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Modulation of the Frequency Response of *Shaker* Potassium Channels by the Quiver Peptide Suggesting a Novel Extracellular Interaction Mechanism

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Abstract

Recent studies have indicated that the *Shaker* potassium channel regulates sleep in *Drosophila*. The *Drosophila quiver* (*qvr*) gene encodes a novel potassium channel subunit that modulates the *Shaker* potassium channel. The Qvr peptide contains a signal sequence for extracellular localization. Qvr may regulate a unique feature of the *Shaker* I_A current that confers special neuronal excitability patterns. Studies of the *Shaker* channel properties in the *qvr* mutation background should provide an opportunity to uncover physiologic modulation of potassium channels. We have begun to investigate the impact of *qvr* protein on the *Shaker* channel properties and its implications in synaptic function *in vivo*. We studied synaptic transmission at the larval neuromuscular junction and characterized the transient potassium current I_A in larval muscles. We identified two different functional states of I_A in *qvr* larval muscles, as reflected by two distinct components, I_{AF} and I_{AS}, differing in their kinetics of recovery from inactivation and sensitivity to a K^+ channel blocker. Correspondingly, *qvr* mutant larvae exhibit multiple synaptic discharges following individual nerve stimuli during repetitive activity.

INTRODUCTION

Potassium channels are ubiquitous in organisms from bacteria to humans (Hille, 2001). The demand for appropriate membrane excitability is met by a large repertoire of potassium channels individually distinct in current amplitude, as well as temporal dynamics of activation, inactivation and recovery from inactivation. There are several well-established mechanisms to generate this functional diversity. First, there are multiple genes encoding potassium channel subunits with different properties, some of which are gated by membrane potential, while others gated by ligand binding, e.g. to calcium or cyclic nucleotides. Second, the coding region of some potassium channel genes exhibits alternative splicing, yielding isoforms of a subunit. Third, different pore-forming subunits can form heteromultimeric assembly. Furthermore, auxiliary subunits can interact with the channel assembly to modulate potassium channel properties under different cellular conditions. Much of the insight into the functional diversity of potassium channels has been gained from *in vivo* studies of potassium channel mutations in conjunction with *in vitro* heterologous expression of single or multiple potassium channel subunits.

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Studies of several *Drosophila* mutants that exhibit the peculiar leg-shaking phenotype have identified: *Shaker* (*Sh*) and *ether a go-go* (*eag*) as genes encoding the pore-forming α subunit of potassium channels, as well as *Hyperkinetic* (*Hk*) as a gene encoding the auxiliary β subunit capable of interacting with *Sh* and *eag* subunits (Chouinard, Wilson, Schlimgen, & Ganetzky, 1995; Kamb, Tseng-Crank, & Tanouye, 1988; Kaplan & Trout, 1969; Pongs et al., 1988; Schwarz, Tempel, Papazian, Jan, & Jan, 1988; Wang & Wu, 1996; Warmke, Drysdale, & Ganetzky, 1991). Genetic dissections have revealed the contribution of each subunit to the biophysical properties of a potassium channel and its role in controlling membrane excitability (Wu & Ganetzky, 1992). Computer assisted behavioral analysis reveals quantifiable distinctions in defects of larval locomotion behaviors caused by such K^+ channel mutations (Wang, Soll, & Wu, 2002; Wang et al., 1997). Physiological experiments have shown that mutations of these three genes impair the transient K^+ current (I_A) in *Drosophila* larval muscles (Salkoff & Wyman, 1981; Wang & Wu, 1996; Wu, Ganetzky, Haugland, & Liu, 1983; Wu & Haugland, 1985; Zhong & Wu, 1991a) and neurons (Baker & Salkoff, 1990; Tanouye & Ferrus, 1985; Yao & Wu, 1999; Zhao, Sable, Iverson, & Wu, 1995). Furthermore, these channel mutations enhance synaptic transmission at the larval neuromuscular junctions (Ganetzky & Wu, 1983; Jan, Jan, & Dennis, 1977; Stern & Ganetzky, 1989; Ueda & Wu, 2009a; Wu et al., 1983), suggesting that these K^+ channel subunits play a role in terminating neurotransmitter release at presynaptic terminals. Studies of additional *Drosophila* mutants with phenotypes similar to *Sh*, *eag* and *Hk* should generate more insight into the specific biophysical properties of the potassium channel that are crucial for individual behavioral phenotypes.

In a previous study, we demonstrated that another *Drosophila* leg-shaking mutant displays enhanced neurotransmission at the larval neuromuscular junction, reduced and slower I_A current in larval muscles, which is caused by mutation in the gene named *quiver* (*qvr*) (Wang, Humphreys, Phillips, Hilliker, & Wu, 2000). Two recent studies have indicated that normal I_A current is necessary for regulating sleep in *Drosophila*. First, *Sh* mutant flies exhibit reduction in sleep amount (Cirelli et al., 2005). Second, a large scale genetic screen for abnormal sleep behavior in *Drosophila* has identified an extreme mutant called *sleepless* which turns out to be an allele of *qvr* (Koh et al., 2008). The *qvr* gene encodes a putative glycosylphosphatidylinositol (GPI)-anchored membrane peptide enriched in fly brain. The Qvr peptide contains a signal sequence for extracellular localization, which is confirmed by immunostaining of cultured cells expressing *qvr* (Koh et al., 2008). These results immediately suggest that physiologic states such as wakefulness may have a functional link to the biophysical properties of the *Sh* channel via Qvr. Furthermore, through this unprecedented interaction with the *Sh* channel from an extracellular domain, Qvr may regulate a unique feature of I_A current that confer special neuronal excitability patterns.

