approaches in identifying new K^+ channel genes that have not yet been isolated on the basis of sequence similarity. Finally, just as mammalian homologs have been found for *Drosophila* K^+ channel genes in the *Sh* superfamily, we anticipate the identification of mammalian counterparts to *eag*.

REFERENCES AND NOTES

1. A. Kamb, L. E. Iverson, M. A. Tanouye, *Cell* **50**, 405 (1987); D. M. Papazian, T. L. Schwarz, B. L. Tempel, Y. N. Jan, L. Y. Jan, *Science* **237**, 749 (1987); B. L. Tempel, D. M. Papazian, T. L. Schwarz, Y. N. Jan, L. Y. Jan, *ibid.*, p. 770; O. Pongs *et al.*, *EMBO J.* **7**, 1087 (1988).
2. A. Baumann, A. Grupe, A. Ackermann, O. Pongs, *EMBO J.* **7**, 2457 (1988); B. L. Tempel, Y. N. Jan, L. Y. Jan, *Nature* **332**, 837 (1988); A. Butler, A. Wei, K. Baker, L. Salkoff, *Science* **243**, 943 (1989); M. J. Christie, J. P. Adelman, J. Douglass, R. A. North, *ibid.* **244**, 221 (1989); D. McKinnon, *J. Biol. Chem.* **264**, 8230 (1989); W. Stuhmer *et al.*, *EMBO J.* **8**, 3235 (1989); A. Kamb *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 4372 (1989); G. C. Frech, M. J. Van Dongen, G. Schuster, A. M. Brown, R. H. Joho, *Nature* **340**, 642 (1989); A. Wei *et al.*, *Science* **248**, 599 (1990); K. G. Chandy *et al.*, *ibid.* **247**, 973 (1990); T. McCormack, E. C. Vega-Saenz de Miera, B. Rudy, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5227 (1990); R. Swanson *et al.*, *Neuron* **4**, 929 (1990).
3. B. Ganetzky and C.-F. Wu, *Trends Neurosci.* **8**, 322 (1985); C.-F. Wu and B. Ganetzky, in *Neurotox '88: Molecular Basis of Drug and Pesticide Action*, G. Lunt. Ed. (Elsevier, Amsterdam, 1986), pp. 311–323.
4. W. D. Kaplan and W. E. Trout, *Genetics* **61**, 399 (1969).
5. B. Ganetzky and C.-F. Wu, *J. Neurogenet.* **1**, 17 (1983).
6. L. Salkoff and R. Wyman, *Nature* **293**, 228 (1981); C.-F. Wu and F. N. Haugland, *J. Neurosci.* **5**, 2626 (1985).
7. C.-F. Wu, B. Ganetzky, F. N. Haugland, A. X. Liu, *Science* **220**, 1076 (1983).
8. Y. Zhong and C.-F. Wu, *ibid.* **252**, 1562 (1991).
9. R. Drysdale, J. Warmke, R. Kreber, B. Ganetzky, *Genetics* **127**, 497 (1991).
10. We isolated the cDNA CH20 by screening a head-specific library provided by B. Hamilton and E. Meyerowitz [M. Palazzolo *et al.*, *Gene (Amsterdam)* **88**, 25 (1990)], using the cDNA T18 (*9*) as a probe. CH20 is 1.3 kb larger than T18 and extends farther in both the 5′ and 3′ directions. After subcloning into pBluescript vectors (Stratagene), we sequenced both strands of CH20 with a Sequenase Kit, version 2 (United States Biochemical Corporation).
11. The nucleotide sequence preceding the first ATG in cDNA CH20 is CAAAATG. The consensus for the translational start in *Drosophila* is (C,A)AA(A,C)-ATG [D. Cavener, *Nucleic Acids Res.* **15**, 1353 (1987)].
12. The GenBank, the European Molecular Biology Laboratory, and the National Biomedical Research Foundation data banks were searched with the use of the sequence analysis software package obtained from the Genetics Computer Group [J. Devereux, P. Haeberli, O. Smithies, *ibid.* **12**, 387 (1984)].
13. W. A. Catterall, *Science* **242**, 50 (1988).
14. R. MacKinnon and G. Yellen, *ibid.* **250**, 276 (1990); R. MacKinnon, L. Heginbotham, T. Abramson, *Neuron* **5**, 767 (1990); G. Yellen, M. E. Jurman, T. Abramson, R. MacKinnon, *Science* **251**, 939 (1991); H. A. Hartmann *et al.*, *ibid.*, p. 942; A. J. Yool and T. Schwarz, *Nature* **349**, 700 (1991).
15. R. MacKinnon, *Nature* **350**, 232 (1991).
16. Consensus recognition sequences were as follows: NX(S,T) for potential sites of glycosylation [C. Hubbard and R. J. Ivatt, *Annu. Rev. Biochem.* **50**, 555 (1981)], (R,K)(R,K)XS or (R,K)(R,K)XXS for potential sites of adenosine 3′,5′-monophosphate (cAMP)–dependent phosphorylation (X indicates an unspecified amino acid) [B. E. Kemp, D. J. Graves, E. Benjamini, E. G. Krebs, *J. Biol. Chem.* **252**, 4888 (1977)], and (S,T)X(R,K) for protein kinase C (PKC)–dependent phosphorylation [J. R. Woodget, K. L. Gould, T. Hunter, *Eur. J. Biochem.* **161**, 177 (1986); A. Kishimoto *et al.*, *J. Biol. Chem.* **260**, 12492 (1985)].
17. J. Kyte and R. F. Doolittle, *J. Mol. Biol.* **157**, 105 (1982).
18. L. Y. Jan and Y. N. Jan, *Nature* **345**, 672 (1990).

