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Facilitation of membrane electrical excitability in *Drosophila*

(potassium currents/membrane biophysics/insect muscle)

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ABSTRACT **Prior electrical activity in the indirect flight muscles of *Drosophila* facilitates membrane excitability. The mechanism of facilitation involves the inactivation of an early, fast, transient outward current by prior membrane depolarization. In the facilitated state the calcium-dependent spike-like response has a decreased current and voltage threshold. The facilitated state persists for 1.5 sec after a membrane active response. A single nerve-driven spike is sufficient to facilitate membrane excitability.**

Most known cases of changes in neural function with use depend on synaptic mechanisms. There are some examples, however, showing that the excitable membrane itself can be plastic in its response to excitation (1–5). As a prelude to genetic analysis of excitable membranes in *Drosophila melanogaster*, physiological analysis of the dorsal longitudinal flight muscle (DLM) was undertaken. In the course of these studies we found striking changes in the excitability characteristics of the membrane, depending on prior use. For example, it was found that a single prior spike may evoke a large active membrane response to a stimulus that previously elicited no active response at all. The mechanism that is apparently responsible for these phenomena involves the inactivation of a transient inhibitory outward current similar to the "A" current first analyzed by Conner and Stevens (6) in molluscan neurons.

METHODS AND MATERIALS

Adult female *D. melanogaster* of wild-type strain Canton-S or the temperature-sensitive paralytic mutant stock *shi(ts-1)* were used in these experiments. Muscle membrane properties were found to be the same for these strains. In experiments requiring decreased transmitter release, the *shi(ts-1)* strain was used. The *shi(ts-1)* mutation confers a presynaptic temperature-sensitive defect that suppresses neurotransmitter release at temperatures higher than 29°C (7, 8). Thus, the use of this mutant together with appropriate temperature controls permits the experimental regulation of the size of the excitatory junction potentials (ejps). The techniques for evoking muscle responses via nerve stimulation are published (8).

For recording and current injection, a single muscle fiber was impaled with two 3 M KCl-filled glass microelectrodes. Current was measured with a Ag-AgCl ground electrode by monitoring the voltage across a 10 kΩ resistor to ground. Flies were mounted so that the spiracles had access to air, and the dorsal thorax was opened and exposed to saline (128 mM NaCl/4.7 mM KCl/1.8 mM $CaCl_2$/1.0 mM phosphate, pH 6.9). For experiments requiring low sodium and the complete removal of calcium, 1.4 mM [ethylenebis(oxyethylenenitrilo)]tetraacetic acid was added and NaCl was partially replaced with choline chloride (to 114 mM). Magnesium chloride (14 mM) was added for membrane stability. Muscle fibers had resting membrane potentials ranging from 60 to 90 mV. All fibers with resting potentials within this range showed the excitability changes described here. Within this range those with higher resting potentials required less current to produce spike-like responses and these responses were of greater amplitude. Experiments were performed at 22°C with Canton-S flies unless otherwise noted. Higher temperatures affected membrane response thresholds and membrane resistance as reported (7), but the changes in membrane excitability noted in this report were observed over the full temperature range of these experiments. The voltage clamp equipment used was the same as that described by Dionne and Stevens (9).

RESULTS

The excitability increase in the DLM could be demonstrated by injecting two sequential current pulses of equal intensity and duration directly into a single muscle fiber. The second of two such pulses produced a much larger response than the first (Fig. 1*A*). The current pulses had to exceed a certain intensity to show this potentiation effect. Two pulses below this threshold level evoked identical responses even though they were applied in rapid succession (Fig. 1*B*). A single nerve-driven spike also was sufficient to potentiate the effect of a subsequent current pulse to the muscle (Fig. 1*C*). The very rapid time course of the nerve-driven spike in Fig. 1*C* contrasts with the variety of spike-like wave forms induced by injected current. The nerve-induced response is mostly a large, extremely fast-rising ejp with a smaller added active component.

