PROPERTIES OF POTASSIUM CHANNELS ALTERED BY
MUTATIONS OF TWO GENES IN *DROSOPHILA*

Chun-Fang Wu

Department of Zoology, University of Iowa, Iowa City, Iowa 52242

Barry Ganetzky

Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706

Mutations that alter membrane currents are especially useful for studying the molecular identity and mechanism of channels, e.g., K^+ channels, that lack specific, high-affinity toxins to facilitate biochemical analysis. Previous voltage-clamp studies in *Drosophila* larval muscles demonstrated that mutations of two genes, *Sh* and *eag*, eliminate or diminish two types of K^+ currents (1). Here we report on the analysis of additional mutations of these genes, which alter the voltage sensitivity and to the current density. The results support the idea that these genes take part in controlling the synthesis or structure of potassium channels.

RESULTS

Muscle fibers of third-instar larvae were analyzed by conventional two-microelectrode voltage-clamp techniques as described previously (1). These "electrically short" fibers are each identifiable and meet isopotential requirements (1, 2). In these cells, two K^+ currents, a fast transient current (I_A) and a steady, delayed-rectification current (I_K) (Fig. 1 A), have been observed and are separable by differences in voltage thresholds and inactivation properties (1). Several *Sh* alleles, including Sh^{KS133} and Sh^{102}, were shown to eliminate I_A completely but did not affect I_K.

The effect of Sh^5 on I_A is different from that of the alleles described above. In this mutant, I_A is still present but greatly diminished in amplitude (Fig. 1 B). In addition, Sh^5 displays abnormal current-voltage (*I-V*) characteristics, the activation of I_A apparently requiring a more depolarized membrane potential (Fig. 1 C). Because the remaining I_A has a small amplitude, precise measurement of its kinetics is difficult. However, the rise time (~20 ms to peak at −15 mV, 8°C) and the half time of decay (~40 ms at −15 mV, 8°C) of the remaining I_A are not significantly different from the normal values of 20 ms and 45 ms. As in other alleles, I_K is not affected, as indicated by the *I–V* relation (Fig. 1 C), the reversal potential, and the kinetics of tail currents (not shown).

Contrasting results have been observed for the effects of Sh^5 in adult flight muscles, where the amplitude and

voltage dependence of I_A activation were like control values but the kinetics of inactivation was abnormally rapid (3). It will be of interest to elucidate the means by which a specific mutation can alter channel properties in contrasting ways at different developmental stages.

Our previous results with eag^1 showed that I_K was significantly reduced and that I_A was also affected, but to a lesser extent (1). This result suggested that the *eag* gene
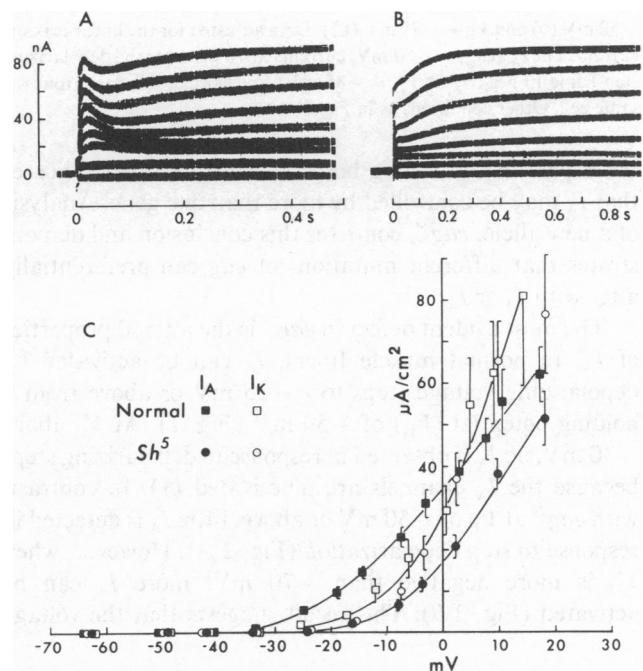

