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Unmasking of a Novel Potassium Current in *Drosophila* by a Mutation and Drugs

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The delayed rectifier potassium current plays a critical role in cellular physiology. This current (I_K) in *Drosophila* larvae is believed to be a single current. However, a likely null mutation in the *Shab* K^+ channel gene (*Shab*^{3}) reduces I_K but does not eliminate it. This raises a question as to whether or not the entire I_K passes through channels encoded by one gene. Similarly, an incomplete blockade of I_K by high concentrations of quinidine, a selective I_K blocker, raises a question as to whether I_K consists of two components that are differentially sensitive to quinidine. We have addressed these questions by a combined use of genetics, pharmacology, and physiology. The current component removed by the *Shab*^{3} mutation differed from the remaining component in activation kinetics, inactivation kinetics, threshold of activation, and voltage dependence. The two components showed strong differences in sensitivity to quinidine. Physiological properties of the current component removed by the *Shab*^{3} mutation were similar to those of the quinidine-sensitive fraction of I_K. Complementary to this, properties of the current component remaining in the *Shab*^{3} mutant muscles were similar to those of the quinidine-resistant fraction of I_K. These observations strongly suggest that, in contrast to the current belief, I_K consists of two components in *Drosophila*, which are genetically, pharmacologically, and physiologically distinct. These components are being called I_{KS} and I_{KF}. I_{KS} is carried via *Shab*-encoded channels. I_{KF} defines a new voltage-activated K^+ current in *Drosophila*.

Key words: Drosophila*; K^+ channels;* Shab*; delayed rectifier; larval muscles; quinidine*

Diversity of K^+ channels provides a basis for a wide spectrum of physiological properties among excitable and nonexcitable cells. For example, K^+ channels with diverse characteristics play a vital role in several phenomena, including repolarization of membrane potential, cardiac and neuronal pacemaker activity, repetitive firing, sensory receptor potentials, secretion, fertilization, and learning (Rudy, 1988; Colatsky, 1990; Cook, 1990; Hille, 1992; Wu and Ganetzky, 1992; Jan and Jan, 1997; Armstrong and Hille, 1998). Our understanding of diversity of K^+ channels, as well as of properties and function of a variety of K^+ channels, has been advanced greatly by single gene mutations of *Drosophila*. Combining mutations that selectively disrupt channels with drugs that block specific channels has helped in resolving various ionic currents and in determining the role of specific currents in excitability of nerve and muscle cell membranes (Salkoff, 1983; Wu et al., 1983; Gho and Mallart, 1986; Elkins and Ganetzky, 1988; Singh and Wu, 1989; Singh and Wu, 1990; Gho and Ganetzky, 1992). Voltage-activated K^+ current in the larval muscles of *Drosophila* has been resolved into two components, a fast transient current (I_A) and a delayed sustained current (I_K) (Salkoff, 1983; Wu et al., 1983; Wu and Haugland, 1985; Singh and Wu, 1989).

Among the two voltage-activated K^+ currents, I_A is disrupted by mutations in the *Shaker* gene, which codes for the structure of the I_A channels (Kaplan and Trout, 1969; Kamb et al., 1987; Papazian et al., 1987; Pongs et al., 1988). This current is blocked by 4-aminopyridine (4-AP) (Wu and Haugland, 1985; Wu and Ganetzky, 1992). Whereas it has been possible to partially block I_K with quinidine, a cinchona alkaloid used as an antiarrhythmic agent in humans (Singh and Wu, 1989; Kraliz et al., 1998), a mutational analysis of this current has not been possible because of absence of mutations that disrupt this current. However, a recently identified mutation (*Shab*^{1}) in the *Shab* gene selectively reduces I_K without affecting other known ionic currents in the larval muscles (Chopra, 1994; M. Chopra, G.-G. Gu, and S. Singh, unpublished observations). Mutations at the *Shab* locus, including a likely null allele (*Shab*^{3}), enable us to ask questions about this current that have not been possible before.

Until now, I_K in *Drosophila* has been believed to be one homogenous current (Wu and Haugland, 1985; Singh and Wu, 1989, 1999; Wu and Ganetzky, 1992; Tsunoda and Salkoff, 1995b). However, the current is not completely removed by *Shab*^{3}, which appears to be a genetically null allele of the gene that codes for the structure of the channels (Hegde et al., 1999). This raises a question as to whether I_K is indeed a single current or whether it consists of two components, only one of which is carried by the *Shab*-encoded channels. The current is also not blocked completely by high concentrations of quinidine and its analogs, which selectively block I_K. The data presented here strongly argue that the delayed sustained current in the larval muscles of *Drosophila* may consist of two distinct components. Identification of a new current component (I_{KF}) in these experiments raises important questions on the identity of the gene that codes for the channels carrying I_{KF}, *in vivo* physiological role of I_{KF} in muscle excitability, and pharmacological specificity of the current.

