A Novel Leg-Shaking Drosophila Mutant Defective in a Voltage-Gated K⁺ Current and Hypersensitive to Reactive Oxygen Species

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1,1’-Dimethyl-4,4’-bipyridinium dichloride (methyl viologen; paraquat), an herbicide that causes depletion of NADPH and generates excessive reactive oxygen species (ROS) in vivo, has been used to screen for ROS-sensitive Drosophila mutants. One mutant so isolated, named quiver1 (qvr1), has a leg-shaking phenotype. Mutants of the Shaker (Sh), Hyperkinetic (Hk), and ether a-go-go (eag) genes, which encode different K⁺ channel subunits that regulate the A-type K⁺ current (Iₐ) in different ways, exhibit leg shaking under ether anaesthesia and have heightened metabolic rates and shortened life spans. We found that Sh, Hk, and eag mutant flies were all hypersensitive to paraquat. Double-mutant combinations among the three channel mutations and qvr1 had drastically enhanced sensitivity to paraquat. Systolic transmission in the larval neuromuscular junction was increased in the qvr1 mutant to the level of Sh mutants. Similar to eag Sh double mutants, double mutants of eag and qvr1 showed striking enhancement in synaptic transmission and a wings-down phenotype, the hallmarks of extreme hyperexcitability. Voltage-clamp experiments demonstrated that the qvr1 mutation specifically disrupted the Sh-dependent Iₐ current without altering the other currents (Iₐ,K, Ca²⁺-activated fast (Iₐ,CF ) and slow (Iₐ,CS ) currents, and Iₐ,CS in larval muscles. Several deficiency strains of the qvr locus failed to complement qvr1 and confirmed that ether-induced leg shaking, reduced Iₐ current, and paraquat hypersensitivity map to the same locus. Our results suggest that the qvr gene may encode a novel K⁺ channel-related polypeptide and indicate a strong link between a voltage-activated K⁺ current and vulnerability to ROS.

Key words: Shaker; Hyperkinetic; ether a-go-go; quiver; potassium channel; synaptic transmission; paraquat; free radical

A set of well studied mutations has defined a suite of phenotypes associated with defective K⁺ channels in Drosophila. In different ways, mutations of Shaker (Sh), ether a-go-go (eag), and Hyperkinetic (Hk) impair the transient A-type K⁺ current (Iₐ) in Drosophila muscles (Salkoff and Wyman, 1981; Wu et al., 1983; Wu and Haugland, 1985; Zhong and Wu, 1991; Wang and Wu, 1996) and neurons (Tanouye and Ferrus, 1985; Baker and Salkoff, 1990; Saito and Wu, 1993; Zhao et al., 1995; Yao and Wu, 1999). These genes encode either the pore-forming or auxiliary subunits of Sh-dependent K⁺ channels (Kamb et al., 1988; Pongs et al., 1988; Schwarz et al., 1988; Warmke et al., 1991; Chouinard et al., 1995; Chen et al., 1996). These channel mutations enhance synaptic transmission at the larval neuromuscular junction (Jan et al., 1977; Ganetzky and Wu, 1983, 1985; Wu et al., 1983; Stern and Ganetzky, 1989), suggesting that the Sh-dependent Iₐ current has a functional role in terminating neurotransmitter release in the presynaptic terminal. Behavioral analysis has demonstrated that Sh-dependent K⁺ channels are crucial for the control of the peri-staltic locomotion in Drosophila larvae (Wang et al., 1997).