A single maintained depolarizing stimulus that exceeded a certain threshold elicited a progressively increasing membrane oscillation (Fig. 2*A*). A maintained depolarizing pulse of somewhat greater intensity produced a series of spike-like responses that showed an initial increase in amplitude (Fig. 2*B*).

This growth of spike height also could be demonstrated by successive nerve-evoked responses. Fig. 2*C* shows that ejps below a certain threshold produced only passive membrane responses. Note that facilitation of neurotransmitter release did not occur in this system. Fig. 2*D* shows that, above that threshold, a train of nerve impulses produced spike-like responses that grew in amplitude.

The time course of the altered excitability following prior membrane electrical activity was investigated. Small test current pulses were applied to the muscle, one with and one without the prior application of a suprathreshold priming pulse. The effect of the priming pulse could be seen as the difference between the membrane response to the solitary test pulse and the response to the test pulse following the priming pulse. The time interval between the priming pulse and the test pulse was varied in each trace. Membrane responses to pulses applied within 1.5 sec of the priming pulse showed an increase in

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Abbreviations: ejp, excitatory junction potential; DLM, dorsal longitudinal flight muscle.

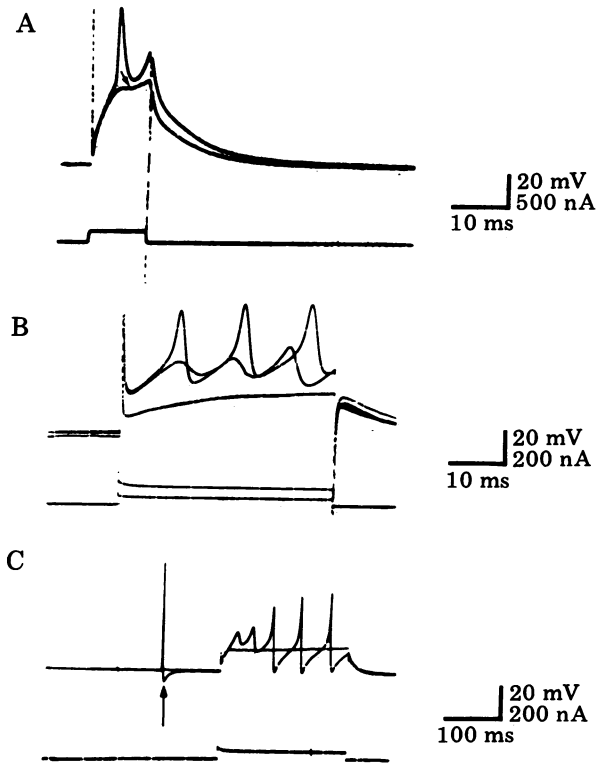

FIG. 1. Muscle membrane responses to double pulses of current injection. (*A*) Two identical current pulses were delivered 66 msec apart (superimposed lower traces). The first pulse caused only a small active response; the second pulse caused a large spike-like response (upper traces). See text for explanation of arrow. A = *shi(ts-1)* at 30°C. (*B*) Four traces are shown. Two identical current pulses (66 msec apart) were delivered at the lower intensity. They produced identical (superimposed) passive responses. Two more current pulses (66 msec apart) were applied at the higher intensity. Both pulses produced active responses (upper two traces) but the second response was much greater. (*C*) Change in membrane response when the direct current pulse was preceded by a nerve-driven spike (arrow). Two sweeps are shown, both with identical current pulses applied to the muscle but one with and one without the nerve-driven spike. The sweeps were approximately 10 sec apart. The sweep without the muscle spike shows only passive depolarization. The sweep with the spike shows active responses. (The undershoot seen in the nerve-driven spike occurs in fibers having low resting potentials.)