Studies of the *Sh* channel properties in the *qvr* mutation background should provide an opportunity to uncover physiologic modulation of potassium channels. We have begun to investigate the impact of *qvr* protein on the *Sh* channel properties and its implications in synaptic function *in vivo*. We studied synaptic transmission at the larval neuromuscular junction and characterized the properties of transient potassium current I_A in larval muscles. We identified two different functional states of I_A in *qvr* larval muscles, as reflected by two distinct components, I_{AF} and I_{AS}, differing in their kinetics of recovery from inactivation and sensitivity to a K^+ channel blocker. Correspondingly, *qvr* mutant larvae exhibit multiple synaptic discharges following individual nerve stimuli during repetitive activity. This work has been described in part in a Ph.D. thesis (Wang, 1997).

METHODS

Drosophila Mutants

All flies were raised at room temperature (20–23°C) and fed with standard medium. The parental stock, qvr^{+}; ry^{+5}, for generating the qvr^{1} mutant, was originally derived from Oregon-R strain and used in this study as a control. $qvr^{\Delta 43\text{-}1}$ is a homozygous lethal allele of *qvr*, generated by mobilization and imprecise excision of a nearby P-element P[17en43] (Humphreys, 1996).

Sh^{5}, Sh^{M}, *g sd* Sh^{rKO120} (abbreviated as Sh^{120} in the text), Hk^{1} and eag^{1} were originally from the collection of Dr. Seymour Benzer at the California Institute of Technology, California. Sh^{M} is a null allele (Zhao et al., 1995) and eliminates I_A in larval muscles (Wu & Haugland, 1985). Sh^{5} is a point mutation in the S4–S5 linker (Gautam & Tanouye, 1990), and alters the voltage dependence of I_A (Gautam & Tanouye, 1990; McCormack et al., 1991; Wu & Haugland, 1985). eag^{4pm} has been identified as a spontaneous mutation in the original stock Sh^{rKO120} (Ganetzky & Wu, 1983). Sh^{rKO120} has a reduced I_A current in larval muscles (Haugland & Wu, 1990), and produces a detectable level of *Sh* polypeptide (Zhao et al., 1995); its mutation site may lie in the 5' portion of the constant region (Gautam & Tanouye, 1990). Compound mutants were all confirmed by scoring leg-shaking phenotype and electrophysiological experiments.

Synaptic Transmission

As previously described, excitatory junctional currents (EJCs) were recorded intracellularly from muscles of abdominal segment 3–5 in third-instar larvae at 16°C in standard saline containing 4 mM $MgCl_2$ (Zhong & Wu, 1991a). Larval dissection was performed in Ca^{2+}-free saline to minimize muscle contraction. For wild-type control, the saline contained 0.2 mM Ca^{2+}. Because of a drastically increased transmission caused by *qvr* mutations (Wang et al., 2000), $[Ca^{2+}]_o$ was lowered from 0.2 to 0.1 mM to allow a quantitative analysis of the altered synaptic mechanism in the qvr^{1} mutant. Importantly, synaptic transmission at wild-type neuromuscular junctions did not show frequency-dependent enhancement either at 0.2 mM Ca^{2+} (Fig. 1) or at 0.1 mM Ca^{2+} (data not shown).

Muscle fibers were maintained at −80 mV with two-electrode voltage clamp. A suction pipette with a tip opening of about 1 μm was employed to stimulate the segmental nerve to evoke synaptic transmission. Stimulations with a duration of 0.1 ms were delivered at a low repetition rate from 0.1 to 0.5 Hz with a Grass stimulator (Model S88). Normally, two discrete EJCs were evoked at two different thresholds, representing signals generated by Is and Ib boutons (Ueda & Wu, 2009b). In all the experiments presented in this study, a stimulus voltage slightly higher than the upper threshold was used. Signals were low-pass filtered at 2 kHz (Model 3202R, Krohn-Hite, Avon, MA). Temperature deviation from room temperature was controlled by a Peltier stage (Cambion, Cambridge, MA).

Voltage-gated K^{+} Currents in Larval Muscles

The two-electrode voltage clamp technique for measuring I_A has been described previously (Haugland & Wu, 1990; S. Singh & Wu, 1989; Wang & Wu, 1996). In brief, third instar larvae were dissected to make body wall muscles accessible, and the voltage-gated I_A and I_K were recorded in Ca^{2+}-free standard saline containing 128 mM NaCl, 2 mM KCl, 14 mM MgCl2, 35 mM sucrose, 5 mM EGTA, and 5 mM HEPES (pH 7.1) at 11°C. A two-second pre-conditioning pulse to −20 mV from a holding potential of −80 mV inactivates I_A but does not affect I_K. The subtraction of the current with pre-conditioning pulse from the one without produces I_A. Data acquisition was performed with an IBM-compatible computer equipped with PClamp software (Version 5) in conjunction with a Master-8 programmable

stimulator (AMPI) for generating depolarizing voltage. Data were analyzed off-line on Macintosh computers with AxoGraph 2.0 software (Axon Instrument). For measuring the sensitivity of I_A to 4-aminopyridine (4-AP, from Sigma), muscle fibers were incubated in saline containing the indicated concentrations of drug for at least 15 min.