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Alteration of Four Identified K^+ Currents in *Drosophila* Muscle by Mutations in *eag*

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Voltage-clamp analysis of *Drosophila* larval muscle revealed that *ether à go-go (eag)* mutations affected all identified potassium currents, including those specifically eliminated by mutations in the *Shaker* or *slowpoke* gene. Together with DNA sequence analysis, the results suggest that the *eag* locus encodes a subunit common to different potassium channels. Thus, combinatorial assembly of polypeptides from different genes may contribute to potassium channel diversity.

A VARIETY OF K^+ CHANNELS PARTICIPATE in many cellular functions and contribute to the signaling capacity of excitable cells (*1*). However, the genetic basis that underlies this diversity still remains to be fully explored (*1–3*). Studies have shown that several distinct K^+ currents in *Drosophila* muscle are selectively affected by mutations in different genes (*4–6*). We investigated the effect of mutations in the *eag* gene (*7*) on all identified K^+ currents in *Drosophila* larval muscle. These include the transient (I_A) and delayed (I_K) voltage-activated K^+ currents and the fast (I_{CF}) and slow (I_{CS}) Ca^{2+}-activated K^+ currents (*8–10*).

A voltage-clamp analysis with two microelectrodes was performed on the body-wall muscle fibers in *Drosophila* third instar larvae (*10–12*). We measured the voltage-activated I_A and I_K in Ca^{2+}-free saline, which eliminated the inward Ca^{2+} current (I_{Ca}), as well as the Ca^{2+}-activated I_{CF} and I_{CS} (*8–10*). I_A and I_K could be separated by their kinetic differences in inactivation and recovery (*11*). A previous inactivating pulse to −20 mV from the holding potential (V_H = −80 mV) inactivated most I_A but left I_K virtually intact (Fig. 1A). The difference between corresponding traces with and without the inactivating pulse was I_A (*11*).

Current-voltage (*I-V*) relations were determined for the peak I_A and the maximum I_K at different voltages for several *eag* alleles (Fig. 1). I_K was reduced in eag^{X6} and, to a lesser extent, in eag^{4PM} and eag^1 (Fig. 1C). I_A decreased in eag^{X6} even though it was not significantly affected in eag^{4PM} and eag^1 (*13*). The extreme phenotype of eag^{X6} is consistent with molecular data that indicate that the *eag* transcript is truncated in this mutant. Therefore, eag^{X6} is likely to exhibit the null phenotype (*14*).

Measurements of Ca^{2+}-dependent currents requires the presence of Ca^{2+} in saline. As in other invertebrate species, the inward current in *Drosophila* muscle fibers is carried by Ca^{2+}, which is required for contraction (*15*). Although it is desirable to measure isolated I_{Ca} with pharmacological elimination of all K^+ currents, such experiments are prevented by the resulting contraction of all muscle fibers in the larval preparation. We instead determined the current mediated by Ca^{2+} channels by replacing Ca^{2+} with Ba^{2+}, which permeates Ca^{2+} channels and blocks K^+ channels but does not initiate muscle contraction (*9, 16*). These experiments indicated that the I_{Ba}'s in eag^{4PM} and eag^{X6} larvae were not different from that found in normal larvae (*17*).