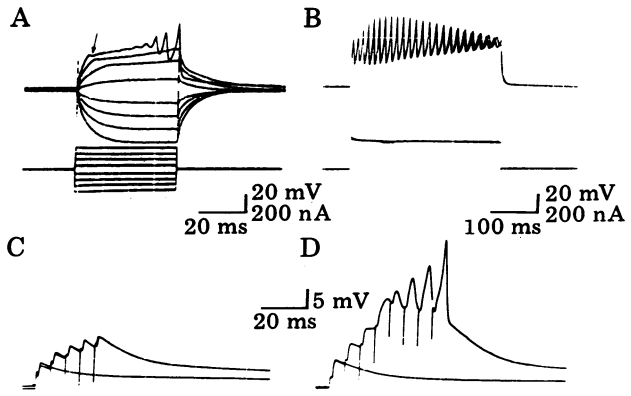

FIG. 2. Passive and active membrane responses. (*A*) Membrane responses (upper traces) to depolarizing and hyperpolarizing current pulses (lower traces). The time interval between sweeps was approximately 10 sec. Note the initiation of membrane oscillation and the growth of the spike-like responses at the largest depolarization. See text for explanation of arrow. (*B*) Membrane response to a sustained, depolarizing, suprathreshold pulse. Spikes show an initial growth in height, followed by decay. Two current pulses were applied to the membrane (separated by a time interval of many seconds). Note the precision with which the membrane recapitulated the initial response. (*C* and *D*) Nerve-evoked membrane responses. (*C*) ejps summate but show only passive membrane responses. (Note that ejps do not show facilitation.) (*D*) ejps as in *C*, but with continuing nerve stimulation, evoked a progressive growth of active spike-like responses. Neurotransmitter release was decreased in this preparation (see *Methods and Materials*). *A*, *C*, and *D* were *shi(ts-1)* at 30°C.

membrane excitability (Fig. 3*A*). Membrane responses recorded 2 sec or more after the priming pulse were identical to membrane responses without the priming pulse (not shown).

The effect of the priming pulse as shown in Fig. 3*A* may be interpreted in different ways: the priming pulse may be increasing membrane resistance, or the priming pulse may be changing the voltage threshold for active membrane responses, or both. If the effect of the priming pulse is to increase overall membrane resistance, the change should be observed for hyperpolarizing as well as depolarizing test pulses. The experiments shown in Fig. 3*B* were an attempt to detect a membrane resistance increase by passing hyperpolarizing test current pulses into the muscle. A membrane resistance increase was not detected in these experiments. A slight depolarizing shift of the baseline resulted from the priming pulse. The response to a hyperpolarizing test pulse (measured from the appropriate baseline) was the same with and without a priming pulse. The depolarizing test pulse in Fig. 3*B*, delivered without a priming pulse, produced only a small passive depolarization; the same test pulse applied after the priming pulse elicited a train of spikes.

Fig. 3*C* shows that, without prior activity, a high level of depolarization can be evoked without setting off spike-like responses. However, after activation a much smaller depolarization is sufficient to elicit spike-like responses. Thus, the effect of prior activity is to lower the threshold for further active responses.

The mechanism of enhanced excitability was further investigated by using preparations in which spiking responses were eliminated by removing Ca^{2+} and decreasing Na^+ in the saline [note that Ca^{2+} is responsible for the fast depolarizing phase of the spiking response (7)]. A purely passive membrane should show an exponential rise to a plateau in response to a current pulse. Such passive behavior was seen in response to hyperpolarizing pulses (Fig. 4*A*). The response to depolarizing current first departed from an exponential by the appearance of a repolarizing deflection (at arrow). This repolarization also was present in the standard saline (arrows, Figs. 1*A* and 2*A*). Thus, even with Ca^{2+} present the net active response induced by an initial depolarization is a repolarizing response which retards development of any regenerative active response. The early repolarizing response, however, disappeared when a second current pulse was applied shortly after the first (Fig. 4*B*). This experiment cannot be repeated in the standard saline because the second pulse would elicit regenerative spikes as in Fig. 1*A*.