RESULTS

Abnormal Synaptic Transmission in *qvr*^{1} Mutant Larvae

The larval neuromuscular junction is easily accessible to electrophysiological measurements, which has been well established to reveal the importance of a given gene in controlling neurotransmission (DiAntonio & Schwarz, 1994; Umbach et al., 1994; Wu & Ganetzky, 1992; Yoshihara, Adolfsen, Galle, & Littleton, 2005; Zhong & Wu, 1991b). We found that the *qvr*^{1} mutant displayed an abnormal form of frequency-dependent enhancement in synaptic transmission (Figure 1). In the wild-type control, excitatory junctional currents (EJCs) were very regular with little fluctuation when the segmental nerve fiber was stimulated at a rate of 0.8 Hz. As can be seen in the inset of Figure 1A, the EJCs in response to the first, 10th, 20th, and 30th stimuli were approximately the same in their size and kinetics. In contrast, the amplitude of EJCs in *qvr*^{1} mutant larvae increased progressively as the nerve was stimulated repetitively, which led to multiple releases as indicated by multi-peak EJCs. Figure 1B inset presents an example of EJCs in *qvr*^{1} mutant larvae when the nerve was stimulated at a rate of 0.8 Hz. The first stimulus generated a small EJC, followed with a larger EJC for the 10th stimulus, and multiple-peaked responses for the 20th and the 30th stimuli. The multiple peaks seen in *qvr*^{1} EJCs require an integration of EJCs over time to appropriately measure the amount of neurotransmitter release. As shown in Figure 1B, the rate of synaptic enhancement was higher when the nerve was stimulated at higher frequency.

Fast- and Slow-Recovery Components in *Sh* I_A Currents Revealed by Mutation in the *qvr* Gene

Most invertebrate muscles, including that of *Drosophila*, do not express Na^+ channel, and rely on Ca^{2+}-mediated action potentials for muscle contraction (Schwartz & Stuhmer, 1984). A step depolarizing potential generates five major currents in *Drosophila* larval muscles. Four outward K^+ currents including the voltage-gated transient I_A and the delayed rectifier I_K, and the Ca^{2+}-dependent fast I_{CF} and the slow I_{CF}, plus an inward Ca^{2+} current (S. Singh & Wu, 1989). Genetic and pharmacological studies have shown that some of these currents consist of distinct components (Gielow, Gu, & Singh, 1995; A. Singh & Singh, 1999). In a previous study, we showed that the *qvr* mutations affect only the transient I_A, but not I_K, I_{CF}, I_{CS} or the calcium current (Wang et al., 2000).

When ten episodes of a depolarizing step to +10 mV were applied to a *qvr* mutant muscle, the first outward transient current (I_A) was more than twice that of the subsequent nine currents, but not as large as the wild-type current. The subsequent nine currents were almost the same size in amplitude (Figure 2A) with the same kinetics, suggesting that there were two components of I_A in *qvr* mutant muscles. This contrasts with the use-dependent inactivation of a homogeneous component, which should display a gradual decay in the current amplitude upon repetitive depolarization. The first and the average of the subsequent nine I_A were shown in Figure 2B for *qvr*^{1} and *qvr*$^{\Delta 43\text{-}1}$/*qvr*^{1}, in comparison with the I_A amplitude of the wild-type control *qvr*$^{+}$. As shown in Figure 2, the first and the subsequent I_A currents displayed the same time to peak and the same inactivation kinetics, which could be visualized when the two traces were normalized (not shown). The fast- and slow-recovery components are therefore named I_{AF} and I_{AS}, respectively. Operationally, the first I_A in Figure 2 is assumed to represent the sum of I_{AF} and I_{AS}, and the average of subsequent

nine I_A currents includes only I_{AF}. The voltage dependence of I_{AF} and I_{AS} for *qvr*^{1} and *qvr*$^{\Delta 43\text{-}1}$/*qvr*^{1} is presented in Figure 2B. The similarity in I-V curve between *qvr*^{1} and *qvr*$^{\Delta 43\text{-}1}$/*qvr*^{1} suggests the observed phenotype is attributable to the *qvr* locus and not likely an effect of an unidentified second-site mutation in the background.

Differential Sensitivity of I_{AF} and I_{AS} to 4-AP

I_A but not I_K is blocked by 4-AP at micromolar concentrations in *Drosophila* larval muscles (Haugland & Wu, 1990). It is known that the binding affinity and the mode of action of a channel blocker often depend on the conformational state of the channel (Hille, 2001). Previous experiments have shown that mutations in the S4–S5 linker of the *Sh* α subunit (Haugland & Wu, 1990; Kirsch, Shieh, Drewe, Vener, & Brown, 1993; McCormack et al., 1991) or in the *Hk* β subunit (Wang & Wu, 1996; Yao & Wu, 1999) confer abnormal sensitivity to 4-AP, which has been shown to bind to the cytoplasmic pore region of the *Sh* channel. As shown in Figure 3, I_{AF} was more sensitive to 4-AP than I_{AS}, resulting in a smaller ratio of I_{AF}/I_{AS} when the concentration of 4-AP increased. The ratio I_{AF}/I_{AS} was about 0.3 when 100 μM 4-AP was applied, in contrast to a ratio of 0.58 ± 0.05 (n = 7) before drug treatment. The decreasing ratio of I_{AF} to I_{AS} at higher concentrations of 4-AP indicates that I_{AF} and I_{AS} have differential sensitivity to 4-AP and that they might have different channel conformations.

