *slo* double mutants was no more abnormal than for Sh^{KS133} alone (Fig. 4). These results indicate that in newly eclosed adults, in contrast to mature adults, I_A does play an important role in repolarization of DLM spikes (cf. Salkoff, 1985), while I_C is unnecessary. In addition, the DLM responses in newly eclosed flies differ from those of mature adults in that the delay in excitation is not observed in normal flies (Fig. 4).

These results are consistent with the observation that I_C is not fully present in pupal muscles but appears only gradually in the DLM membrane over a period of hours following eclosion (Salkoff, 1985). Apparently, while I_A is necessary and sufficient to repolarize the DLM spikes in newly eclosed adults, I_C must develop fully before there is a delay in excitation.

Role of I_K and Ca^{2+} inactivation in spike repolarization and refractory period

No mutants are available that specifically eliminate I_K in DLMs. Nor is it possible pharmacologically to remove this current in DLMs without affecting other K^+ currents as well. Nonetheless, it is possible to infer the role of I_K in spike repolarization by comparing DLM responses under conditions where I_A, I_C, and I_K are all blocked with the responses obtained when only I_A and I_C are missing. In voltage-clamp experiments 100 mM TEA was found to block all 3 outward currents completely, whereas the inward Ca^{2+} current remained intact (not shown). In the presence of 100 mM TEA under current-clamp conditions, normal
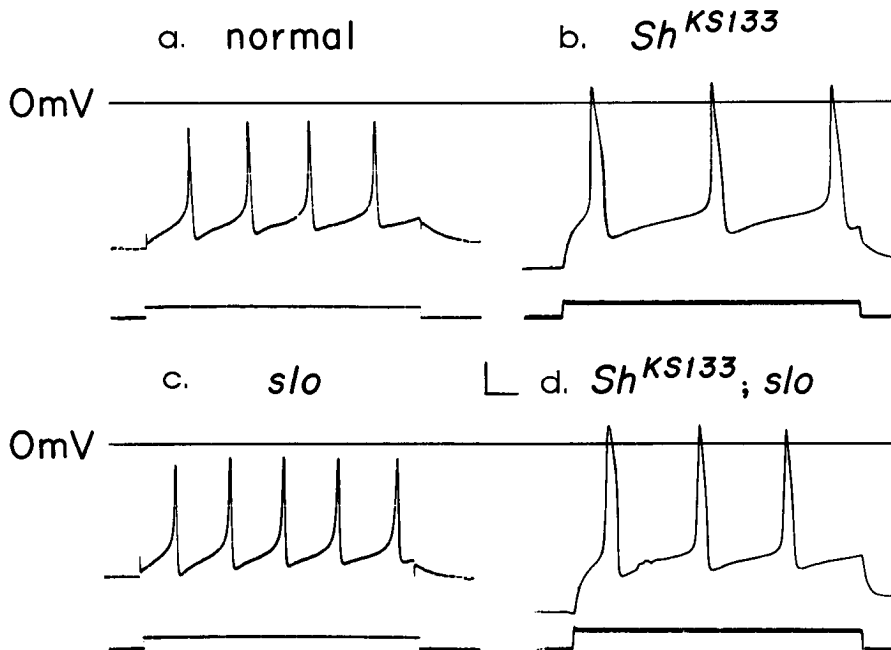
Figure 4. Membrane potential changes in DLMs of newly eclosed normal and mutant adults produced by constant depolarizing current pulses. Age of flies in each case is one hour posteclosion. *a*, Normal, $n = 6$. *b*, Sh^{KS133}, $n = 10$. *c*, *slo*, $n = 10$. *d*, Sh^{KS133}; *slo* double mutant, $n = 5$. Calibration: 20 mV, 100 nA, 20 msec.

Figure 5. Membrane potential changes in normal DLMs produced by constant depolarizing current pulses in the presence of K⁺-channel blockers. *a,* Normal in saline containing 100 mM TEA (*n* = 20). The responses from two consecutive current pulses spaced 1.5 sec apart are superimposed. *b,* Normal in saline containing 0 mM Ca^{2+} and 10 mM Ba^{2+} (*n* = 20). Calibration: 20 mV, 100 nA, 0.1 sec in *a* and 0.5 sec in *b*.
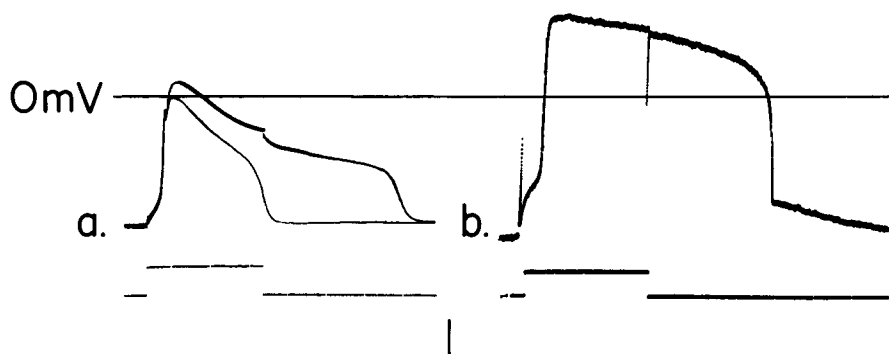
DLMs produced very long-lasting spikes up to 4 sec in duration (Fig. 5*a*). The amplitude of these spikes was greater than normal but comparable to that previously observed in *slo* or *Sh*^KS133; *slo*. If a second current pulse was applied soon after repolarization of the first response, the second response that was evoked usually had less than half the duration of the first (Fig. 5*a*). One possible reason for this result is that the prolonged Ca^{2+} influx during the first spike results in an increased level of Ca^{2+} current inactivation, which is capable of terminating the second spike.