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MATERIALS AND METHODS

Flies were grown on a standard cornmeal medium at 21°C (Chopra and Singh, 1994). K^+ currents were recorded from body-wall muscles 12 and 13 (Gu and Singh, 1997) of wandering third instar larvae by two-microelectrode voltage clamping (Wu and Haugland, 1985). Larvae were dissected from dorsal side, and internal organs were removed. All recordings were completed within 30 min from the start of the dissection (Gielow et al., 1995). Electrodes were made from thin-walled borosilicate glass capillaries with an outside diameter of 1.0 mm (World Precision Instruments, Sarasota, FL). The voltage electrode was filled with 2.5 M KCl and the current electrode with a 3:1 mixture of KCl and potassium citrate (Wu and Haugland, 1985). Resistances of both electrodes were in the range of 10–15 MΩ.

All recordings were made in a Ca^{2+}-free bath solution. This prevents the activation of the two Ca^{2+} currents and the two Ca^{2+}-activated K^+ currents (Wu and Haugland, 1985; Singh et al., 1989). These recording conditions produce only the voltage-activated fast transient (I_A) and the delayed sustained (I_K) currents. Currents were elicited by 500 msec voltage steps from a holding potential of −80 mV to potentials between -40 and +40 mV, in 10 mV increments. In some experiments, as mentioned in the figure legends, a prepulse of 2 sec duration, to −20 mV, was used to inactivate I_A (Wu and Haugland, 1985).

The recording solution contained (in mM): NaCl 77.5, KCl 5, $MgCl_2$ 20, $NaHCO_3$ 2.5, trehalose 5, sucrose 115, EGTA 0.5, and HEPES 5 (Stewart et al., 1994; Gu and Singh, 1997). In addition, the recording solution in some experiments, as explained in Results, also contained quinidine, 4-AP, and tetraethylammonium (TEA). The pH was adjusted to 7.1 with NaOH.

Voltage stimuli for eliciting the currents were generated with the help of a Macintosh IISi computer through a 12-bit digital-to-analog converter (MacADIOS II/16 board; GW Instruments, Somerville, MA). The resulting current was recorded with the help of an amplifier (TEC 01C/02/03; NPI Electronic GmbH, Haeldenstrasse, Germany) connected to the computer. Data were converted from analog to digital form with the help of a 16-bit analog to digital converter (MacADIOS II/16 board; GW Instruments). Currents were sampled every 500 μs for digital conversion, except during capacitance transients (every 100 μs), which were used for measuring cell capacitance. The digitized data were analyzed off-line with the help of a program written in "C" language.

Control recordings were performed independently for each set of experiments, and digital subtraction was performed between data obtained during the same set of experiments. The current measurements are given as current density (nanoampere per nanofaraday) to avoid differences attributable to fiber size. The values are given as mean ± SE.

RESULTS

The voltage-activated delayed sustained current (I_K) recorded from the normal and the *Shab*^{3} mutant muscles is shown in Figure 1, *A* and *B*, respectively. Under the recording conditions used in this experiment (see Materials and Methods), the voltage-clamp traces show a fast transient peak (I_A) and the delayed sustained current (I_K). Figure 1*C* shows the current–voltage (*I–V*) relationships for I_K recorded from the normal and the mutant muscles. The *Shab*^{3} mutation has two small deletions in the N-terminal region of the channel protein upstream of the first transmembrane domain (Hegde et al., 1999). The first deletion of 24 base pairs removes nucleotides 508–531. The second deletion removes nucleotides 656–1011, in turn shifting the reading frame and introducing a stop codon 74 bases downstream of the mutation. The *Shab*^{3} protein is thus expected to be truncated before the S1 segment, which starts at amino acid 436. The truncated protein is also expected to lack the N-terminal tetramerization region before the S1 segment (Li et al., 1992; Shen et al., 1993) and is thus not likely to act in a dominant-negative manner. *Shab*^{3} is thus most likely a null mutation in the gene. If the entire I_K current passes through *Shab*-encoded channels, the *Shab*^{3} mutation is expected to completely eliminate I_K. However, *Shab*^{3} removes the current only partially (Fig. 1), reducing it by ~65% of the total current. One possibility raised by these data are that the sustained K^+ current may consist of two distinct current components, only one of which is carried by channels encoded by the *Shab* gene.

