Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 1133–1137, February 1992
Neurobiology

Overexpression of an *Aplysia* Shaker K^+ channel gene modifies the electrical properties and synaptic efficacy of identified *Aplysia* neurons

(expression vector/microinjection/action potential/synaptic transmission)

BONG-KIUN KAANG*†, PAUL J. PFAFFINGER*‡, SETH G. N. GRANT*, ERIC R. KANDEL*, AND YASUO FURUKAWA*

*Howard Hughes Medical Institute, Center for Neurobiology and Behavior, and †Integrated Program in Cellular, Molecular Biology, and Biophysical Studies, Columbia University College of Physicians and Surgeons, 722 West 168th Street, New York, NY 10032

Contributed by Eric R. Kandel, October 21, 1991

ABSTRACT **Although potassium channels play a variety of roles in shaping the electrical properties of neurons, little is known about how these channels are constituted in neurons. To examine the assembly and physiological function of A-type K^+ channels in mature differentiated neurons, we have developed a highly efficient gene transfer method for *Aplysia* neurons that has allowed us to express about 10^7 copies of the cloned *Aplysia* Shaker (*Sh*) K^+ channel (AK01a) in single identified cells. We find that expression of AK01a phenocopies one of the native transient K^+ currents (I_{Adepol}), suggesting that the native channel carrying I_{Adepol} is assembled as a homooligomer of AK01a. Overexpression of AK01a has substantial effect on the action potential, shortening its duration, enhancing its hyperpolarizing afterpotential, and depressing by more than half the amount of transmitter release by the action potential from the terminals. Thus, the AK01a channel not only contributes to the firing properties within a given neuron but also can regulate the signaling between interconnected cells.**

Potassium channels are important regulators of the resting potential, the action potential, and the excitability of neurons (1). In addition, they have a role in certain forms of procedural learning. For example, in the marine molluscs *Aplysia* (2–5) and *Hermissenda* (6–9) modulation of different species of K^+ currents in sensory neurons contributes to several learned modifications of simple reflex behaviors. The cloning of the Shaker (*Sh*) locus in *Drosophila* (10–13) has now made it possible to study the functions of various K^+ channels on the molecular level. However, K^+ channels have so far not been successfully transfected into mature, differentiated nerve cells in the intact nervous system. As a result, it has not been possible to study cloned K^+ channels in their native cellular environment and to determine, by detailed comparison of cloned and endogenous channels, the subunit composition of any endogenous K^+ channel. To overcome this problem, we have developed an expression vector that allows expression of genes in the identified neurons of the adult nervous system in the sea hare *Aplysia*. With this expression vector, the reporter gene *lacZ* from *Escherichia coli* is expressed in 80% of injected cells. Using this vector, we have been able to analyze the properties and physiological roles of a cloned *Aplysia* Shaker K^+ channel (AK01a) (14). We find that AK01a phenocopies the native K^+ current I_{Adepol}, suggesting that this native K^+ channel is assembled as a homooligomer of AK01a. In addition, our experiments indicate that overexpression of this single species of K^+ channel permits one to redesign the electrical properties of a neuron so as to alter the waveform of its action potential and to modify the effectiveness of its synaptic actions on its population of follower cells.

MATERIAL AND METHODS

Construction of Plasmids. To make the expression vector, pNEX [plasmid for neuronal expression, 3.0 kilobases (kb)], the *Eco*RI–*Xmn* I (0.8 kb) restriction fragment of pUC19 was replaced by the same fragment containing the simian virus 40 (SV40) polyadenylylation signal after deletion of the *Nae* I–*Bam*HI fragment from 4×AP-1 RSV-lacZ [kindly provided by R. H. Goodman (Portland, OR) and S. Fink (Charlestown, MA); RSV, Rous sarcoma virus], and then *Hin*dIII fragment containing the 4×AP-1 repeat and RSV promoter/enhancer was inserted into the *Hin*dIII site of pUC19. *E. coli* β-galactosidase gene was excised from pNAssβ (kindly provided by G. R. MacGregor, Houston, TX) as a *Bam*HI fragment (3.7 kb) and inserted into the *Bam*HI site of pNEX, and the orientation was determined. To make pNEX-AK01a, the full-length cDNA fragment (1.8 kb) of *Aplysia* Shaker (AK01a) having *Cla* I and *Xmn* I sites at 5′ and 3′ ends, respectively, was inserted into *Acc* I- and *Sma* I-digested pNEX.