Genetic Dissection of I_{AF} and I_{AS}

We next used a repertoire of I_A channel mutants in *Drosophila* to further investigate channel assembly in double mutants with *qvr*. The sum of I_{AF} and I_{AS} of the double mutants, when compared with the I_A of the corresponding single mutants, might yield information about the nature or constraints of interactions with the *qvr* product within the channel (see Figure 4). $I_{AF} + I_{AS}$ in *Sh*^{5} *qvr*^{1} double mutant was about 5.0 ± 0.2 nA/nF, similar to the amplitude of I_A in *Sh*^{5} mutant muscles (4.5 ± 0.5 nA/nF). In contrast, $I_{AF} + I_{AS}$ in *Sh*^{120} *qvr*^{1} double mutant was only around 3.9 ± 0.2 nA/nF, significantly smaller than the observed 6.2 ± 0.6 nA/nF in *Sh*^{120} mutant muscles. Notably, as shown in Figure 4, $(I_{AF} + I_{AS})/I_A$ reduction fell consistently in the range of 44–70% in *Hk*^{1}*qvr*^{1}, *eag*^{1}*qvr*^{1} and *eag*^{4pm}*qvr*^{1}, as seen in *Sh*^{120}*qvr*^{1} double mutants, compared with nearly unchanged for *Sh*^{5} *qvr*^{1} double mutants. Apparently, *Sh*^{5} mutation prevented the amplitude reduction of I_A current in *qvr*^{1} mutant larvae. This discrepancy in the reduction of conductance conferred by the *qvr*^{1} mutation in different molecularly identified mutants may stem from the structural differences between *Sh*^{5} and *Sh*^{120}, which have been shown to be a point mutation in the S4–S5 linker and a mutation with defect in the 5' portion of the constant region, respectively (Gautam & Tanouye, 1990).

DISCUSSION

The involvement of the *qvr* K^+ channel subunit in sleep regulation and its potential interaction with the *Sh* K^+ channel from extracellular domain (Koh et al., 2008) call for more biophysical studies of the Qvr function. The present study shows that Qvr is important for the maintenance of neuronal excitability. Frequent stimulations of the motor axons in *qvr* mutant larvae generate discretely increased neuromuscular transmission. In a detailed biophysical characterization of the *Sh* potassium channel, we found that the transient I_A current in *qvr* mutant muscles exhibits two discrete components, I_{AF} and I_{AS}, which are not observed in normal muscles. I_{AF} displays faster kinetics of recovery from inactivation and more sensitivity to 4-AP than I_{AS}. Furthermore, analysis of I_{AF} and I_{AS} in double mutants, *Sh qvr*, *eag qvr* and *Hk qvr*, identified a potential conformational change in the *Sh* K^+ channel conferred by Qvr.

Our previous study shows that *qvr* mutations affect only the I_A channel in both conductance and kinetics, without altering I_K, I_{CF}, I_{CS}, and I_{Ca}, suggesting that the Qvr assumes a role in modulating the α-subunit of the I_A channel (Wang et al., 2000). Its phenotypic similarities to the three known K^+ channel mutants *Sh*, *Hk* and *eag* provide hints that the *qvr* gene might code for a distinct K^+ channel subunit. The Qvr protein is predicted to contain a GPI-attachment site and the GPI anchor can be cleaved by PLC (Koh et al., 2008). We have investigated the potential conformational change conferred by the interaction between Qvr and the *Sh* K^+ channel. First, we used a pharmacological approach. 4-AP has been well established as a *Sh* channel blocker that binds to the cytoplasmic pore region of the channel (Haugland & Wu, 1990; Kirsch et al., 1993; McCormack et al., 1991). The sensitivities to 4-AP of I_{AF} and I_{AS} showed an IC_{50} of 60 and 200 μM, respectively, which were much higher than the IC_{50} of only 7 μM in wild-type muscles (Wang & Wu, 1996). The differential 4-AP sensitivities in I_{AF} and I_{AS} imply different conformation in the cytoplasmic pore region. We then used several K^+ channel mutants to identify potential site of conformational change. The Sh^5 mutation, which has an amino acid replacement in the S4–S5 linker of the *Sh* polypeptide (Gautam and Tanouye, 1990), caused an increase in 4-AP sensitivity (Haugland & Wu, 1990). Among several different double mutants, Sh^5qvr^1 exhibited a special property: a sum amplitude of I_{AF} and I_{AS} is on par with the I_A amplitude in Sh^5 mutant muscles. These results suggest that the interaction between Qvr and the *Sh* channel confers conformational change at the Sh^5 mutation site.