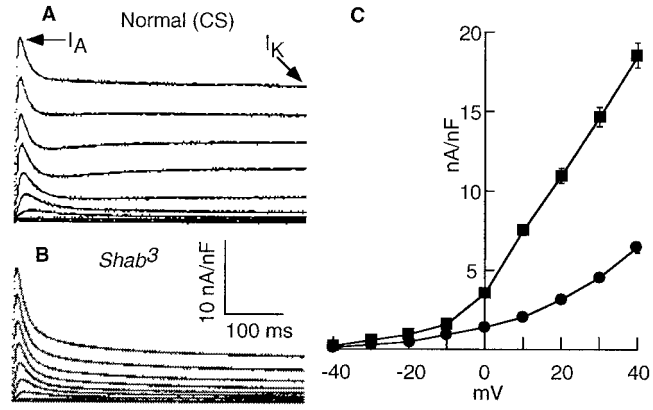

Figure 1. The *Shab*^{3} mutation reduces I_K only partly. Membrane currents recorded from the normal (*A*, ■) and the mutant (*B*, ●) muscles are shown. Under the recording conditions used (see Materials and Methods), only I_A (the fast transient peak in *A* and *B*) and I_K (the sustained current) are seen. *C*, Current–voltage relationships for the sustained current, as measured at the end of the 500 msec pulse. For *A*, number of larvae (L) = 9; number of fibers (F) = 19. For *B*, L = 10; F = 33.

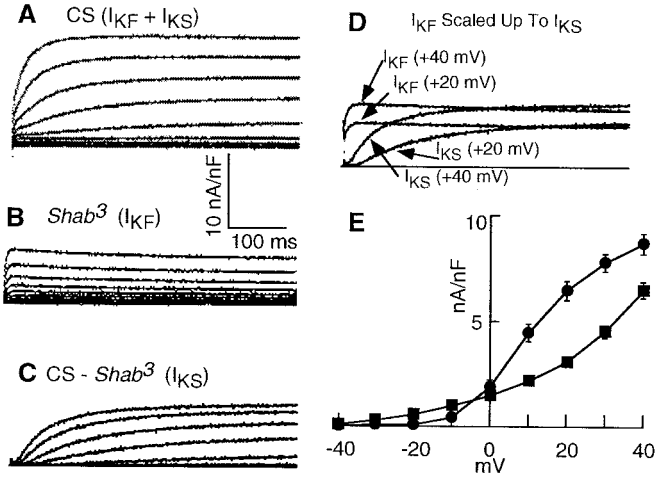

Figure 2. Properties of the two components of I_K. I_A was inactivated by a prepulse (2 sec, −20 mV). *A*, Total current (I_{KF} + I_{KS}) as recorded from the normal (CS) muscles. *B*, Current remaining in the *Shab*^{3} mutant muscles (I_{KF}). *C*, Current component (I_{KS}) affected by the *Shab*^{3} mutation as obtained by subtracting the current shown in *B* from that shown in *A*. *D*, I_{KF} digitally scaled up for comparison, so as to bring its maximum value to the maximum value of I_{KS}. To avoid clutter, current traces are shown for voltage pulses to +20 and +40 mV only. *E*, *I–V* relationships for I_{KF} (■) and I_{KS} (●). *D* shows relative currents, and the current scale does not apply to it. For *A*, L = 3; F = 7. For *B*, L = 3; F = 10.

To examine whether properties of the current component eliminated by *Shab*^{3} differed from those of the remaining component, the two components were compared for their activation and inactivation kinetics (Fig. 2). To enable this comparison, the fast transient current (I_A), which masks the rise of I_K, needs to be removed. I_A was inactivated by using a 2 sec prepulse to −20 mV. Under these conditions, only I_K is observed (Wu and Haugland, 1985). I_K was recorded from wild-type [Canton-S (CS)] and *Shab*^{3} muscles. The component removed by the *Shab*^{3} mutation (Fig. 2*C*) was obtained by digitally subtracting the mutant current

(Fig. 2*B*) from the wild-type current (Fig. 2*A*). Figure 2*D* compares the kinetics of the two current components. In this figure, the *Shab*-independent component was scaled vertically to bring its maximal value to the level of the maximal value of the *Shab*-affected component. Traces are shown for voltage steps to +20 and +40 mV. Activation kinetics of the *Shab*-independent component were faster than those of the *Shab*-affected component. In addition, the *Shab*-independent component showed slight inactivation after reaching the maximum value, whereas the *Shab*-affected component did not show inactivation until the end of the pulse. Kinetic differences between the *Shab*-affected and the *Shab*-independent components lend support to the possibility of I_K consisting of two distinct components. For the following discussion, the current component eliminated by *Shab*^{3} (presumably representing channels encoded by the *Shab* gene) is designated as I_{KS} (for "slow" activation). The current component unaffected by the *Shab*^{3} mutation (presumably representing channels not encoded by the *Shab* gene) is designated as I_{KF} (for "fast" activation).

