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 (\(i_\text{K}\)) in *Drosophila* larvae is believed to be a single current. However, a likely null mutation in the *Shab* \(K^+\) channel gene (*Shab*\(^3\)) reduces \(i_\text{K}\) but does not eliminate it. This raises a question as to whether or not the entire \(i_\text{K}\) passes through channels encoded by one gene. Similarly, an incomplete blockade of \(i_\text{K}\) by high concentrations of quinidine, a selective \(i_\text{K}\) blocker, raises a question as to whether \(i_\text{K}\) 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 \(i_\text{K}\). 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 \(i_\text{K}\). These observations strongly suggest that, in contrast to the current belief, \(i_\text{K}\) consists of two components in *Drosophila*, which are genetically, pharmacologically, and physiologically distinct. These components are being called \(i_{\text{KF}}\) and \(i_{\text{KS}}\). \(i_{\text{KF}}\) is carried via *Shab*-encoded channels. \(i_{\text{KF}}\) 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*\(^3\)) 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 homogeneous 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 (\(i_{\text{KF}}\)) in these experiments raises important questions on the identity of the gene that codes for the channels carrying \(i_{\text{KF}}\), in vivo physiological role of \(i_{\text{KF}}\) 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 KC1 and the current electrode with a 3:1 mixture of KC1 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 Ca²⁺-free bath solution. This prevents the activation of the two Ca²⁺-currents and the two Ca²⁺-activated K⁺ currents (Wu and Haugland, 1985; Singh et al., 1989). These recording conditions produce only the voltage-activated fast transient (IF) and the delayed sustained (IK) 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 IK (Wu and Haugland, 1985).

The recording solution contained (in mM): NaCl 77.5, KCl 5, MgCl₂ 20, NaHCO₃ 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 quindine, 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 (IK) recorded from the normal and the Shab3 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 (IA) and the delayed sustained current (IK). Figure 1C shows the current–voltage (I–V) relationships for IK recorded from the normal and the mutant muscles. The Shab3 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 Shab3 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. Shab3 is thus most likely a null mutation in the gene. If the entire IK current passes through Shab-encoded channels, the Shab3 mutation is expected to completely eliminate IK. However, Shab3 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.

To examine whether properties of the current component eliminated by Shab3 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 (IA), which masks the rise of IK, needs to be removed. IA was inactivated by using a 2 sec prepulse to -20 mV. Under these conditions, only IK is observed (Wu and Haugland, 1985). IK was recorded from wild-type [Canton-S (CS)] and Shab3 muscles. The component removed by the Shab3 mutation (Fig. 2C) was obtained by digitally subtracting the mutant current...
From the wild-type current (Fig. 2A). Figure 2D 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 (presumably representing channels encoded by the Shab gene) is designated as $I_{KS}$ (for “slow” activation). The current component unaffected by the Shab (presumably representing channels not encoded by the Shab gene) is designated as $I_{KF}$ (for “fast” activation).

Figure 2E 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 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 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 muscles in the presence of $100 \mu M$ quinidine. Currents recorded from CS and Shab in the absence of quinidine were similar to those shown in Figure 2, A and B, respectively, and are not shown here. Figure 3A shows representative $I_K$ (i.e., $I_{KF} + I_{KS}$) not affected by quinidine. Figure 3B represents residual $I_K$ not affected by quinidine. Data shown in Figure 3B were digitally subtracted from data shown in Figure 3A to obtain residual $I_{KS}$ not affected by quinidine (Fig. 3C). Figure 3D shows $I-V$ plots for $I_{KS}$ and $I_{KF}$ in the presence of $100 \mu M$ quinidine, with $I-V$ plots for $I_{KS}$ and $I_{KF}$ in quinidine-free solution shown for comparison as dotted and dashed lines, respectively. The bar graph in Figure 3E 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 $\sim 35\%$ and $I_{KS}$ by $\sim 89\%$. Thus, the two current components showed a strong difference in their blockade by quinidine. The data indicate that the Shab mutation and quinidine affect the same component of the total current, whereas the component left unaffected by the Shab 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, 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 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 $100 \mu M$ 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 3A, respectively, and are not shown here. Figure 4E 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 $B$, $L = 4$; $F = 13$.
slightly less than $I_{KF}$ and the quinidine-sensitive fraction being slightly more than $I_{KS}$ (Fig. 4D).

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$^3$-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 $I_{KF}$, which might provide an excellent preparation in which all current components can be studied individually.

I$K_F$, 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 1A, 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 $I_{CS}$) (Ghosh 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...
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. 6E), 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, \( I_{KS} \) 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.
REFERENCES