Microinjection of Plasmids into *Aplysia* Neurons. From *Aplysia californica* weighing 10–100 g (furnished by the Howard Hughes Medical Institute, Miami Facility) the abdominal ganglion was dissected and treated with protease IX (Sigma) at 10 mg/ml in L15 medium (15) for 1 hr at 34.5°C. The ganglion was then pinned on a Sylgard plate and desheathed carefully to expose the neurons to the L15 medium containing an equal volume of *Aplysia* hemolymph or artificial seawater (ASW) that had the following composition: 460 mM NaCl/10 mM KCl/11 mM $CaCl_2$/55 mM $MgCl_2$/10 mM Hepes, pH 7.6. In a few experiments, neurons in primary cell culture were used. Primary cell culture techniques and media have been described (15). Within 1–24 hr after desheathing, the neurons were injected by using the air pressure system (Pico-Injector, PLI-100; Medical Systems, Greenvale, NY) with a DNA solution, consisting of plasmid DNA at 0.5–1.0 μg/μl, 0.05% fast green, 10 mM Tris·HCl at pH 7.3, and 100 mM NaCl in a volume of 0.01–1 nl, depending on the cell size. The impedance of the microelectrode was 5 MΩ when it was filled with 3 M KCl.

Detection of β-Galactosidase. The ganglia and cultured cells were fixed within 4–48 hr after microinjection with ice-cold 2% paraformaldehyde/0.05% glutaraldehyde/0.1 M sodium phosphate, pH 7.3, for 5 min. To visualize the expression of

Abbreviations: SV40, simian virus 40; RSV, Rous sarcoma virus; 4-AP, 4-aminopyridine.

‡Present address: Division of Neuroscience, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030.

β-galactosidase, the fixed preparations were stained with 0.1 M sodium phosphate, pH 7.3/1.3 mM $MgCl_2$/3 mM $K_3Fe(CN)_6$/3 mM $K_4Fe(CN)_6$ containing 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) at 1 mg/ml at 37°C for 5 min to 12 hr.

Electrical Measurements. *Xenopus laevis* oocytes were prepared, injected with the synthesized AK01a complementary RNA, and voltage clamped 2–3 days after the injection as previously described (14). The external solution was ND-96 (96 mM NaCl/2 mM KCl/1.8 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes, pH 7.6). Expression of AK01a in *Aplysia* neurons was measured 7–24 hr after injection of pNEX-AK01a, using the same voltage-clamp method for oocyte experiments. The external solution was Na^+- and Ca^{2+}-free ASW (460 mM Tris/10 mM KCl/66 mM $MgCl_2$/10 mM Hepes, pH 7.6). Because the AK01a-channel current (I_{AK01a}) was not blocked by tetraethylammonium (TEA) (14), 30–50 mM TEA was added to block the native delayed K^+ currents. Although the treatment was routinely done to see the inactivation of I_{AK01a} with less contamination, it was not necessary in many cases because I_{AK01a} was overwhelming compared with the native membrane currents (see Fig. 3*A*). I_{Adepol} in neuron R15 was also measured in Na^+- and Ca^{2+}-free ASW containing TEA, as described previously (14, 31). The membrane currents of neuron L10 were recorded in the ASW.

The recordings of the action potential of L10 and the synaptic potentials of its follower cells were made in ASW containing 60 mM Ca^{2+} (16). Five to 8 hr after the control recording, pNEX-AK01a was injected into L10. Second recordings were made 14–18 hr after the injection. If the resting potential of L10 was more than −40 mV in the second recording, and if the postsynaptic cells showed no significant change in the resting potential compared with the first recording, the preparation was considered to be healthy and accepted. The resting potential of L10 was reset to a control value if there was any change, because the resting potential level of L10 greatly affects the transmitter release (17).

RESULTS AND DISCUSSION

We microinjected plasmid vectors that contained different promoter sequences driving the *E. coli* β-galactosidase reporter gene and defined regulatory sequences capable of driving high-level expression of the reporter in *Aplysia* neurons (see *Acknowledgments*). We obtained the highest levels of expression with RSV long terminal repeat (18) or human c-*fos* regulatory sequences (19). A number of other promoters had little or no detectable activity. These included other viral promoters (SV40 early region, herpes simplex virus thymidine kinase, adenovirus 2 major late, cytomegalovirus immediate early gene) (20); vertebrate cellular gene promoters [human β-actin (21), human vasoactive intestinal peptide (22), human proenkephalin (23)]; and the *Aplysia* R14 neuropeptide promoter (24).