Figure 2*E* shows that voltage-dependence of the I_{KF} component is different from that of the I_{KS} component. Activation threshold of the two components is also different, with I_{KF} activating at approximately −30 mV and I_{KS} at approximately −10 mV. This further strengthens arguments for the distinct nature of the two current components.

The possibility that I_K may consist of two distinct components is also raised by another independent set of experiments. Quinidine and its analogs, which selectively block I_K in larval muscles (Singh and Wu, 1989, 1990) do not block I_K completely, even at high concentrations (Kraliz and Singh, 1997; Kraliz et al., 1998). One possibility raised by these data is that I_K may consist of two components, only one of which is sensitive to blockade by quinidine. To determine whether there was any correlation between the two likely current components resolved by quinidine and the two likely components resolved by the *Shab*^{3} mutation, we examined the blockade of I_{KF} and I_{KS} by quinidine (Fig. 3). As in the experiments mentioned above, I_{KF} was recorded from *Shab*^{3}, and I_{KS} was obtained by digitally subtracting I_{KF} from the total current obtained in the wild-type (CS). Figure 3, *A* and *B*, respectively, show recordings from the CS and the *Shab*^{3} muscles in the presence of 100 μM quinidine. Currents recorded from CS and *Shab*^{3} in the absence of quinidine were similar to those shown in Figure 2, *A* and *B*, respectively, and are not shown here. Figure 3*A* represents residual I_K (i.e., $I_{KF} + I_{KS}$) not affected by quinidine. Figure 3*B* represents residual I_{KF} not affected by quinidine. Data shown in Figure 3*B* were digitally subtracted from data shown in Figure 3*A* to obtain residual I_{KS} not affected by quinidine (Fig. 3*C*). Figure 3*D* shows *I–V* plots for I_{KF} and I_{KS} in the presence of 100 μM quinidine, with *I–V* plots for I_{KF} and I_{KS} in quinidine-free solution shown for comparison as *dotted* and *dashed lines*, respectively. The bar graph in Figure 3*E* shows percentages of I_{KF} and I_{KS} obtained during a pulse to +40 mV, remaining in the presence of quinidine. Quinidine reduced I_{KF} by ~35% and I_{KS} by ~89%. Thus, the two current components showed a strong difference in their blockade by quinidine. The data indicate that the *Shab*^{3} mutation and quinidine affect the same component of the total current, whereas the component left unaffected by the *Shab*^{3} mutation is the one that is less sensitive to quinidine.

According to the above interpretation, properties of the current carried by the *Shab*-encoded channels (I_{KS}) are expected to be similar to those of the quinidine-sensitive fraction of the current,