The Ca^{2+}-activated I_{CF} and I_{CS} were studied at a lower temperature, and the external Ca^{2+} concentration was reduced to 0.9 mM from the standard 1.8 mM (*8, 11*). Under these conditions, muscle contraction was minimized, but the two currents could still be measured (Fig. 2A). We removed the voltage-activated I_A by addition of 4-aminopyridine (*18*) and the voltage-activated I_K by addition of quinidine (*10*). The remaining net current was outward; the inward I_{Ca} was masked by the I_{CF} and I_{CS}. The inward tail currents (Fig. 2A) that followed the depolarization-induced outward currents were characteristic of Ca^{2+}-activated K^+ currents and distinct from I_K, which lacks inward tails at a V_H of −80 mV (Fig. 1C).

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Further simple physiological separation of I_{CF} and I_{CS} is not yet feasible. However, as indicated by studies involving genetic elimination of I_{CF} (*6, 10*), I_{CF} contributes mainly to the early outward transient current, and I_{CS} contributes to the delayed plateau (Fig. 2A).

The *I-V* relations for the plateau at the end of pulses (Fig. 2B) and for the transient peak (Fig. 2C) were determined. Because I_{Ca} does not appear to be affected by various *eag* alleles, the differences between normal and mutant currents represent reductions in I_{CF} and I_{CS}. Both I_{CF} and I_{CS} were reduced in eag^1 and eag^{X6} larvae, whereas only I_{CS} was decreased in eag^{4PM} larvae (Fig. 2).

Our results demonstrate that all K^+ currents in larval muscle were affected by *eag* mutations, unlike *Sh* (*Shaker*) and *slowpoke* (*slo*) mutations, which specifically eliminate I_A and I_{CF}, respectively (*4–6, 8–10*). This observation raises the possibility that the *eag* gene contributes a subunit common to different K^+ channels. Indeed, sequence analysis of *eag* shows that this gene defines a putative K^+ channel polypeptide that is related to but distinct from the I_A channel proteins encoded by the *Sh* superfamily (*14*). Recombinant heteromultimeric K^+ channels have been expressed in *Xenopus* oocytes from the assembly of different splicing products of the *Sh* locus or of the products of genes in the *Sh* family (*19, 20*). We suggest that a combinatorial assembly of polypeptides from different genes contributes to K^+ channel diversity in vivo (see also *3, 8, 20*).

None of the *eag* alleles examined completely eliminated any of the K^+ currents in *Drosophila* larval muscle. The severity of the defects in eag^{X6} larvae likely represents the null phenotype of this locus. Similar defects have been seen in a different allele, eag^{sc29}, which also truncates *eag* transcripts and is thus also likely to cause a null phenotype (*14*). The fact that none of the currents were eliminated even in *eag* null alleles and that individual species of *Sh* transcripts can produce I_A-like currents in the oocyte expression system (*21*) indicate either that the *eag* product is not essential to channel activation or that functional channels can form with other channel subunits substituted for the *eag* subunit.

The notion that the *eag* protein participates directly in channel operation is strengthened by its allele-dependent effects. The mutation eag^1 affected I_{CF} and I_{CS} to the same extent as did the null allele eag^{X6} but left I_A intact (Figs. 1 and 2). In eag^{4PM}, only I_{CS} was substantially reduced (Figs. 1 and 2). Furthermore, allele-specific interactions that could result from physical contact between two gene products (*22*) were observed in *eag Sh* double-mutant combinations; defects of I_A not seen for either the *eag* or *Sh* allele alone were detected in certain double-mutant combinations (*23*). Such phenotypes might arise from the compounding effects of structural alterations in the *Sh* and *eag* polypeptides in the I_A channel. The allele-dependent effects on individual K^+ currents may reflect the association of specific domains of the *eag* protein with the function of a particular channel. In *Paramecium*, separate domains of calmodulin are responsible for the activation of two different currents, and mutants associated with different domains demonstrated allele-dependent effects on the currents (*24*).

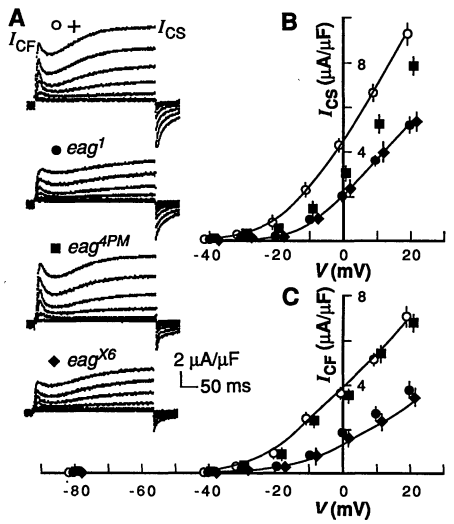

Fig. 2. Effects of *eag* mutations on I_{CF} and I_{CS}. Families of currents were recorded in saline containing Ca^{2+}, 4-aminopyridine, and quinidine (*12*) at 5°C. (**A**) The traces represent Ca^{2+}-activated outward I_{CF} and I_{CS}, which masked the inward I_{Ca}. (**B**) *I-V* relations at the I_{CS} plateau determined at the end of the 400-ms test pulse. (**C**) *I-V* relations at the I_{CF} peak. *I-V* relations were determined without subtraction of I_{Ca}. The number of fibers was 10, 10, 8, and 8 from three to five larvae for +, eag^1, eag^{4PM}, and eag^{X6}, respectively.