Sh, eag, and Hk mutants are well known for their leg-shaking phenotype (Kaplan and Trout, 1969). However, little attention has been given to the observations that oxygen consumption is increased by Sh, eag, and Hk mutations and longevity is inversely related to the enhancement of metabolic rate in these mutant flies (Trout and Kaplan, 1970). Drosophila, like other aerobic organisms, uses several enzymes for reactive oxygen species (ROS) homeostasis (Campbell et al., 1986; Mackay and Bewley, 1989; Phillips et al., 1989; Staveley et al., 1990). The superoxide radical is catalytically reduced by superoxide dismutase (SOD) to hydrogen peroxide, which in turn is catalytically reduced to water by catalase (Fridovich, 1995). Genetic tools are available in Drosophila to investigate ROS homeostasis and relevant pathways (Phillips and Hilliker, 1990). 1,1’-Dimethyl-4,4’-bipyridinium dichloride (methyl viologen; paraquat) is an herbicide that generates superoxide in vivo at the expense of NADPH when oxygen is available. Susceptibility to millimolar concentrations of paraquat has been used successfully in screening for mutants in the ROS pathway (Phillips et al., 1989; Humphreys et al., 1993, 1996).

We demonstrated that like quiver (qvr) mutants, Drosophila K⁺ channel mutants Sh, eag, and Hk were also hypersensitive to paraquat challenge. The EMS-induced qvr1 mutation, along with several deficiency lines, reduced the amplitude and slowed the kinetics of Iₐ, like several previously isolated leg-shaking mutants. These results elucidate the physiological roles of the qvr polypeptide and revealed functional similarities among qvr and the known Iₐ, K⁺ channel mutants. Sh-dependent K⁺ channels are known to be modulated not only by second messenger-dependent processes (Zhong and Wu, 1993b) but also by oxidoreduction (Schlief et al., 1990; Gulbis et al., 1999; J. Chen et al., 2000), which may provide a means to regulate synaptic efficacy. This study may initiate work toward a comprehensive understanding of qvr and K⁺ channel mutants to shed light on the link between ROS and K⁺ currents.

MATERIALS AND METHODS

Fly stocks. All flies were raised at room temperature (20–23°C) and fed with standard Drosophila medium. The parental stock qvr1; ry¹ was used for generating the qvr1 mutant, which was originally derived from the wild-type strain Oregon-R and was used in this study as the control. The Canton-S (CS) wild-type strain, used for comparison, is not significantly different from Oregon-R in many physiological aspects examined in this study. The qvr locus was mapped previously to 48A (Humphreys et al., 1996). Df(2R)en-SFX31/CyO (48A1; 48B5-7) and w; Df(2R)en-B, b¹ pr¹/CyO (47E3-6; 48A4-B2) were provided by the Bloomington Stock Center (Bloomington, IN). These two deficiency lines are homozygous lethal and...
failed to complement the qvr1 mutation in leg-shaking behavior and paraquat hypersensitivity (Humphreys et al., 1996). qvr1, qvr2, and qvr3 are homologous lethal deficiency lines generated by mobilization and imprecise excision of a nearby P-element P[17en43] (Humphreys, 1996). Except for qvr1, all P-element mutagenesis lines failed to complement the qvr1 mutation in leg-shaking behavior in vivo. P[17en43] and P[17en43] were kindly provided by Dr. Judy Kassiel at the Food and Drug Administration Center for Biologics Evaluation and Research, Sh1, Sh2, Sh3 and Sh5 (abbreviated Sh120 in the text; see Table 1), Hk, eag, and nap were originally from the collection of Dr. Seymour Benzer at the Cold Spring Harbor Laboratory. Sh1 is a null allele (Zhao et al., 1995) and eliminates I\(_K\) in larval muscles (Wu and Haugland, 1985). Sh5 is a point mutation in the S4–S5 cytoplasmic linker (Gautam and Tanouye, 1990) and alters the voltage dependence of I\(_K\) (Wu and Haugland, 1985). The HK mutation was originally described in Kaplan and Trapp (1978) and is kindly provided by Dr. Rodney Williamson at the Beckman Research Institute of the City of Hope. The compound mutants eag1 Sh120, Hk1 eag, and eag1 Sh120 nap are the same stocks used in previous studies (Budnik et al., 1990). Other compound mutants were generated for this study. Compound mutants were all confirmed by scoring leg-shaking phenotype and electrophysiological phenotype in larval muscles. The semicolon for indication of mutations on separate chromosomes is omitted in the text for simplicity. nap1 is an EMS-induced mutation (Wu et al., 1978), which reduces the expression of sodium channels and is allelic with mle mutations (Kernan et al., 1991). Flies bearing this mutation become paralyzed at 37°C or higher because of the blocking of nerve action potentials.