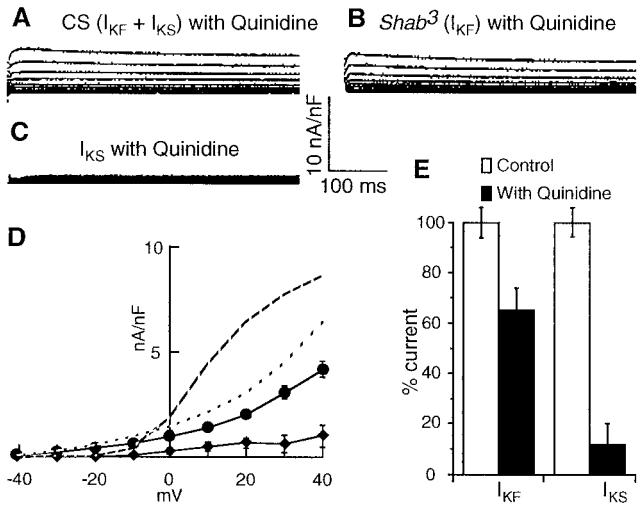

Figure 3. Differential sensitivity of I_{KF} and I_{KS} to blockade by quinidine. A prepulse was used to inactivate I_A. Currents recorded from CS (representing I_{KF} + I_{KS} in quinidine-free solution) and *Shab*^{3} (representing I_{KF} in quinidine-free solution) were similar to those shown in Figure 2, *A* and *B*, respectively, and are not shown here. *A*, Currents recorded from CS in the presence of 100 μM quinidine. This represents fraction of (I_{KF} + I_{KS}) not blocked by quinidine. *B*, Currents recorded from the *Shab*^{3} muscles in the presence of quinidine. This represents the fraction of I_{KF} not blocked by quinidine. *C*, The current obtained by digitally subtracting the current seen in *B* from that seen in *A*. This provides the fraction of I_{KS} not blocked by quinidine. *D*, *I–V* plots for I_{KS} without quinidine (*dashed line*), I_{KF} without quinidine (*dotted line*), I_{KF} with quinidine (●), and I_{KS} with quinidine (♦). *E*, Bar graph shows percentages of I_{KF} and I_{KS} remaining in the presence of quinidine for a voltage step to +40 mV. For *A*, L = 8; F = 22. For *B*, L = 4; F = 13.

and properties of the *Shab*-independent current (I_{KF}) are expected to be similar to those of the quinidine-resistant fraction of the current, with only a minor deviation attributable to some blockade of I_{KF} by quinidine. Figure 4 compares the properties of I_{KF} and I_{KS} with those of the quinidine-resistant and the quinidine-sensitive fractions, respectively. As described for Figure 2 above, I_{KF} was measured in *Shab*^{3} muscles, and I_{KS} was obtained by subtracting I_{KF} from the CS current. Similarly, quinidine-resistant fraction was obtained by measuring current from CS in the presence of 100 μM quinidine, and the quinidine-sensitive fraction was obtained by subtracting quinidine-resistant fraction from the total CS current. I_{KF}, I_{KS}, and the quinidine-resistant fraction of total I_K were as shown in Figures 2, *B* and *C*, and 3*A*, respectively, and are not shown in Figure 4. The quinidine-sensitive fraction of total I_K is shown in Figure 4*A*. Figure 4, *B* and *C*, compares the activation and inactivation kinetics of the two current components as resolved by the two methods. To avoid clutter, current traces are shown for voltage pulses to +20 and +40 mV only. Traces for quinidine-resistant fraction were scaled up to traces for I_{KF} for comparison of kinetics. Similarly, traces for I_{KS} were scaled up to those for the quinidine-sensitive fraction for comparison. Activation and inactivation kinetics of I_{KF} were similar to those of the quinidine-resistant fraction (Fig. 4*B*). Similarly, kinetics of I_{KS} were similar to those of the quinidine-sensitive fraction (Fig. 4*C*). Figure 4*D* shows that voltage dependence of I_{KS} and I_{KF} (as seen in Fig. 2*E* and shown here for comparison as *dashed* and *dotted lines*, respectively) were similar to those of quinidine-sensitive and quinidine-resistant fractions, respectively. Partial blockage of I_{KF} by quinidine is reflected in the quinidine-resistant fraction being

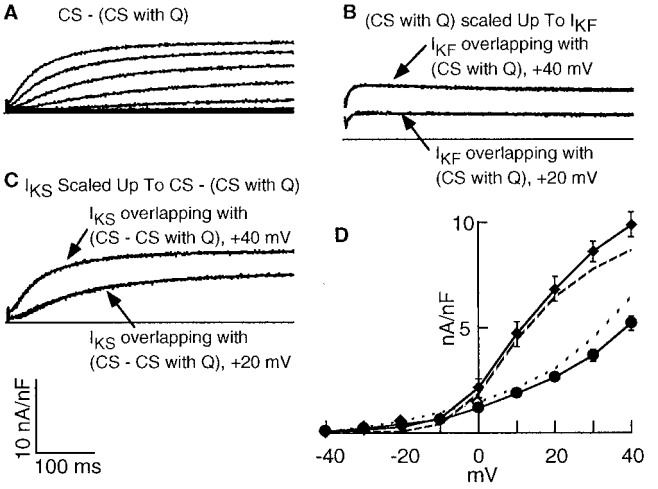

Figure 4. Comparison of the properties of different current components. I_A was inactivated by a prepulse. I_{KF}, I_{KS}, and the quinidine-resistant fraction of I_K (i.e., $I_{KF} + I_{KS}$) were as shown in Figures 2, *B* and *C*, and 3*A*, respectively, and are not shown here. *A*, This panel shows I_K recorded in the presence of quinidine subtracted from I_K recorded in quinidine-free solution. This gives quinidine-sensitive fraction of the total current ($I_{KF} + I_{KS}$). *B*, Kinetics of the quinidine-resistant fraction compared with those of I_{KF}. For comparison of kinetics, the quinidine-resistant fraction was digitally scaled up so as to bring its maximum value to the maximum value of I_{KF}. Each of the two traces seen here (and in *C*) are a near overlap of two currents, which annot be distinguished in the traces. *C*, Kinetics of the quinidine-sensitive current compared with those of I_{KS}. I_{KS} was digitally scaled up to bring its maximum value to the maximum value of the quinidine-sensitive current. *D*, Comparison of *I–V* plots for I_{KF} (*dotted line*) and I_{KS} (*dashed line*) with those of quinidine-resistant (●) and quinidine-sensitive (♦) fractions, respectively. *B* and *C* show relative currents, and the current scale does not apply to them. For *A*, L = 8; F = 22.