FIGURE 1 Membrane currents measured with a two-voltage clamp in larval muscle fibers at 8°C. To eliminate inward Ca^{2+} current, saline (1, 2) contained 0 mM $CaCl_2$, 0.5 mM EGTA and 15 mM $MgCl_2$. A, Normal (Canton-S) muscle. Membrane currents for voltage steps from a holding potential (V_H) of −50 mV to −65, −35, −18, −11, −4, 2, 9, 15, and 21 mV. Note that the transient I_A was activated at more negative membrane potentials than the delayed rectification, I_K. B, Sh^5 muscle. V_H = −50 mV. Voltage steps to −65, −35, −17, −10, −3, 3, 10, and 16 mV. Note that I_A was greatly diminished whereas I_K was not affected. C, Current-voltage relations for active currents at peak I_A (filled symbols) and steady-state I_K (open symbols) in normal (squares) and Sh^5 (circles) larval muscle fibers at V_H = −50 mV. Calculations of active current density (1, 2) are based on the measured surface area of each fiber and subtraction of the linear leakage current from the total current, such as the ones shown in A and B. Pooled data (mean ± SEM) from 14 normal fibers and 6 Sh^5 fibers.
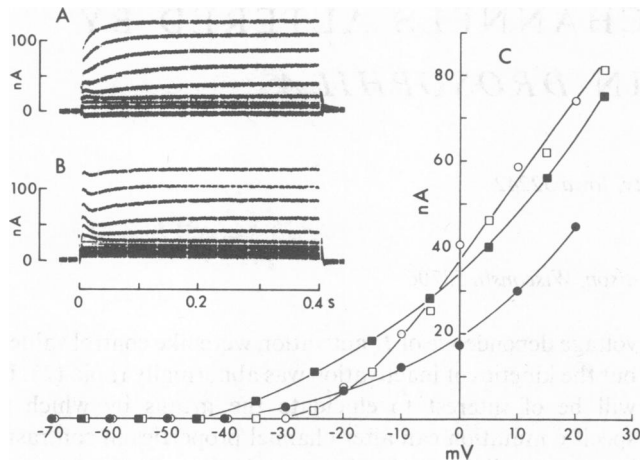
FIGURE 2 Membrane currents in eag^{24} at 7°C. *A*, V_H = −50mV. Voltage steps ranging from −70 to +30 mV in 10 mV increments. *B*, V_H = −85 mV. Step depolarizations ranging from −75 to +25 mV in 10 mV increments. Note that I_A was inactivated at V_H = −50 mV but not at V_H = −85 mV. *C*, Voltage-current relations for active currents at peak I_A at V_H = −50 mV (•) and V_H = −85 mV (■) and steady-state I_K at V_H = −50 mV (○) and V_H = −85 mV (□). Data adjusted for the linear leakage current. For I_A at V_H = −50 mV, currents at 10 ms after the depolarizing step (time to peak I_A at V_H = −85 mV) are plotted. All data from the same cell. Other conditions as in Fig. 1.

takes part in controlling both I_A and I_K. It also indicates that I_A may be controlled by more than one gene. Analysis of a new allele, eag^{24}, confirms this conclusion and demonstrates that different mutations of *eag* can preferentially alter with I_A or I_K.

The most evident defect in eag^{24} is the altered properties of I_A. In normal muscle fibers, I_A can be activated by depolarizing voltage steps to ~−25 mV or above from a holding potential (V_H) of −50 mV (Fig. 1). At V_H above −30 mV, no I_A is observed in response to depolarizing steps because the I_A channels are inactivated (1). In contrast, with eag^{24} at V_H of −50 mV or above, little I_A is detected in response to step depolarization (Fig. 2 *A*). However, when V_H is more negative than −70 mV, more I_A can be activated (Fig. 2 *B*). This result suggests that the voltage

dependence of the steady-state inactivation has been shifted such that in the mutant, inactivation occurs at more negative values of membrane potential (Fig. 2 *C*).

It is of interest to determine whether the voltage dependence of I_A inactivation can be separately altered without affecting other properties of the channels. The voltage dependence of I_A activation and steady-state I_K amplitude (Fig. 2 *C*) do not indicate drastic differences from normal. However, the detailed kinetic properties and ionic selectivities of I_A and I_K channels in eag^{24} remain to be examined. Furthermore, the channel properties can be investigated by measuring single-channel currents in dissociated *Drosophila* neurons in culture using patch-clamp techniques (4).

Relating the physiological defects of mutants such as *Sh* and *eag* to the molecular properties and structure of the affected channels remains as a challenge for further investigation. Attempts to identify the gene products and to specify the nature of the mutational alteration using molecular cloning techniques are underway.

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