**RESULTS**

**Increased paraquat sensitivity and excitability in double mutants**

Vigorous leg shaking when ether-anesthetized, a phenotype similar to that of the previously identified K\(^+\) channel mutants Sh, Hk, and eag, was observed in qvr1 mutant flies (Humphreys et al., 1996). This phenotypic similarity led us to perform a comprehensive test of paraquat sensitivity in those molecularly characterized leg-shaking mutants. As seen in Table 1, when exposed to 10 mM paraquat for 48 hr, Sh, Sh120, Hk1, and eag1 mutant flies had 32–48% survival rates, similar to that seen in qvr1 (42%) but much lower than that of each single mutant. These numbers for controls and mutants are consistent with those reported previously for some of these alleles (Humphreys et al., 1996).

Double mutants of eag and Sh are even more hyperexcitable than are eag or Sh single mutants in synaptic transmission at the larval neuromuscular junction (Ganetzky and Wu, 1983, 1985; Zhong and Wu, 1993a) and in the adult flight muscle system (Engel and Wu, 1992). To see whether ROS sensitivity was similarly enhanced in double-mutant combinations, we extended the paraquat-feeding study to include various double combinations among qvr1 and mutations of the three K\(^+\) channel genes. A survival rate of 0% was observed in eag1 Sh120 double-mutant flies fed with 10 mM paraquat, which was much more extreme than that of any single mutant (Table 1; 48% for eag1; 32% for Sh120). Shqvr, Hk1 qvr1, and eag1 qvr1 double-mutant flies showed 0, 2, and 0% survival rates after exposure to paraquat, lower than that of each single mutant. nap1, a mutation reducing the expression of a Na\(^+\) channel and suppressing the hyperexcitability in eag1 Sh120 nap1 triple mutants (Wu and Ganetzky, 1992), lowered the paraquat-induced mortality in eag1 Sh120 nap1 mutants, despite the fact that the nap1 mutant flies showed a significant lower survival rate compared with that of the wild-type controls (Table 1). These results indicate that hyperexcitability is closely correlated with paraquat hypersensitivity.

The dosage dependence of survival rate after exposure to paraquat is shown in Figure 1. A noticeable number of double-mutants flies died even without exposure to paraquat. For example, the survival rates for eag1 qvr1 and Hk1 qvr1 in 0 mM paraquat were 82 and 93%, respectively. This could be attributed to the shorter life span of hyperexcitable flies (Trott and Kaplan, 1970).

A novel phenotype arose from double mutants of eag1 and qvr1, similar to the synergistic effects seen in eag Sh double mutants. Wings of the double mutants pointed downward instead of extending horizontally as in normal flies. This “wings-down” phenotype has been studied previously in eag Sh double mutants (Engel and Wu, 1992). It is a hallmark of hyperexcitable mutants (Ganetzky et al., 1985).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>0 mM Paraquat</th>
<th>10 mM Paraquat</th>
</tr>
</thead>
<tbody>
<tr>
<td>qvr1</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>qvr2</td>
<td>93</td>
<td>94</td>
</tr>
<tr>
<td>qvr3</td>
<td>97</td>
<td>98</td>
</tr>
</tbody>
</table>

**Table 1. Paraquat sensitivity of Drosophila channel mutants and qvr**
and Wu, 1985) and has been used in mutant screening (Stern and Ganetzky, 1992).