The early repolarizing response was further examined under voltage clamp in Ca^{2+}-free saline. The first response to a depolarizing voltage step was a rapidly appearing, but transient outward current (Fig. 5*A*, arrow). The maintained outward current (bracket) had the appearance of the normal (delayed rectification) potassium current in that it did not rapidly inactivate and that conductance increased as membrane depolarization increased (notice the increasing current increments caused by constant voltage step increases). The transient outward current component had similarities to the "A" current of Conner and Stevens (6) which is also carried by K^+. Both de-

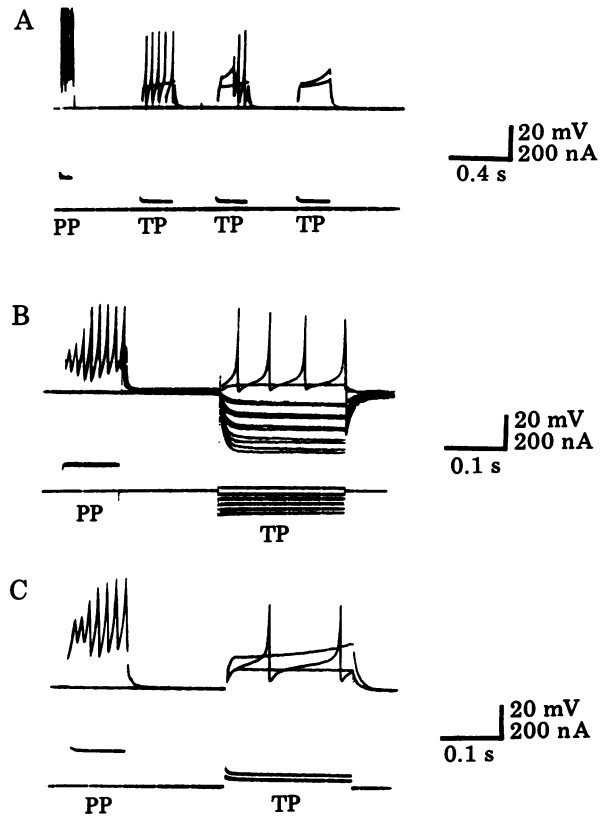

FIG. 3. Time- and voltage-dependence of potentiated responses. (*A*) Current applied is shown in lower traces; membrane responses are in upper traces. For three of the six superimposed sweeps, a large priming current pulse (PP) was delivered to the muscle; each time, spike-like membrane responses were elicited (PP upper trace). At various times after the priming pulse, a smaller test pulse (TP) was delivered to the muscle (each of the three sweeps that begins with a priming pulse also contains a single test pulse). At 0.5- and 1.0-sec delays, the test pulse also elicited spike-like responses. At 1.5 sec, the response was still above control but was not spike-like. As a control, in three sweeps only test pulses were given, with no priming pulse. The three control responses were all equal and small. (*B*) Membrane conductance changes after a priming pulse (and a decrease of the current threshold of the spiking response). Twelve traces are shown. Equal current pulses were applied with and without a priming pulse. Except for a slight depolarizing shift of the baseline, no changes in membrane responses to hyperpolarizing pulses were noted after the priming pulse. Measured from the appropriate baseline, membrane responses to hyperpolarizing test pulses were the same with and without a priming pulse. The single small depolarizing pulse that followed a priming pulse elicited a series of spikes; the equal test pulse without a primer did not. (*C*) Decreasing voltage threshold for the spiking response. Three superimposed sweeps are shown. Only the third sweep had a priming pulse. The first sweep had only the smaller current test pulse shown in the lower trace. The membrane response was small and passive (smallest response in upper trace). The second sweep had a larger test pulse. The response was about twice as large as in the previous sweep but the membrane did not spike. The third sweep had the priming pulse, but the test pulse was returned to the lower current level. The membrane now responded with spikes. Note that the second sweep shows a level of membrane depolarization higher than the spike thresholds seen in the third sweep, yet the second sweep shows no spikes. Thus, in the third sweep the threshold for spiking was decreased by the prepulse.