By using the RSV promoter followed by a polylinker multicloning site and SV40 polyadenylylation sequences, we constructed the pNEX vector (Fig. 1*A*), which allows expression of any coding sequence in *Aplysia* neurons. We first created pNEX-lacZ by subcloning the β-galactosidase coding region in the multicloning site. This reporter gene was expressed in 80% of microinjected neurons of *Aplysia* ganglia as well as in neurons in primary culture (Fig. 1 *B* and *C*). For example, in one experiment, we injected 184 cells in five ganglia and observed β-galactosidase expression in 144 of the injected cells.

We next examined the expression of a specific cloned *Aplysia* K^+ channel (AK01a) (14). Recent studies in *Drosophila* muscle suggest that all of the native K^+ channels are likely to be heterooligomeric and seem to require at least two

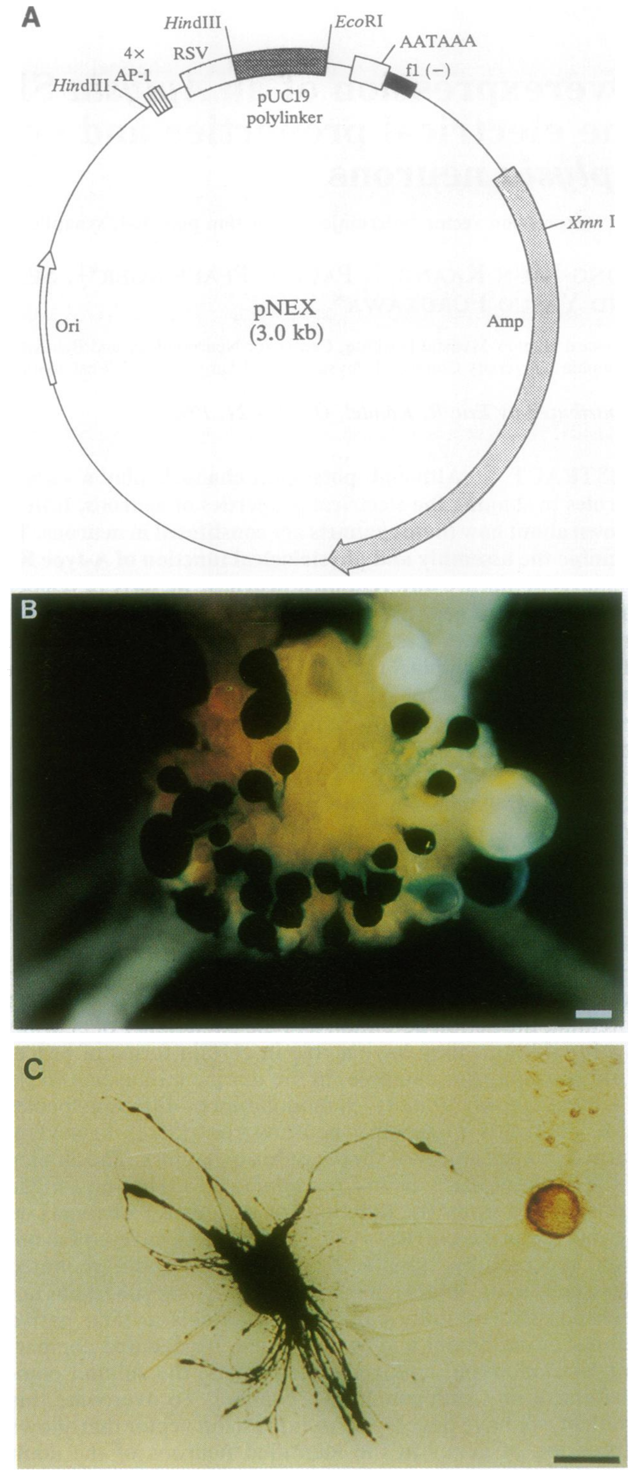

FIG. 1. (*A*) Construction of pNEX (plasmid for neuronal expression). Although this vector contains four copies of mammalian AP-1 binding sequences 5′ to the RSV promoter, vectors lacking the AP-1 sequences exhibit similar efficiency of expression. (*B* and *C*) Histochemical detection of β-galactosidase expression in *Aplysia* neurons. Neurons on the dorsal surface of the abdominal ganglion (*B*) and in primary cell culture (*C*) were injected with pNEX-lacZ and expression was detected by using 5-bromo-4-chloro-3-indolyl β-D-galactoside. Thirty-four out of 40 injected cells in the abdominal ganglion, and only the injected cell in primary culture, showed blue color. (White scale bar, 200 μm; black scale bar, 100 μm.)

distinct types of subunits (25). We therefore were interested in determining whether any of the native K^+ channels in *Aplysia* neurons are assembled as homooligomers or whether all are heterooligomers, as is thought to be the case in *Drosophila*.