As can be seen in Figure 2, nearly 100% of eag qvr 4pm double-mutant flies showed the wings-down phenotype. Hk1 qvr 1 and eag 4pm qvr 1 double mutants had 10 and 13% of the flies, respectively, exhibiting the wings-down phenotype. No wings-down flies were observed in Sh5 qvr 1, ShH4 qvr 1, or Sh120 qvr 1, although leg shaking was more vigorous in these double mutants than in Sh or qvr 1 alone. Furthermore, no wings-down flies were seen in Hk1 Sh5 and Hk1 Sh120 double mutations, in contrast to the 10% wings-down frequency seen in the Hk1 qvr stock. The sequence of potency for causing the wings-down phenotype is eag 1 > eag 4pm > Hk1 > Hk2 > Sh5 (Fig. 2).

Figure 1. Paraquat hypersensitivity. Adult flies (24–48 hr old) were fed with paraquat for 48 hr at 25°C in darkness, and the survival rate was determined at the end of the 48 hr period. K+ current mutants eag 1 and Hk1 were as sensitive to paraquat as was the qvr 1 mutant. The double mutants eag 1 qvr 1 and Hk1 qvr 1 were more sensitive to paraquat than were any of the single mutants, indicating synergistic interactions between the qvr 1 mutation and the K+ current mutations. For qvr 1, n = 259, 259, 293, and 340 (from 0 to 10 mM paraquat); for qvr 1, n = 238, 257, 268, and 445; for Hk1, n = 219, 189, 190, and 326; for eag 1, n = 207, 250, 250, and 269; for eag 1 qvr 1, n = 33, 44, 37, and 97; for Hk1 qvr 1, n = 40, 47, 49, and 140.

Figure 2. Wings-down frequency in hyperactive mutants. Nearly 100% of the double-mutant eag qvr 1 flies were wings-down. See Materials and Methods for the determination of wings-down frequency. Error bars represent SD. SD = √(p(1 − p)/n), where p is the wings-down frequency and n is the number of flies. The number of flies examined is indicated above each bar in parentheses.

Figure 3. Enhancement of nerve activity by the qvr 1 mutation. In wild-type neuromuscular junctions of third-instar larvae, MEJPs were observed without nerve stimulation (top trace) as a result of spontaneous quantal release. The eag 1 mutant displayed MEJPs similar to that of the wild-type control (second trace from top). However, the amplitude and frequency of the spontaneous EJPs (recognized by amplitudes larger than quantal size) seen in eag 4pm mutants were both drastically increased by the qvr 1 mutation in the eag 4pm qvr 1 double mutant (bottom two traces). Experiments were done at room temperature (23°C) in HL3 saline containing 1 mM CaCl2 and 20 mM MgCl2.

Synaptic transmission at the larval neuromuscular junction

A unique property of eag mutants is that they display spontaneous EJPs, caused by spontaneous firing in the hyperexcitable motor axons (Ganetzky and Wu, 1982), which are different in amplitude and frequency from miniature EJPs (MEJPs). The frequency of spontaneous EJPs is higher in eag 1 than in eag 4pm (Ganetzky and Wu, 1983). This is correlated to the degree in hyperexcitability conferred by different eag mutations, with eag 1 affecting K+ currents in larval muscles more than eag 4pm (Zhong and Wu, 1991). The frequency and amplitude of the spontaneous EJPs were drastically increased by the qvr 1 mutation in eag 4pm qvr 1 double mutants (Fig. 3). However, the qvr 1 mutation itself did not cause any noticeable alteration in the amplitude, time course, or frequency of MEJPs. Similar synergistic interaction has been observed between Sh and eag in double-mutant combinations (Ganetzky and Wu, 1983). This suggests that a presynaptic rather than a postsynaptic alteration conferred by the qvr 1 mutation is responsible for the enhancement of the spontaneous EJP phenotype of eag.