In voltage-clamp experiments, 10 mM Ba^{2+} resembled TEA in its effects on blocking outward currents (not shown). Ba^{2+} was found to reduce I_K severely in DLMs, as it does in squid axon (Eaton and Brodwick, 1980), and blocked I_A almost completely as well. In addition, Ba^{2+} either blocked or failed to activate I_C. However, in contrast to TEA-treated muscle fibers, Ba^{2+} also greatly reduced inactivation of the inward current. In saline containing 10 mM Ba^{2+} and 0 mM Ca^{2+}, the DLM response evoked under current clamp remained depolarized for periods ranging from several seconds to more than a minute and reached membrane potentials of greater than +30 mV (Fig. 5*b*). Similar responses to these were observed with *slo* DLMs in the presence of Ba^{2+} (not shown).

These data suggest that when I_C is absent, as in *slo* flies, repolarization of the DLM spike is eventually accomplished by I_K. Consequently, blocking of I_K along with I_C and I_A results in spikes that can persist for several seconds or more.

Repolarization of synaptically evoked spikes

The prolonged DLM spikes observed in *slo* mutants were elicited under current-clamp conditions. It was thus of interest to ask whether loss of I_C would have a similar effect under experimental conditions that more closely resembled normal flight.
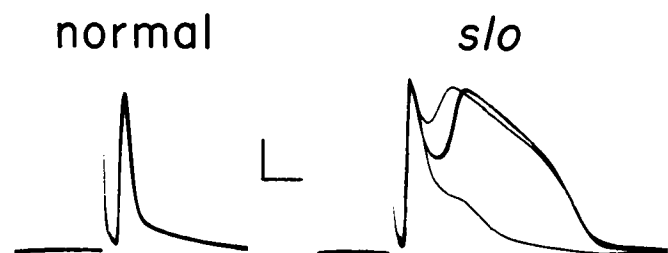
During normal flight, the firing frequency of the DLMs is about 5 Hz (Harcombe and Wyman, 1978; Koenig and Ikeda, 1980). In Figure 6 we show the response of normal and *slo* DLMs to repetitive stimulation at a frequency of 5 Hz. In normal flies, each spike repolarized within 2 msec (Fig. 6*a*). In contrast, in *slo* flies the first spike repolarized with a normal time course, but subsequent spikes were about 20 msec in duration (Fig. 6*b*). These results are the same as those observed in the current-clamp experiments. It therefore seems very likely that I_C plays the major role in repolarization of DLM spikes during normal flight. This conclusion is consistent with our previous observation that *slo* mutants fly very poorly or not at all (Elkins et al., 1986).

Discussion

In these experiments we have used mutations in *Drosophila* in a manner similar to specific pharmacological agents to dissect the physiological roles for the different K⁺ currents in flight muscles. Despite their similar amplitude and time course in voltage-clamp experiments, I_A and I_C apparently have very different functions in DLMs. In other systems, delayed excitation is dependent upon the function of I_A (Conner and Stevens, 1971a, b; Byrne, 1980; Getting, 1983; Shimahara, 1983; Segal et al., 1984). In *Drosophila* DLMs, however, elimination of I_A by *Sh*^KS133 has no detectable effect on delayed excitation. In contrast, specific elimination of I_C by *slo* results in the complete loss of delayed excitation. Similarly, repolarization of the DLM spikes is essentially normal in the absence of I_A but prolonged by a factor of 10 when I_C is blocked. In addition, the interspike interval is increased from 20 to 40 msec in *slo* but is normal in *Sh*. Thus, at least under current-clamp conditions, I_C plays the major role in controlling these aspects of the electrical activity of DLMs.