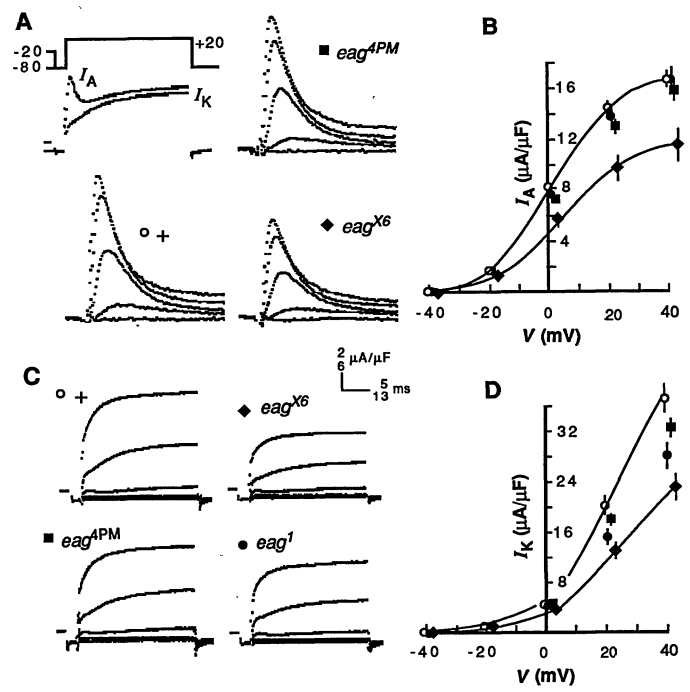

Fig. 1. Effects of *eag* mutations on I_A and I_K in *Drosophila* larval muscle. (**A**) The voltage paradigm for separating I_A and I_K and the isolated I_A. As shown in the inset (top left), a conditioning pulse (2 s) to −20 mV inactivated I_A but left I_K intact (*11*). Without the pulse, both I_A and I_K were activated by the test pulse (60 ms). The families of traces demonstrate I_A extracted from the currents with and without the conditioning pulse. (**B**) *I-V* relations of I_A for normal (+) and mutant alleles. (**C**) Traces of I_K from normal and mutant alleles. I_A was inactivated by the conditioning pulse. (**D**) *I-V* relations for I_K in normal and mutant alleles. All experiments were performed at 16°C, in Ca^{2+}-free saline (*11*). The number of fibers was 10, 9, 8, and 8 from three to six larvae for +, eag^1, eag^{4PM}, and eag^{X6}, respectively. Scale bars, 2 μA/μF (vertical), 5 ms (horizontal) for I_A; 6 μA/μF and 13 ms for I_K. For this figure and Fig. 2, data were collected from muscle fibers 6 and 12 in abdominal segments 3 to 5 (*11*) at a V_H of −80 mV. In both figures, traces represent current density (normalized to membrane capacitance in microamperes per microfarad) averaged from the number of fibers specified for each allele, error bars in *I-V* relations indicate SEM, and curves are fit by eye to data from normal and eag^{X6} fibers.

REFERENCES AND NOTES

1. B. Rudy, *Neuroscience* **25**, 729 (1988); B. Hille, *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA, 1984).
2. M. A. Tanouye, C. A. Kamb, L. E. Iverson, L. B. Salkoff, *Annu. Rev. Neurosci.* **9**, 255 (1986); B. Ganetzky and C.-F. Wu, *Annu. Rev. Genet.* **20**, 13 (1986).
3. C.-F. Wu and B. Ganetzky, in *Ion Channels in Neural Membranes*, J. M. Ritchie, R. D. Keynes, L. Bolis, Eds. (Liss, New York, 1986), pp. 407–423; L. Y. Jan and Y. N. Jan, *Trends Neurosci.* **13**, 415 (1990).
4. L. B. Salkoff and R. Wyman, *Nature* **293**, 228 (1981); C. K. Solc, W. N. Zagotta, R. W. Aldrich, *Science* **236**, 1094 (1987).
5. C.-F. Wu, B. Ganetzky, F. N. Haugland, A. X. Liu, *Science* **220**, 1076 (1983).
6. T. Elkins, B. Ganetzky, C.-F. Wu, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8451 (1986).
7. W. D. Kaplan and W. E. Trout, *Genetics* **61**, 399 (1969); B. Ganetzky and C.-F. Wu, *J. Neurogenet.* **1**, 17 (1983).
8. C.-F. Wu and F. N. Haugland, *J. Neurosci.* **5**, 2626 (1985).
9. M. Gho and A. Mallart, *Pfluegers Arch.* **407**, 526 (1986); L. Salkoff, *Nature* **302**, 249 (1983); A. Wei