EJCStudy serve as a quantitative measurement of synaptic transmission, because muscles are held at a constant membrane potential by the voltage-clamp technique to provide a constant driving force and thus avoid nonlinear summation of multiple quantal release in EJP recordings. As described in other species, the amplitude of EJCs follows a fourth-power relationship with external Ca2+ concentration in the Drosophila larval neuromuscular system (Zhong and Wu, 1991; Stewart et al., 1994; Wang et al., 1994). At an external Ca2+ concentration of 0.4 mM, the increase in EJC amplitude caused by the qvr 1 mutation, [27.2 ± 7.4 nA (EJC ± SD); n = 6], was no greater than that by a null mutation, ShM (32.2 ± 9.1; n = 5), and was not further enhanced in qvr 1 ShM double mutants (34.6 ± 3.4; n = 5), suggesting that qvr and Sh share the same pathway in the regulation of synaptic transmission. The difference between qvr 1 and qvr 4pm control is proportionally greater at 0.5 than at 1.0 mM [Ca2+]. (Fig. 4). At these Ca2+ concentrations, the EJC amplitudes of qvr 1 and qvr 1/qvr 4pm mutants did not follow the fourth-power relationship, indicating that defective K+ currents in
these mutants could weaken membrane repolarization and cause transmitter release approaching the saturation level at relative lower concentrations. This could be caused by approaching saturation of the glutamate receptors on the postsynaptic membrane at the higher $[\text{Ca}^{2+}]_0$, which sets the ceiling of EJCs.

**qvr mutations specifically affect the $I_A$ current**

Outward $K^+$ currents in Drosophila larval muscles can be separated into at least four different components: two voltage-dependent currents, a transient ($I_A$) and a delayed rectifier ($I_K$) current, and two $Ca^{2+}$-activated currents, a fast ($I_{CF}$) and a slow ($I_{CS}$) current (Singh and Wu, 1989). Invertebrate muscles generally do not express $Na^+$ channels, and their inward currents are mediated by $Ca^{2+}$ channels (Schwartz and Stühmer, 1984), which is also true for Drosophila muscles. We first examined $Ca^{2+}$ channels for possible defects in qvr$^1$ because of their important role in neurotransmitter release. Quinidine, 4-AP, and TEA were used to block $I_A$ and $I_K$ (Gielow et al., 1995). Ba$^{2+}$ ions, which pass through $Ca^{2+}$ channels with high permeability, were used here as the charge carrier to avoid activating the $Ca^{2+}$-activated $K^+$ currents $I_{CF}$ and $I_{CS}$. Figure 5 shows that the $Ca^{2+}$ current in larval muscle was not affected by the qvr$^1$ mutation.

$K^+$ channels are thought to terminate synaptic transmission by a rapid membrane repolarization (Hille, 1992). The paraquat hypersensitivity and the enhanced synaptic transmitter release seen in both qvr mutants and $K^+$ channel mutants suggest that the qvr polypeptide might have a functional role in the modulation of $K^+$ channels. All four $K^+$ currents mentioned above were examined in qvr$^1$ mutant larvae (Figs. 6, 7). The $Ca^{2+}$-activated outward $K^+$ currents $I_{CF}$ and $I_{CS}$ were examined in the presence of 20 mM $Ca^{2+}$, and the saline contained 1 mM 4-AP and 100 $\mu$M quinidine to block the voltage-activated $I_A$ and $I_K$. Under these conditions there were no significant differences in the amplitude or kinetics of the outward currents $I_{CF}$ and $I_{CS}$ induced by membrane depolarization (Fig. 6).