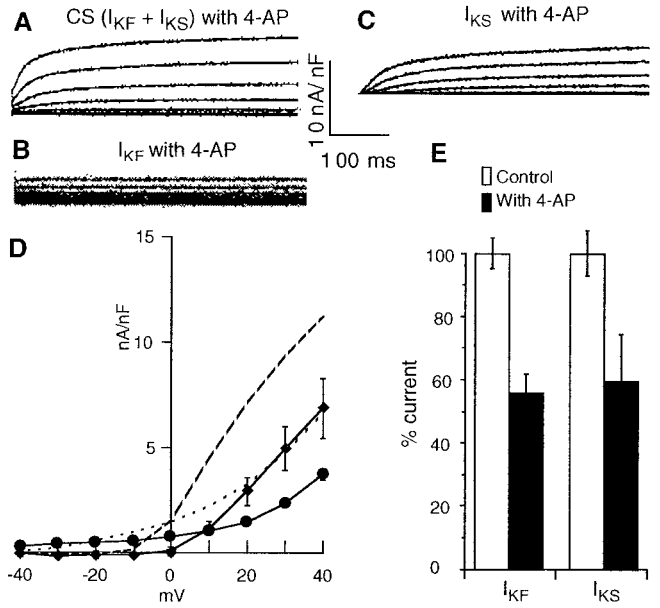

Figure 5. Blockade of the two current components by 4-AP. Fractions of I_{KF} and I_{KS} blocked by 4-AP were calculated in the same way as for quinidine in Figure 3. Currents recorded from CS (representing I_{KF} + I_{KS} in 4-AP-free solution) and from *Shab*^{3} (representing I_{KF} in 4-AP-free solution) were similar to those shown in Figure 1, *A* and *B*, respectively, and are not shown here. *A*, Currents recorded from CS in the presence of 5 mM 4-AP. This represents fraction of ($I_{KF} + I_{KS}$) not blocked by 4-AP. No I_A (the fast transient current) is seen in this figure because 5 mM 4-AP blocks I_A. *B*, Currents recorded from the *Shab*^{3} muscles in the presence of 5 mM 4-AP. This represents the fraction of I_{KF} not blocked by 4-AP. *C*, The current obtained by digitally subtracting the current seen in *B* from the current seen in *A*. This provides the 4-AP-resistant fraction of I_{KS}. *D*, *I–V* plots for I_{KS} without 4-AP (*dashed line*), I_{KF} without 4-AP (*dotted line*), I_{KF} with 4-AP (●), and I_{KS} with 4-AP (♦). *E*, Bar graph showing percentages of I_{KF} and I_{KS} remaining in the presence of 4-AP for a voltage step to +40 mV. For *A*, L = 2; F = 5. For *B*, L = 6; F = 20.

slightly less than I_{KF} and the quinidine-sensitive fraction being slightly more than I_{KS} (Fig. 4*D*).

Shab^3 and quinidine affect the current by very different mechanisms. *Shab*^{3} is expected to eliminate the channel protein itself, whereas quinidine blocks the channels present in the membrane. The above data showing similar effects of quinidine and the *Shab*^{3} mutation on the amplitude, activation kinetics, inactivation kinetics, threshold of activation, and voltage dependence of two current components suggest that the delayed sustained current consists of two distinct components, one carried by *Shab*-encoded channels (which are blocked by quinidine) and the other carried by a different set of channels (which are relatively less sensitive to blockade by quinidine).