and L. Salkoff, *Science* **233**, 780 (1986).
10. S. Singh and C.-F. Wu, *Neuron* **2**, 1325 (1989).
11. F. N. Haugland and C.-F. Wu, *J. Neurosci.* **10**, 1357 (1990); C. F. Wu *et al.*, *J. Exp. Biol.* **147**, 21 (1989).
12. The voltage-clamp setup and protocol for data collection and analysis have been described (*11*). I_A and I_K were recorded in Ca^{2+}-free saline that contained 5 mM Hepes (pH 7.1), NaCl (128 mM), KCl (2 mM), sucrose (35.5 mM), $MgCl_2$ (14 mM), and EGTA (0.5 mM). For recording I_{CF} and I_{CS}, the saline solution contained $CaCl_2$ (0.9 mM) and $MgCl_2$ (4 mM) but no EGTA. In addition, we used 4-aminopyridine (100 μM) and quinidine (100 μM) to eliminate I_A and I_K. Third instar larvae were dissected in Ca^{2+}-free saline at room temperature.
13. A voltage-clamp study (*5*) of eag^1 also showed a similar decrease in I_K and a small but statistically significant reduction in amplitude at peak I_A. This study was performed without physiological separation of currents (*11*) (Fig. 1), and the peak I_A might have been contaminated by I_K.
14. R. Drysdale, J. Warmke, R. Kreber, B. Ganetzky, *Genetics* **127**, 497 (1991); J. Warmke, R. Drysdale, B. Ganetzky, *Science* **252**, 1560 (1991).
15. D. J. Aidley, *The Physiology of Excitable Cells* (Cambridge Univ. Press, New York, ed. 3, 1989), pp. 289–293.
16. S. Hagiwara and L. Byerly, *Annu. Rev. Neurosci.* **4**, 69 (1981); M. G. Gorczyca and C.-F. Wu, *J. Membr. Biol.* **121**, 237 (1991).
17. The measurement of Ba^{2+} currents was conducted at 16°C in the saline used for I_{CF} and I_{CS} recording (*12*), except that Ca^{2+} was replaced by Ba^{2+} (10 mM).
18. F. N. Haugland and C.-F. Wu, *Soc. Neurosci. Abstr.* **13**, 150 (1987).
19. T. L. Schwarz, B. L. Tempel, D. M. Papazian, Y. N. Jan, L. Y. Jan, *Nature* **331**, 137 (1988); A. Kamb, J. Tseng-Crank, M. A. Tanouye, *Neuron* **1**, 421 (1988); O. Pongs *et al.*, *EMBO J.* **7**, 1087 (1988).
20. E. Y. Isacoff, Y. N. Jan, L. Y. Jan, *Nature* **345**, 530 (1990); J. P. Ruppersberg *et al.*, *ibid.*, p. 535; K. McCormack *et al.*, *Biophys. J.* **57**, 209a (1990); A. M. Covarrubias, A. Wei, D. McKinnon, L. Salkoff, *Soc. Neurosci. Abstr.* **16**, 3 (1990).
21. L. C. Timpe *et al.*, *Nature* **331**, 143 (1988); L. E. Iverson, M. A. Tanouye, H. A. Lester, N. Davidson, B. Rudy, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5723 (1988); T. Hoshi, W. N. Zagotta, R. W. Aldrich, *Science* **250**, 533 (1990).
22. T. C. Huffaker, M. A. Hoyt, D. Botstein, *Annu. Rev. Genet.* **21**, 259 (1987).
23. For example, a clear shift to positive potentials in steady-state inactivation was seen in $eag^{4PM}Sh^{rK0120}$, and a drastic decrease in I_A amplitude was recorded in $eag^{24}Sh^5$. Double-mutant combinations of these less severe *Sh* alleles with other *eag* alleles did not produce such nonadditive effects on I_A (Y. Zhong and C.-F. Wu, unpublished data).
24. A. K. John *et al.*, *Cell* **62**, 165 (1990).
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