When $Ca^{2+}$-free saline is used, only $I_A$ and $I_K$ are activated by a step of depolarizing voltage. $I_A$ and $I_K$ can be separated physiologically by their different responses to a 2 sec conditioning pre-pulse from a holding potential of $-80$ to $-20$ mV, which inactivates
Figure 7. The transient $I_A$ and delayed $I_K$ currents in $qvr$ mutant muscles. Larval preparations were dissected and recorded in Ca$^{2+}$-free standard saline containing 14 mM MgCl$_2$ and 5 mM EGTA. The membrane potential was held at −80 mV. A. Right, The amplitude of $I_A$ was drastically reduced by $qvr$ mutations. Left, The activation kinetics of $I_A$ was slower in $qvr$ mutants as shown in the representative current traces generated by membrane depolarization to +10 mV (see also Table 2). B. The amplitudes of the transient current $I_K$ were 2.5 nA/nF (see also Table 2). Time to peak. Recordings during the first 3 msec show a capacitive transient and have been omitted for clarity. B, $I_K$ was not altered by $qvr$ mutations. $I–V$ curves show $I_K$ measured at the end of the depolarization pulse (between 190 and 200 msec after the onset of depolarization) when a plateau was reached. Data are the mean ± SEM.

$I_A$ but leaves $I_K$ intact when they are assessed by a test pulse delivered 10 msec later (Haugland and Wu, 1990). Figure 7 shows that only the $I_A$ current was affected by the mutation. The $I_A$ current in $qvr^+$ mutants appeared to have a very unstable component that inactivated easily and recovered slowly and incompletely (J. W. Wang and C.-F. Wu, unpublished observations). For simplicity, only the stable and fast-recovery component is presented here. The amplitude of the transient $I_A$ was greatly reduced at various membrane potentials as seen in the $I–V$ curve, and the kinetics of $I_A$ was slower in the $qvr$ mutations as the time to peak $I_A$ was lengthened (Table 2; Fig. 7, representative traces). When larval muscles were depolarized to +10 mV from a holding potential of −80 mV, the average amplitude of $I_A$ for the $qvr^+$ mutant larvae was 2.5 ± 0.3 nA/nF, only 20% of the wild-type $I_A$ current (12.3 ± 0.8 nA/nF) in $qvr^+$ larvae.

The EMS mutagenesis of the second chromosome yielded only one paraquat-hypersensitive leg-shaking allele, $qvr^+$. To attribute the observed physiological phenotype to the $qvr$ locus defined on the basis of paraquat hypersensitivity, we examined two deficiencies, $Df(2R)en-B$ and $Df(2R)en-SFX31$, that cover a chromosome region that contains $qvr^+$. In addition, we generated five new deficiency lines from the mobilization of two P-elements that map near the $qvr$ locus. All of these deficiency lines are homozygous lethal. Four deficiency lines designated $qvr^{3a-1,qvr^{3a-2,qvr^{3a-3,qvr^{3a-4}$ and $qvr^{3a-4}$ were obtained by mobilizing the P-element in $P[17en1]$. The $qvr^{3a-4}$ mutation was obtained by mobilizing the P-element in $P[17en43]$. All of these deficiency lines except $qvr^{3a-1}$ failed to complement the $qvr^+$ mutation in the leg-shaking behavioral test. As shown in Table 2, heterozygotes between these deficiency lines and $qvr^+$ showed a reduction in $I_A$ amplitude and slower $I_A$ kinetics as indicated by the time to peak $I_A$. These heterozygotes, except $qvr^{3a-4}$/$qvr^+$, did not show a significantly different amplitude of $I_A$ from that of the $qvr^+$ mutation. These results establish that the physiological phenotype comaps with the $I_A$ and $I_K$ hypersensitivity to the $qvr$ locus.

Table 2. Alteration of $I_A$ in $qvr$ deficiency lines

<table>
<thead>
<tr>
<th>Genotype</th>
<th>$I_A$ (nA/nF)</th>
<th>Time to peak (msec)</th>
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<tr>
<td>$qvr^+$</td>
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<td>8</td>
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<tr>
<td>$qvr^+$</td>
<td>2.5</td>
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</tr>
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<td>10.8</td>
<td>2</td>
</tr>
<tr>
<td>$qvr^{3a-3,qvr^+}$</td>
<td>1.6</td>
<td>10.6</td>
<td>2</td>
</tr>
<tr>
<td>$qvr^{1/2,qvr^+}$</td>
<td>1.3</td>
<td>11.3</td>
<td>2</td>
</tr>
<tr>
<td>$qvr^{3a-4,qvr^+}$</td>
<td>1.8</td>
<td>11.6</td>
<td>5</td>
</tr>
<tr>
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<td>10.4</td>
<td>2</td>
</tr>
<tr>
<td>$Df(2R)en-SFX31/qvr^+$</td>
<td>1.9</td>
<td>11.7</td>
<td>2</td>
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</table>