The excitability control of the motor neurons can be attributed to the function of the *Sh* channel. It is interesting to note that the 4-AP sensitive I_A current plays an important role in the gating of action potentials in hippocampal CA3 pyramidal neurons (Debanne, Guerineau, Gahwiler, & Thompson, 1997). Action potentials in the pyramidal neurons can be blocked by the activation of the I_A current with a brief hyperpolarization a few milliseconds before the induction of an action potential. The discrete increase in EJC at the neuromuscular junction in *Drosophila* larvae suggests that Qvr is important in the controlling the propagation of action potentials. This result is in accord with previous findings that efficient membrane repolarization is required to suppress supernumerary action potentials in the motor axon (Ueda & Wu, 2006).

Sleep is thought as a physiological state that increases the efficiency of behavior by regulating its timing and energy use (Siegel, 2009). Studies of rat cerebral energy consumption suggest that about half of the brain energy is used to drive signals along axons and across synapse (Laughlin & Sejnowski, 2003). Our study suggests that modulation of the *Sh* K^+ channel by Qvr is a potential gating mechanism for the generation of action potentials in the nervous system. One can speculate that physiologic modulation of Qvr function plays an important role in controlling action potentials in relevant neural circuit for sleep. However, *qvr* expression does not fluctuate with the circadian cycle (Koh et al., 2008). Therefore, future experiments to demonstrate whether and how wakefulness/sleep states modulate the biophysical properties of the *Sh* K^+ channel, reminiscent of the comprehensive studies in the mammalian thalamocortical systems (McCormick & Bal, 1997), will be important to provide a mechanistic link between sleep and K^+ channel function. Alternatively, it is possible that wakefulness/sleep states do not modulate *Sh* K^+ channel and the abnormal sleep phenotype in K^+ channel mutant flies is due to their hypersensitivity to sensory stimulations and a hypersensitive motor system. Future studies of Qvr function in *Drosophila* central brain with optical imaging may shed light on the role of Qvr in sleep regulation (Root et al., 2008; Wang et al., 2003).

REFERENCES

Baker K, Salkoff L. The Drosophila Shaker gene codes for a distinctive K+ current in a subset of neurons. Neuron. 1990; 4(1):129–140. [PubMed: 2310571]

Chouinard SW, Wilson GF, Schlimgen AK, Ganetzky B. A potassium channel beta subunit related to the aldo-keto reductase superfamily is encoded by the Drosophila hyperkinetic locus. Proc Natl Acad Sci U S A. 1995; 92(15):6763–6767. [PubMed: 7542775]

Cirelli C, Bushey D, Hill S, Huber R, Kreber R, Ganetzky B, et al. Reduced sleep in Drosophila Shaker mutants. Nature. 2005; 434(7037):1087–1092. [PubMed: 15858564]

Debanne D, Guerineau NC, Gahwiler BH, Thompson SM. Action-potential propagation gated by an axonal I(A)-like K+ conductance in hippocampus. Nature. 1997; 389(6648):286–289. [PubMed: 9305843]

DiAntonio A, Schwarz TL. The effect on synaptic physiology of synaptotagmin mutations in Drosophila. Neuron. 1994; 12(4):909–920. [PubMed: 7909234]

Ganetzky B, Wu CF. Neurogenetic analysis of potassium currents in Drosophila: synergistic effects on neuromuscular transmission in double mutants. J Neurogenet. 1983; 1(1):17–28. [PubMed: 6100303]

Gautam M, Tanouye MA. Alteration of potassium channel gating: molecular analysis of the Drosophila Sh5 mutation. Neuron. 1990; 5(1):67–73. [PubMed: 2369521]

Gielow ML, Gu GG, Singh S. Resolution and pharmacological analysis of the voltage-dependent calcium channels of Drosophila larval muscles. J Neurosci. 1995; 15(9):6085–6093. [PubMed: 7666192]

Haugland FN, Wu CF. A voltage-clamp analysis of gene-dosage effects of the Shaker locus on larval muscle potassium currents in Drosophila. J Neurosci. 1990; 10(4):1357–1371. [PubMed: 2109786]

Hille, B. Ionic channels of excitable membranes. Third ed.. Sinauer; Sunderland: 2001.

Humphreys JM. University of Guelph. 1996

Jan YN, Jan LY, Dennis MJ. Two mutations of synaptic transmission in Drosophila. Proc R Soc Lond B Biol Sci. 1977; 198(1130):87–108. [PubMed: 20636]

Kamb A, Tseng-Crank J, Tanouye MA. Multiple products of the Drosophila Shaker gene may contribute to potassium channel diversity. Neuron. 1988; 1(5):421–430. [PubMed: 3272175]

Kaplan WD, Trout WE 3rd. The behavior of four neurological mutants of Drosophila. Genetics. 1969; 61(2):399–409. [PubMed: 5807804]

Kirsch GE, Shieh CC, Drewe JA, Vener DF, Brown AM. Segmental exchanges define 4-aminopyridine binding and the inner mouth of K+ pores. Neuron. 1993; 11(3):503–512. [PubMed: 8398143]

Koh K, Joiner WJ, Wu MN, Yue Z, Smith CJ, Sehgal A. Identification of SLEEPLESS, a sleep-promoting factor. Science. 2008; 321(5887):372–376. [PubMed: 18635795]

Laughlin SB, Sejnowski TJ. Communication in neuronal networks. Science. 2003; 301(5641):1870–1874. [PubMed: 14512617]

McCormack K, Tanouye MA, Iverson LE, Lin JW, Ramaswami M, McCormack T, et al. A role for hydrophobic residues in the voltage-dependent gating of Shaker K+ channels. Proc Natl Acad Sci U S A. 1991; 88(7):2931–2935. [PubMed: 2011602]