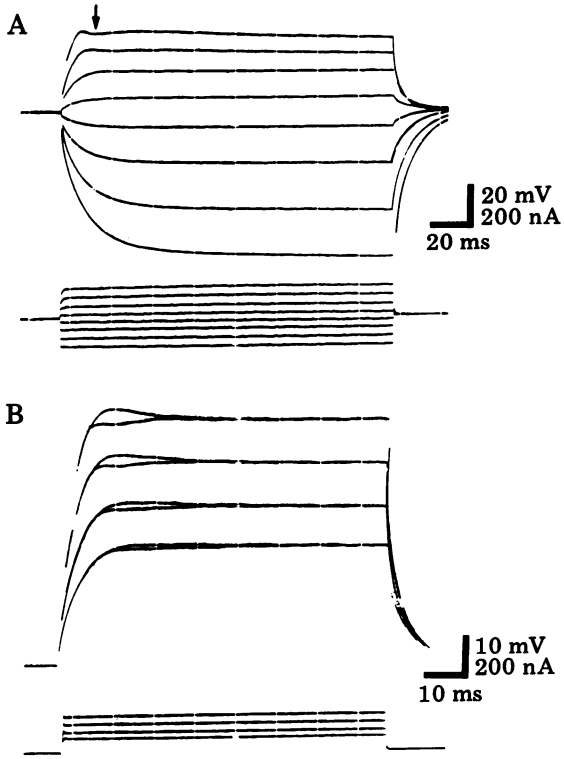

FIG. 4. Active membrane responses in low sodium, calcium-free saline. (*A*) Membrane responses to constant current injection of hyperpolarizing and depolarizing pulses. Note the downward (repolarizing) deflection that is an early response to depolarizing pulses (arrow). Also note that delayed rectification is evident in these records. (*B*) Responses to double pulses at different current levels. At each current level the downward deflection disappeared after the first pulse. The time interval between pulses was 125 msec. Current is shown in lower traces and membrane responses are in upper traces.

layed rectification and an early outward current carried by potassium are apparently present in other insect systems (10, 11). Although the ionic basis for the currents in this system requires further investigation, both outward currents were blocked by the potassium-blocking agent tetraethylammonium chloride at 50 mM (unpublished data).

The transient component decreased markedly when a long-lasting voltage step was repeated after a short time interval (Fig. 5*B*). A depolarizing step of shorter duration only partially eliminated the current; repeated presentation of short depolarizations successively diminished the current (Fig. 5*C*).

Thus, the suppression of the transient current appears to depend on the length of time that the membrane remains depolarized. This, as well as the voltage dependence of suppression, was tested in the experiments shown in Fig. 6. In these experiments, long (approximately 160 msec) command voltage steps were applied to the membrane, and shorter test step pulses were applied during the course of this longer pulse to test for the suppression of the current at various time intervals after the initiation of the longer pulse (each record shows three superimposed sweeps). Below a threshold (pulses more negative than −55 mV), a progressive steady-state inactivation did not appear. This is shown by the fact that long clamp pulses more negative than −55 mV did not measureably suppress activation of the transient current (Fig. 6*A*). However, during more positive voltage steps, activation of the current was progressively suppressed during the time course of the command pulse (Fig. 6*B*). At even more positive voltage steps (Fig. 6*C*), suppression of the current occurred even sooner. Hence, both the level of depolarization and the time spent in the depolarized state are important in determining the degree of suppression of the transient current. This might explain the facilitation of membrane excitability by a single nerve driven spike as in Fig. 1*C*. Although the duration of the spike was very short, the degree of depolarization was very great. At near-threshold depolarizations the time course for inactivation of the transient current