Loss of I_C probably alters DLM responses during flight in a manner similar to that observed in current-clamp experiments, consistent with the diminished flight ability of *slo* flies (Elkins et al., 1986). For example, we observed that DLM spikes evoked by endplate potentials in response to stimulation of the motorneuron at frequencies corresponding to that of normal flight were as prolonged as those observed under current-clamp conditions. It will be of interest to determine whether such prolonged DLM spikes also cause an alteration in the interspike interval during normal flight. It remains unclear whether the phenomenon of delayed excitation has a physiological role in the normal activity of DLMs. The endplate potential during normal flight is very large and fast rising. As a result, it does not produce the graded depolarization that causes delayed excitation as during current-clamp experiments but instead im-

normal   *slo*

Figure 6. Membrane potential changes produced in normal and *slo* DLMs in response to repetitive stimulation of the motorneuron. In each case, 5 consecutive responses are shown superimposed. The motorneuron was stimulated at a frequency of 5 Hz (*n* = 100). Calibration: 20 mV, 20 msec.

mediately activates a full-sized DLM spike (Boettinger, 1951). However, in muscles of some other arthropods, graded spiking can be observed in response both to current injection (Fatt and Katz, 1953) and normal synaptic excitation (Hoyle, 1967). Therefore, in organisms related to flies, there may be an important biological role for graded muscle spiking, and, as in *Drosophila*, the mechanism that produces these graded responses may also depend on the action of a Ca^{2+}-dependent K^+ current.

In newly eclosed flies, in contrast with those several hours older, I_A plays an important role in regulating the duration, amplitude, and frequency of DLM spikes. These functions are apparently taken over within a few hours after eclosion by I_C (cf. Salkoff, 1985). Elimination of I_A in newly eclosed flies has effects on DLM activity similar to those caused by elimination of I_C in mature adults, including overshooting spikes, prolonged spike duration, and increased interspike interval. Thus, DLM responses are normal in newly eclosed *slo* flies but not in *Sh* flies. I_C is known to be the last of the ion currents to appear in DLMs and is not fully mature in newly eclosed adults (Salkoff, 1983b, 1985). At the time it replaces I_A as the repolarizing current, I_C is undergoing a marked increase in amplitude (Salkoff, 1985).

The reason why I_A is insufficient to bring about rapid repolarization of DLM spikes in mature adults is not yet understood. The results suggest some type of age-dependent change in the relative strengths of I_A and the inward Ca^{2+} current. For example, I_A may decrease in amplitude or it may remain constant while the DLM membrane area or Ca^{2+} current increase. The Ca^{2+} current first becomes evident only around the time of eclosion (Salkoff, 1985) and may subsequently increase in amplitude. The possible biological significance of this transition of DLM spike repolarization from a mechanism that is I_A dependent to one that is I_C dependent remains to be examined.

Although the specific defects of the *Sh* and *slo* mutations permit the roles of I_A and I_C in DLM activity to be dissected with some precision, the role of I_K is less clear owing to the lack of any genetic or pharmacological means of blocking this current specifically. Nonetheless, our results indicate that in the absence of I_C, repolarization of the DLM spike is accomplished eventually by I_K. When I_K is eliminated along with I_C, spike duration can be as long as several seconds. We have yet to determine the physiological consequences of eliminating I_K from the DLMs while leaving I_C functionally intact.

When all K^+ currents have been eliminated, Ca^{2+} current inactivation may itself terminate the prolonged muscle spike. The difference we observed in duration and amplitude of the DLM response in Ba^{2+}- versus TEA-containing saline, despite the fact that both treatments greatly reduce or eliminate all outward currents, may then be attributable to the additional block of inward current inactivation in Ba^{2+}-containing saline. The data suggest that inactivation of Ca^{2+} current may also be responsible for the long interspike interval observed in *slo* DLMs. When all K^+ currents are present or when only I_A is missing, the interval between spikes in these experiments was about 20 msec. In the absence of I_C, this interval increased to more than 40 msec. Because Ca^{2+} current inactivation in insect muscle is strongly dependent on internal free Ca^{2+} (Ashcroft and Stanfield, 1981), it may be that the prolonged Ca^{2+} influx in *slo* DLMs transiently increases the cytosolic Ca^{2+} leading to more complete inactivation of Ca^{2+} current. When Ca^{2+} influx was further increased in TEA-treated DLMs, there was an apparent spike

refractory period of a second or more between the very prolonged responses (data not shown).

Without the *slo* mutation, it would have been very difficult to demonstrate directly the roles of I_C in DLM electrical activity for lack of a method to eliminate this current specifically. Charybdotoxin, a peptide toxin from scorpion venom, preferentially blocks I_C channels in various systems (Hermann, 1985; Miller et al., 1985; Obaid et al., 1985), including *Drosophila* DLMs (Elkins et al., 1986). However, at doses required to block I_C as completely as in the *slo* mutant, the toxin also reduced the Ca^{2+} inward current (T. Elkins, unpublished observations). I_C is likely to have important functional roles outside the DLMs. For example, a Ca^{2+}-dependent K^+ current is thought to play a role in the repolarization of the presynaptic terminal of the larval neuromuscular junction (Jan et al., 1977; Ganetzky and Wu, 1982). In addition, a Ca^{2+}-dependent K^+ current has been implicated in the ionic mechanism underlying associative conditioning in another invertebrate, *Hermissenda* (Grover and Farley, 1985). Thus, the *slo* mutation may prove equally useful in the analysis of the physiological roles of Ca^{2+}-dependent K^+ currents in neuronal activity and in more complex neural processes.

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