To examine the sensitivity of I_{KF} and I_{KS} to other K^+ channel blockers, we tested the two currents for blockade by 4-AP and TEA. Figures 5 and 6 show the effect of these drugs on the two currents. 4-AP (5 mM) blocked both I_{KF} and I_{KS} to a similar extent, to ~56 and ~60% of the control current, respectively. Thus, I_{KF} and I_{KS} are much less sensitive to blockade by 4-AP than I_A, which is almost completely blocked by 50 μM 4-AP (Wu and Ganetzky, 1988). This is consistent with the inability of 1 mM 4-AP to affect *Drosophila Shab* channels expressed in *Xenopus* oocytes (Covarrubias et al., 1991). The effect of 10 mM TEA is shown in Figure 6 with I_{KF} and I_{KS} being ~75 and 84% of the control current, respectively. This compares with ~100 mM TEA nearly eliminating I_A and I_{CF} in the larval muscles (Wu and Ganetzky, 1988). We are currently testing other K^+ channel blockers to identify drugs and toxins that can selectively block I_{KF}. Pharmacological agents and mutations that eliminate I_{KF} selectively, and in general a pharmacological profile of the two currents, will be very helpful in analyzing the properties of the two currents, in determining their individual roles in membrane excitability, and in studying the mechanisms underlying their regulation.

With the resolution of I_K into two components, the total voltage-activated K^+ current in the larval muscles of *Drosophila*, as shown in Figure 1*A*, can be now resolved into three distinct components (Fig. 7) in several ways. These include the *Shaker* and the *Shab* mutations, which eliminate I_A and I_{KS}, respectively, and 4-AP and quinidine, which block I_A and I_{KS}, respectively. Differences in physiological properties between the three currents further help in resolving these currents. In combination with a similar resolution of the two Ca^{2+}-activated K^+ currents (I_{CF} and I_{CS}) (Gho and Mallart, 1986; Singh and Wu, 1989, 1990) and two Ca^{2+} currents (the 1,4-dihydropyridine-sensitive and the amiloride-sensitive current) (Gielow et al., 1995), the larval muscles of *Drosophila* provide an excellent preparation in which all known specific current components can now be resolved and studied individually.

DISCUSSION

Experiments described in this report provide a strong argument for the existence of two distinct current components (I_{KF} and I_{KS}) in the slow sustained voltage-activated K^+ current (I_K) in the

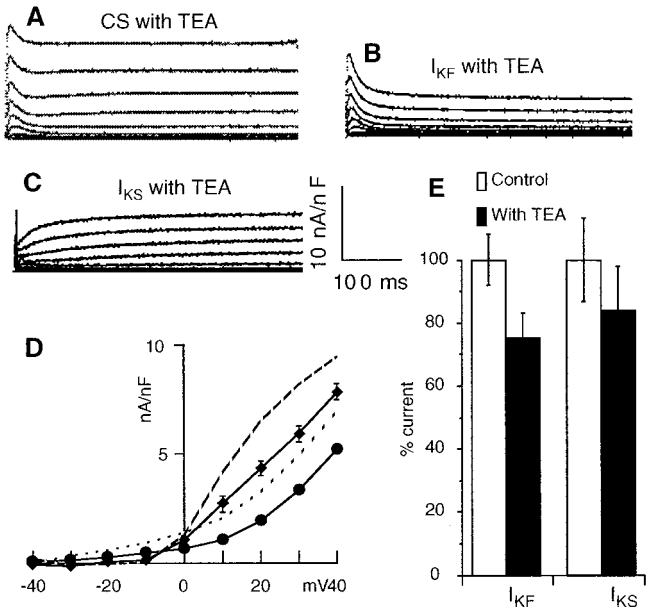

Figure 6. Effect of TEA on I_{KF} and I_{KS}. Fractions of I_{KF} and I_{KS} blocked by TEA were calculated in the same way as for quinidine in Figure 3. Currents recorded from CS and *Shab*^{3} were similar to those shown in Figure 1, *A* and *B*, respectively. *A*, Currents from CS in saline containing 10 mM TEA. *B*, Currents from *Shab*^{3} in saline with 10 mM TEA. *C*, The current obtained by digitally subtracting the current seen in *B* from the current seen in *A*. This provides the TEA-resistant fraction of I_{KS}. *D*, *I–V* plots for I_{KS} without TEA (*dashed line*), I_{KF} without TEA (*dotted line*), I_{KS} with TEA (♦), and I_{KF} with TEA (●). *E*, Bar graph showing percentages of I_{KF} and I_{KS} remaining in the presence of TEA for a voltage step to +40 mV. For *A*, L = 4; F = 11. For *B*, L = 4; F = 17.