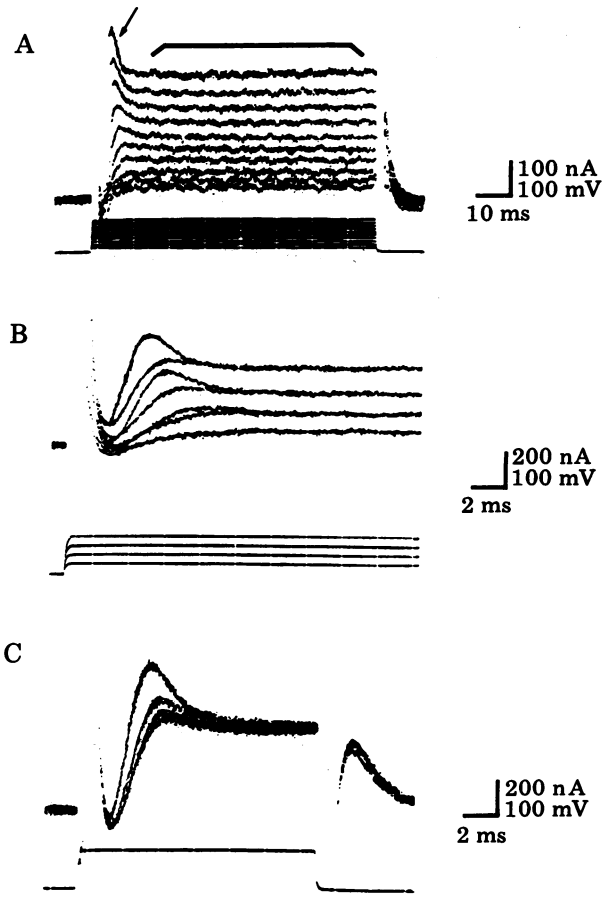

FIG. 5. Voltage clamp experiments showing outward membrane currents associated with depolarizing voltage steps from a constant holding potential of −60 mv (low-sodium, calcium-free saline). Outward current is shown in the upward direction. Current is shown in the upper traces and voltage, in the lower traces. (*A*) Voltage steps with a long time course, showing both the transient outward component (arrow) and delayed rectification which does not inactivate during the time course of the records (bracket). (*B*) Double pulses of voltage steps to various membrane potentials, showing the decrease of the transient component in the second pulse. Note that the sweep speed is faster than in *A*. At each of four voltage levels, an 80-msec command clamp pulse was applied and then reapplied after a gap of 48 msec. At each of the three higher voltages, the first command pulse caused a much larger transient current than the second. The lowest voltage step was below threshold for the transient current. (*C*) Graded suppression of the transient component by repetitive voltage steps of short duration. A 12-msec clamp pulse was applied five times at 15 Hz. The transient outward current decreased on each successive depolarization.