McCormick DA, Bal T. Sleep and arousal: thalamocortical mechanisms. Annu Rev Neurosci. 1997; 20:185–215. [PubMed: 9056712]

Pongs O, Kecskemethy N, Muller R, Krah-Jentgens I, Baumann A, Kiltz HH, et al. Shaker encodes a family of putative potassium channel proteins in the nervous system of Drosophila. Embo J. 1988; 7(4):1087–1096. [PubMed: 2456921]

Root CM, Masuyama K, Green DS, Enell LE, Nassel DR, Lee CH, et al. A presynaptic gain control mechanism fine-tunes olfactory behavior. Neuron. 2008; 59(2):311–321. [PubMed: 18667158]

Salkoff L, Wyman R. Genetic modification of potassium channels in Drosophila Shaker mutants. Nature. 1981; 293(5829):228–230. [PubMed: 6268986]

Schwartz LM, Stuhmer W. Voltage-dependent sodium channels in an invertebrate striated muscle. Science. 1984; 225(4661):523–525. [PubMed: 6330898]

Schwarz TL, Tempel BL, Papazian DM, Jan YN, Jan LY. Multiple potassium-channel components are produced by alternative splicing at the Shaker locus in Drosophila. Nature. 1988; 331(6152):137–142. [PubMed: 2448635]

Siegel JM. Sleep viewed as a state of adaptive inactivity. Nat Rev Neurosci. 2009; 10(10):747–753. [PubMed: 19654581]

Singh A, Singh S. Unmasking of a novel potassium current in Drosophila by a mutation and drugs. J Neurosci. 1999; 19(16):6838–6843. [PubMed: 10436041]

Singh S, Wu CF. Complete separation of four potassium currents in Drosophila. Neuron. 1989; 2(4): 1325–1329. [PubMed: 2516727]

Stern M, Ganetzky B. Altered synaptic transmission in Drosophila hyperkinetic mutants. J Neurogenet. 1989; 5(4):215–228. [PubMed: 2553904]

Tanouye MA, Ferrus A. Action potentials in normal and Shaker mutant Drosophila. J Neurogenet. 1985; 2(4):253–271. [PubMed: 3936906]

Ueda A, Wu CF. Distinct frequency-dependent regulation of nerve terminal excitability and synaptic transmission by IA and IK potassium channels revealed by Drosophila Shaker and Shab mutations. J Neurosci. 2006; 26(23):6238–6248. [PubMed: 16763031]

Ueda A, Wu CF. Effects of social isolation on neuromuscular excitability and aggressive behaviors in Drosophila: altered responses by Hk and gsts1, two mutations implicated in redox regulation. J Neurogenet. 2009a; 23(4):378–394. [PubMed: 19863269]

Ueda A, Wu CF. Role of rut adenylyl cyclase in the ensemble regulation of presynaptic terminal excitability: reduced synaptic strength and precision in a Drosophila memory mutant. J Neurogenet. 2009b; 23(1–2):185–199. [PubMed: 19101836]

Umbach JA, Zinsmaier KE, Eberle KK, Buchner E, Benzer S, Gundersen CB. Presynaptic dysfunction in Drosophila csp mutants. Neuron. 1994; 13(4):899–907. [PubMed: 7946336]

Wang, JW. Electrophysiological and genetic analyses of Drosophila behavioral mutants: the functional roles of voltage-gated potassium channel subunits. University of Iowa; Iowa City: 1997.

Wang JW, Humphreys JM, Phillips JP, Hilliker AJ, Wu CF. A novel leg-shaking Drosophila mutant defective in a voltage-gated K(+)current and hypersensitive to reactive oxygen species. J Neurosci. 2000; 20(16):5958–5964. [PubMed: 10934243]

Wang JW, Soll DR, Wu CF. Morphometric description of the wandering behavior in Drosophila larvae: a phenotypic analysis of K+ channel mutants. J Neurogenet. 2002; 16(1):45–63. [PubMed: 12420789]

Wang JW, Sylwester AW, Reed D, Wu DA, Soll DR, Wu CF. Morphometric description of the wandering behavior in Drosophila larvae: aberrant locomotion in Na+ and K+ channel mutants revealed by computer-assisted motion analysis. J Neurogenet. 1997; 11(3–4):231–254. [PubMed: 10876655]

Wang JW, Wu CF. In vivo functional role of the Drosophila hyperkinetic beta subunit in gating and inactivation of Shaker K+ channels. Biophys J. 1996; 71(6):3167–3176. [PubMed: 8968587]

Wang JW, Wong AM, Flores J, Vosshall LB, Axel R. Two-photon calcium imaging reveals an odor-evoked map of activity in the fly brain. Cell. 2003; 112(2):271–282. [PubMed: 12553914]

Warmke J, Drysdale R, Ganetzky B. A distinct potassium channel polypeptide encoded by the Drosophila eag locus. Science. 1991; 252(5012):1560–1562. [PubMed: 1840699]

Wu CF, Ganetzky B. Neurogenetic studies of ion channels in Drosophila. Ion Channels. 1992; 3:261–314. [PubMed: 1330057]

Wu CF, Ganetzky B, Haugland FN, Liu AX. Potassium currents in Drosophila: different components affected by mutations of two genes. Science. 1983; 220(4601):1076–1078. [PubMed: 6302847]