To study the properties of the AK01a channel and to compare it to native K^+ currents, we first injected the synthesized AK01a cRNA into *Xenopus* oocytes, where we could be fairly sure that it is expressed as a homooligomer. When expressed in *Xenopus* oocytes the AK01a channel carries a transient K^+ current (I_{AK01a}) (14). This current is quite similar to I_{Adepol}, one of the three previously characterized native transient K^+ currents expressed in *Aplysia* neurons (14, 31). However, although I_{Adepol} in neurons resembles I_{AK01a} expressed in oocytes, the native current differs slightly from I_{AK01a} in oocytes. Specifically, the midpoint of the steady-state activation and inactivation profile of the current expressed in oocytes is shifted 10 mV in the depolarized direction compared to the native current I_{Adepol} (Fig. 2 *A* and *B*). In addition, the native current is slightly more sensitive to 4-aminopyridine (4-AP) than I_{AK01a} in oocytes (Fig. 2 *C* and *D*).

We therefore asked: Can I_{AK01a} phenocopy the native current better when expressed in *Aplysia* neurons? To address this question, we created pNEX-AK01a and expressed AK01a in two types of identified *Aplysia* neurons, those that have the native I_{Adepol} (R15 and R2) and those that lack this current (L7 and L11). Seven hours after injection of pNEX-AK01a, we observed a large transient K^+ current in the injected cells. From the size of the maximally expressed current we estimate (from the measured total current of 10 μA and an estimated single channel current of 1 pA) that more than 10^7 copies of the AK01a channel could be expressed in a single cell. The current was the same whether or not the cell expressed I_{Adepol} as a native current, indicating that different neuronal environments do not modify the basic current. In the early stages of expression (7–10 hr after injection) the neurons expressed a rapidly inactivating K^+ current, which strongly resembled the native I_{Adepol}. With time the currents began to manifest a noninactivating component, which seemed to be a later consequence of the progressive expres-