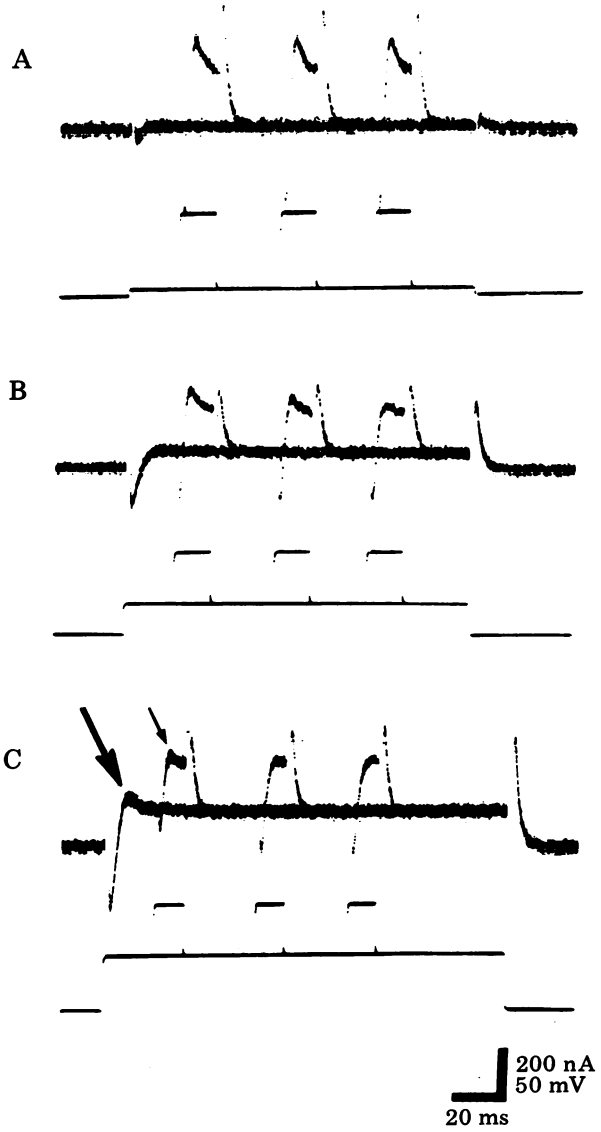

FIG. 6. Suppression of the transient outward current component during the course of long voltage clamp steps (low-sodium, calcium-free saline). At various times during long voltage steps the ability to activate the transient component was tested by applying a second voltage step of shorter duration. Note that each trace shown consists of three superimposed sweeps. A single sweep consists of a long voltage step and a single short test voltage step. The holding potential in *A*, *B*, and *C* was −60 mV. Sweeps were separated in time by approximately 15 sec, allowing the system time to recover fully. (*A*) When the long voltage step was small it had no observable effect on the amplitude of the transient current evoked by the test step. (*B*) When the long voltage step was larger, the transient component evokable by the short step decreased during the time course of the long pulse. (*C*) Similar to *B*, but with an even larger long-term voltage step. Activation of the transient component is evident at the beginning of the long voltage step (large arrow). The transient current could be activated again within 15 msec (small arrow) of its initial activation but could not be activated by the end of the long pulse.

was quite slow; this may explain the long delay required before spiking commenced when the membrane was depolarized to near threshold (Fig. 2*A*, top trace).

Fig. 6*C* suggestes that inactivation occurs in two phases, a rapid phase and a slower phase. The current evoked by the initial pulse (large arrow) appeared to be largely inactivated within the first 10 msec. However, subsequent test pulses showed a continued slow inactivation for at least 50 msec. Two-phase inactivation also has been suggested for the sodium current (12).

DISCUSSION

The transient outward current observed in *Drosophila* DLM fibers and its activity-induced depression are apparently responsible for most, if not all, of the membrane excitability changes noted in this report. Confirmation of this must await a more detailed quantitative study of the DLM membrane currents. Conner and Stevens (6) described a transient outward current in Mollusca which has characteristics similar to the current described here. That current, the "A" current, was hypothesized to be due to a second set of potassium channels distinct from those of delayed rectification. This hypothesis has since been supported by the finding that the "A" current could be pharmacologically separated from delayed rectification (13).

In the *Drosophila* DLM the fact that the transient outward current can be suppressed by repetitive depolarizations although delayed rectification remains unchanged may prove to be further evidence for distinct sets of potassium channels.

The transient outward current in this system, the *Drosophila* "A" current, differs from the "A" current described by Conner and Stevens in that the kinetics are more rapid. The time constant of inactivation is about 60 msec in *Anisodoris* but in *Drosophila* it is about an order of magnitude faster. This is not without precedent, however, because a transient outward current with an inactivation time constant of approximately 6 msec was described in the supramedullary cells of the puffer fish (14).

The mechanism of suppressing this *Drosophila* "A" current by depolarizing activity requires further study. In snail neurons, the depression is hypothesized to be due to calcium entry during the initial depolarization (3). In *Drosophila*, however, Ca^{2+}-free saline appears not to interfere with the suppression of this current.

Conner and Stevens (6, 15) showed that, in isolated somata of nudibranch molluscs, the "A" current plays a role in repetitive spiking behavior. Activation of this current delays the expression of a spike and thus determines the interval between spikes. It has also been shown that the "A" current can modulate the efficiency of synaptic transmission by short-circuiting excitatory synaptic potentials (4, 5, 16). In *Drosophila* the "A" current can change the magnitude of spike-like responses. Because the DLM spike-like response is due to an inward calcium current, the *Drosophila* "A" current may be important in modulating calcium availability to the muscles.

Finally, the variety of muscle membrane electrical activities seen in *Drosophila* suggests that at least several different excitable channels are present in the membrane. The isolation of mutants affecting membrane electrogenesis could offer insights into molecular questions pertinent to membrane biophysics. Hence, this system may be ideal for applying the tools both of *Drosophila* genetics and of voltage clamping to the analysis of excitable membranes.

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