DISCUSSION

In this study we present a genetic and physiological characterization of a novel leg-shaking mutation, $qvr^+$. The observed paraquat hypersensitivity in $Sh$, $Hk$, and $eag$ mutant flies may be related to the shorter life span and increased metabolic rate in these hyperactive mutants (Trout and Kaplan, 1970), which could increase ROS production and thus favors paraquat hypersensitivity. The measurement of survival rate in double mutants suggests that the hypersensitivity to paraquat is closely related to membrane hyperexcitability. It should be noted that the Cu/Zn superoxide dismutase mutation cSOD$^{1088}$ or exposure of wild-type flies to 1 mM paraquat did not alter the conductance or kinetics of the $I_A$ current in larval muscles (Wang and Wu, unpublished observations) and that the enzymatic levels of catalase or cSOD are normal in $qvr^+$ mutant flies (Humphreys, 1996). These results suggest that general disturbance in ROS homeostasis per se does not alter $I_A$ currents. Similar to the $eag Sh$ double mutants, double mutants of $eag$ and $qvr^+$ showed a wings-down phenotype, the hallmark of extreme hyperexcitability. However, the mutation cSOD$^{1088}$, when combined with $Sh^+$, $Hk^+$, or $eag^-$, did not generate any wings-down double-mutant flies (J. M. Humphreys, A. J. Hilliker, and J. P. Phillips, unpublished observations), suggesting that the wings-down phenotype may be caused by hyperexcitability instead of an increase in the ROS level. These observations raise an interesting possibility that a defect in $I_A$ $K^+$ channels can disrupt $K^+$ ion homeostasis and in turn result in excessive ROS. This could be confirmed in the future by measuring the ROS level in all of these $K^+$ channel mutants.

Null mutations of the $Sh$ gene eliminate the $I_A$ current (Wu et al., 1983), whereas the major component of $I_K$ in Drosophila muscles is abolished by a mutation in the Shab locus (Tsunoda and Salkoff, 1995; Singh and Singh, 1999). Deletion of the slowpoke gene removes $I_{Ca}$ current (Elkins et al., 1986; Komatsu et al., 1990). In contrast to these mutations of $K^+$ channel $alpha$ subunits, null mutations of the $beta$ subunit modify but do not abolish $I_A$ (Wang and Wu, 1996; Yao and Wu, 1999). Furthermore, the specific effect of $qvr$ mutations on $I_A$ current instead of a more global effect on $K^+$ currents parallels the phenotype of $Hk$ mutations. Mutation of $qvr$ disrupted the modulation of but did not eliminate $I_A$. The phenotypic similarities of physiological hyperexcitability and leg-shaking behavior between $qvr$ and the other $K^+$ channel mutant stocks $Sh$, $Hk$, and $eag$ suggest that the $qvr$ gene might encode a novel $K^+$ channel-related polypeptide. Heterozygotes between several deficiencies and $qvr^+$ showed phenotypes similar to that of the $qvr^+$ homozygote in the amplitudes of $I_A$ and EJC, suggesting that $qvr^+$ may be a null mutation.

The molecular cloning and physiological characterization of the $Sh$, $eag$, and $Hk$ genes have served to point out the complex molecular machinery required for the proper functioning of $K^+$ channels. On the basis of the reduction of all four muscle $K^+$...
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Schwarz TL, Tempel BL, Papazian DM, Jan Y-N, Jan LY (1988) Multiple...