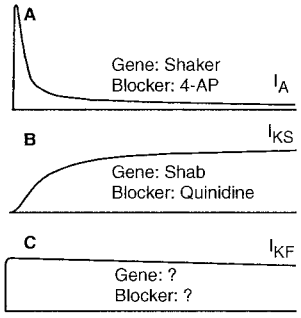

Figure 7. A schematic representation of the three voltage-activated K^+ currents in the larval muscles. *Gene* refers to the gene that codes for the channels carrying a particular current. *Blocker* refers to the drug that selectively blocks the current.

larval muscles of *Drosophila*. Voltage-activated K^+ current in the larval muscles of *Drosophila* has been previously resolved into two distinct currents. With the data presented here, we can now resolve the total voltage-activated K^+ current into three components. Resolution of I_K into I_{KS} and I_{KF} will be particularly helpful in analyzing the properties of these two currents, deciphering the functional role of each current in muscle excitability, and studying mechanisms underlying their function and regulation.

Channels carrying I_{KS} are encoded by the *Shab* gene. I_{KS} shares properties with the current generated by expressing *Shab* channels in *Xenopus* oocytes. These properties include relative resistance to blockade by 4-AP and a relatively slow activation (Covarrubias et al., 1991; Tsunoda and Salkoff, 1995a). However, in contrast to a slight inactivation of the delayed rectifier current recorded from *Xenopus* oocytes expressing *Shab* channels (Salkoff et al., 1992), I_{KS} shows no observable inactivation of I_{KS} during the 500 msec pulse.

The gene that encodes the channels carrying the new current (I_{KF}) identified in this study remains to be identified. Among the channels that give rise to voltage-activated K^+ currents in *in vitro* expression systems, the *Shaker* and the *Shal* channels give rise to fast transient currents, whereas the *Shab* and the *Shaw* channels give rise to slow sustained currents (Iverson et al., 1988; Timpe et al., 1988; Salkoff et al., 1992). One of the two sustained currents (I_{KS}) resolved in this study is carried by the *Shab*-encoded channels. These data raise the possibility that the second channel may be encoded by the *Shaw* gene (Tsunoda and Salkoff, 1995a). However, physiological and pharmacological properties of I_{KF} seen in our recordings differ from those of *Drosophila* and mammalian *Shaw* current observed in *Xenopus* oocyte system (Salkoff et al., 1992; Kirsch and Drewe, 1993; Kanemasa et al., 1995). For example, Kv3.1 channels (a mammalian representative of *Shaw*) are ~150 times more sensitive to 4-AP than Kv2.1 (*Shab*) channels (Kirsch and Drewe, 1993). This contrasts to almost similar blockade of the I_{KF} channels to that of the *Shab*-encoded I_{KS} channels (Fig. 5). Similarly, in contrast to I_{KF} (Fig. 6*E*), Kv3.1 channels are blocked by TEA with a half-blocking dose of ~220 μM (Iverson et al., 1988; Timpe et al., 1988; Salkoff et al., 1992). One possibility is that the observed differences arise from an *in vitro* expression of the channels. On the other hand, I_{KF} may be carried via channels encoded by other genes, such as *seizure* (*sei*) or *ether-a-go-go* (*eag*) (Warmke et al., 1991; Titus et al., 1997; Wang et al., 1997). Mutations at the *eag* locus have been shown to reduce I_K (Zhong and Wu, 1991), although the exact mechanism for this effect is not yet clear. It is not clear at this stage whether I_{KF} is a *Shaw* current with novel properties, whether it is carried via *sei*- or *eag*-encoded channels, or whether it represents an as yet unidentified gene for a voltage-activated K^+ channel in *Drosophila*. It will be very instructive to examine the nature of I_{KF} and identify the gene that codes for the channels carrying this current. Availability of a pharmacological agent that selectively blocks these channels can provide a valuable tool for this purpose. Similarly, single gene mutations that affect the current can also help greatly. Such mutations and pharmacological agents will also be very valuable for a molecular analysis of the I_{KF} channels and their function in *Drosophila*.

The delayed rectifier current is a ubiquitous current present in a large variety of cells in most species. In human cardiac cells, it consists of two components that show differential sensitivity to various antiarrhythmic agents (Sanguinetti and Jurkiewicz, 1990; Singh, 1998). It will be interesting to analyze correlations, if any, between the two components of I_K in *Drosophila* and the two components of the delayed rectifier current in human cardiac cells. There is already some indication of a pharmacological overlap between *Drosophila* and human cardiac K^+ currents. Quinidine, which affects the delayed rectifier K^+ channels in human heart (Roden, 1996), affects heartbeat in *Drosophila* (Gu and Singh, 1995), as well as blocks I_{KS} in *Drosophila* larval muscles (Kraliz et al., 1998). Any correspondence between components of *Drosophila* and mammalian delayed rectifier currents will be very useful in undertaking a genetic analysis of cardiac excitability, particularly with the help of mutations that affect I_{KF} and I_{KS} in *Drosophila*.

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