Wu CF, Haugland FN. Voltage clamp analysis of membrane currents in larval muscle fibers of Drosophila: alteration of potassium currents in Shaker mutants. J Neurosci. 1985; 5(10):2626–2640. [PubMed: 2413182]

Yao WD, Wu CF. Auxiliary Hyperkinetic beta subunit of K+ channels: regulation of firing properties and K+ currents in Drosophila neurons. J Neurophysiol. 1999; 81(5):2472–2484. [PubMed: 10322082]

Yoshihara M, Adolfsen B, Galle KT, Littleton JT. Retrograde signaling by Syt 4 induces presynaptic release and synapse-specific growth. Science. 2005; 310(5749):858–863. [PubMed: 16272123]

Zhao ML, Sable EO, Iverson LE, Wu CF. Functional expression of Shaker K+ channels in cultured Drosophila “giant” neurons derived from Sh cDNA transformants: distinct properties, distribution, and turnover. J Neurosci. 1995; 15(2):1406–1418. [PubMed: 7869107]

Zhong Y, Wu CF. Alteration of four identified K+ currents in Drosophila muscle by mutations in eag. Science. 1991a; 252(5012):1562–1564. [PubMed: 2047864]

Zhong Y, Wu CF. Altered synaptic plasticity in Drosophila memory mutants with a defective cyclic AMP cascade. Science. 1991b; 251(4990):198–201. [PubMed: 1670967]

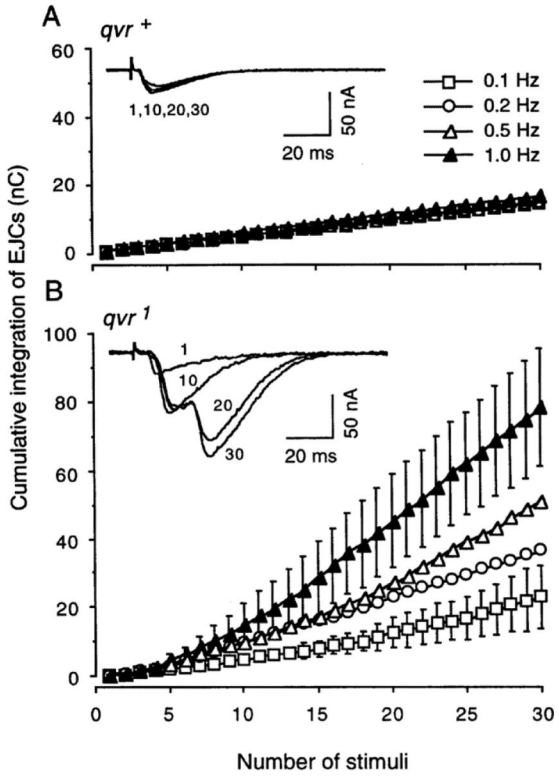

Figure 1.

Frequency-dependent enhancement in synaptic transmission at *qvr*^{1} mutant neuromuscular junctions. The integration of EJCs over time rather than a simple measurement from the peak EJC is more indicative of the amount of transmitter release in this case. Segmental nerve fibers were stimulated at indicated repetition rates for a duration of 0.1 ms with a voltage above the higher of the two thresholds that could evoke synaptic transmission in the two motor axon terminals. Representative traces were obtained at 0.8 Hz. **A**. Larval preparation was immersed in standard saline containing 0.2 mM $CaCl_2$ and 4 mM $MgCl_2$. SEM (n = 4) are smaller than the symbols, therefore not shown. **B**. Repetitive stimulations of mutant neuromuscular junctions gradually led to multiple EJCs. Standard saline contained 0.1 mM $CaCl_2$ and 4 mM $MgCl_2$. Under this condition, the initial EJCs were about the same as those from *qvr*$^{+}$ larvae. Mean ± SEM (n = 4) is shown at 0.1 and 1.0 Hz, and error bars are omitted from plots at 0.2 and 0.5 Hz for simplicity. 16°C.

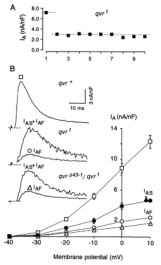

Figure 2.

Two discrete components I_{AF} and I_{AS} in *qvr* mutations. **A**. Ten episodes of +10 mV depolarizing steps were applied to a qvr^{1} muscle at a repetition rate of 0.05 Hz from a holding potential of −80 mV. The first depolarization generated a transient current of 7.2 nA/nF, however the subsequent depolarizations generated only a transient current of 3.0 nA/nF. The first I_A includes I_{AF} and I_{AS}, and the average of the subsequent nine I_A is defined as I_{AF}. **B**. I-V curves for I_{AF} and I_{AS}. I-V curve for the I_A of qvr^{+} is also plotted for comparison. Inset depicts typical traces from qvr^{+}, qvr^{1} and $qvr^{\Delta 43\text{-}1}/qvr^{1}$ when muscles were depolarized to +10 mV. $qvr^{\Delta 43\text{-}1}$ is homozygous lethal and generated from mobilization and imprecise excision of a nearby P element. n = 8, for qvr^{+}. For mutants (qvr^{1} and $qvr^{\Delta 43\text{-}1}/qvr^{1}$), each muscle fiber was used for measurement at only one voltage; each data point was from 4–5 muscle fibers. Values are shown as mean ± SEM. Recordings at approximately the first 3 ms include a capacitance surge and were omitted from the figure. For this and the following figures, experiments were done in standard saline containing 5 mM EGTA and 14 mM $MgCl_2$ (pH 7.1) at 11°C.

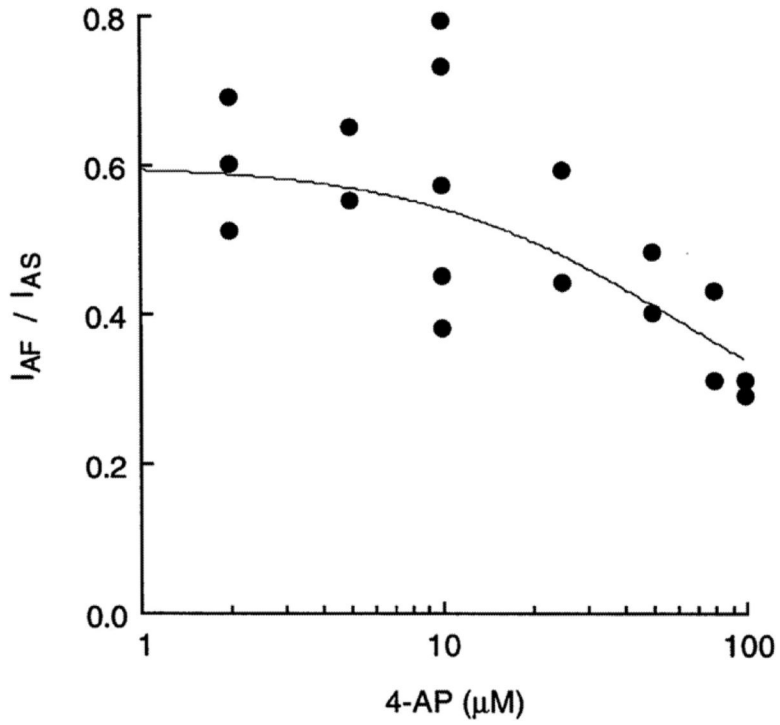

Figure 3.

Differential sensitivity to 4-AP for I_{AF} and I_{AS}. Each data point represents the ratio of I_{AF}/I_{AS} for one muscle fiber. The ratio was 0.58 ± 0.05 (n = 7) before drug treatment; it became smaller at higher concentrations, because I_{AF} was more sensitive than I_{AS} to 4-AP. The curve line indicates IC_{50} = 60 μM for I_{AF}, and IC_{50} = 200 μM for I_{AS}. Muscles were incubated for at least 15 min in saline containing indicated concentrations of 4-AP. Muscles were depolarized to +10 mV from a holding potential of −80 mV.

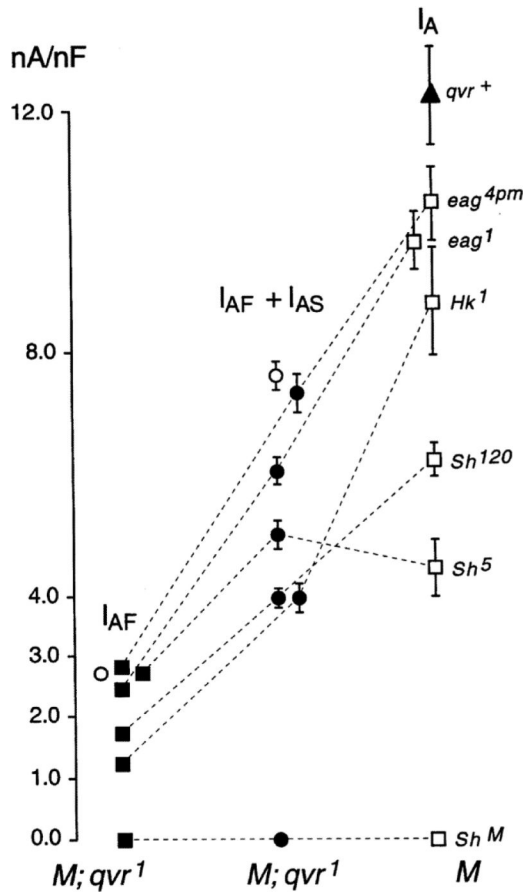

Figure 4.

The peak amplitudes of I_{AF} and I_{AS} were differentially modulated by different K^+ channel subunits. The sum of I_{AF} and I_{AS}, i.e., the I_A current in response to the first depolarization, and I_{AF} of double mutants are plotted in comparison to the I_A currents seen in single mutants of *Sh*, *Hk* and *eag*. Sh^5qvr^1 mutation exhibited no statistically significant reduction in I_{AF} and I_{AS} from the I_A current in Sh^5. Open circles indicate qvr^1; the filled triangle indicates qvr^+ control. Muscles were depolarized to +10 mV from a holding potential of −80 mV. Sample size: qvr^+, n = 8; eag^{4pm}, 4; eag^1, 4; Hk^1, 4; Sh^{120}, 6; Sh^5, 5; qvr^1, 13; $eag^{4pm}qvr^1$, 9; eag^1qvr^1, 12; Sh^5qvr^1, 11; $Sh^{120}qvr^1$, 8; Hk^1qvr^1, 10.