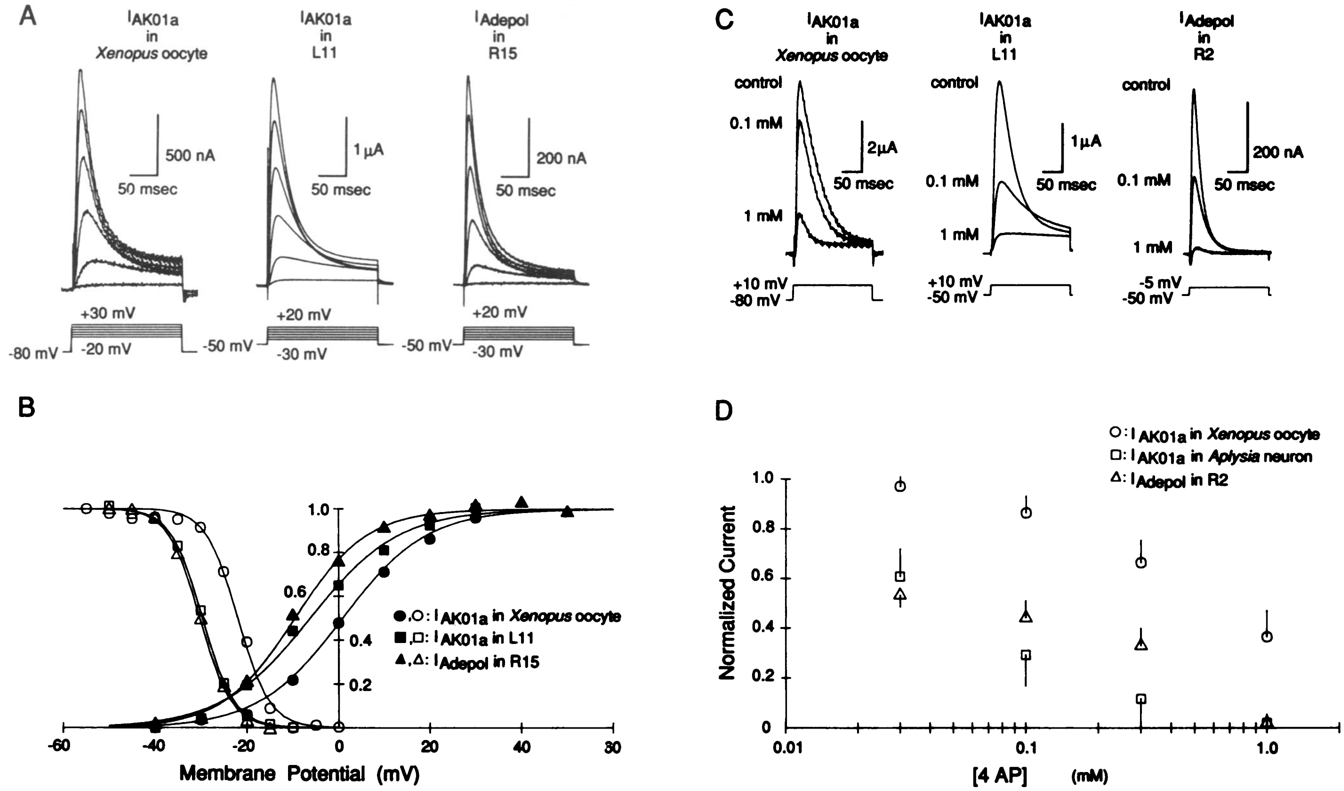

FIG. 2. Comparison between I_{AK01a} in *Xenopus* oocytes, I_{AK01a} in *Aplysia* neurons, and a native transient K^+ current (I_{Adepol}). (*A*) Families of I_{AK01a} in a *Xenopus* oocyte, I_{AK01a} in *Aplysia* neuron L11, and I_{Adepol} in another *Aplysia* neuron, R15. Holding potential in all measurements was −80 mV. Pulse protocols are shown underneath. In the measurements in *Aplysia* neurons, a 1-sec prepulse to −50 mV was applied before the test pulse to inactivate native conventional A-type K^+ currents (14, 31). I_{Adepol} was not present in L11. (*B*) The activation and the steady-state inactivation of three currents shown in *A*. The activation curve (filled symbols) was obtained by plotting the normalized peak conductance against the membrane potential. The peak conductance was calculated by dividing the peak current by the driving force (the difference of the reversal potential and the test potential). The reversal potential (E_{rev}) of I_{AK01a} in *Aplysia* neurons determined by the tail current reversal was −90.0 ± 9.4 mV (n = 11). E_{rev} of I_{AK01a} in oocytes is −83 mV (14), and that of I_{Adepol} is −52.9 mV (31). The apparent depolarized E_{rev} of I_{Adepol} may be due to the contamination by other currents (31). The steady-state inactivation curve (empty symbols) was obtained by plotting the normalized peak current at the test pulse against the prepulse potential. The test potential was either 10 mV (I_{AK01a}) or 0 mV (I_{Adepol}), and the duration of the prepulse was 2 sec. The peak current (or the conductance) was normalized by the maximum value estimated from the least-square fitting of the data to the Boltzmann function of the form $P = M/\{1 + \exp[(E - V_{1/2})/k]\}$. P is the peak value, M is the maximum value, E is the membrane potential, $V_{1/2}$ is the voltage at which $P = M/2$, and k is the slope factor. The smooth lines are the normalized Boltzmann functions. $V_{1/2}$ and k for the activation are as follows (mV): 0.9 and −9.2 (oocyte), −6.2 and −9.7 (L11), and −9.5 and −7.9 (R15). $V_{1/2}$ and k for the inactivation are as follows (mV): −21.9 and 3.4 (oocyte), −29.6 and 3.4 (L11), and −30.2 and 3.4 (R15). (*C*) Effect of 4-AP on three currents. (*D*) Dose responses of 4-AP block of three currents. Each symbol shows the normalized mean current of three or four determinations. Vertical bar imposed on each symbol is SD. To examine the dose–response relationships of 4-AP block the currents were elicited every 1 min and 4-AP was applied by bath perfusion. Quasi-steady-state block could be obtained within 4–8 min. For the experiments on I_{AK01a} in oocytes and neurons, the increasing concentrations of 4-AP (0.03–1 mM) were applied sequentially. In the case of I_{Adepol}, however, only two doses (submaximal and 1 mM) were tested in each experiment because of some run-down of the current during a long-lasting recording. The blocked currents were normalized to the control currents and plotted against the concentration of 4-AP.

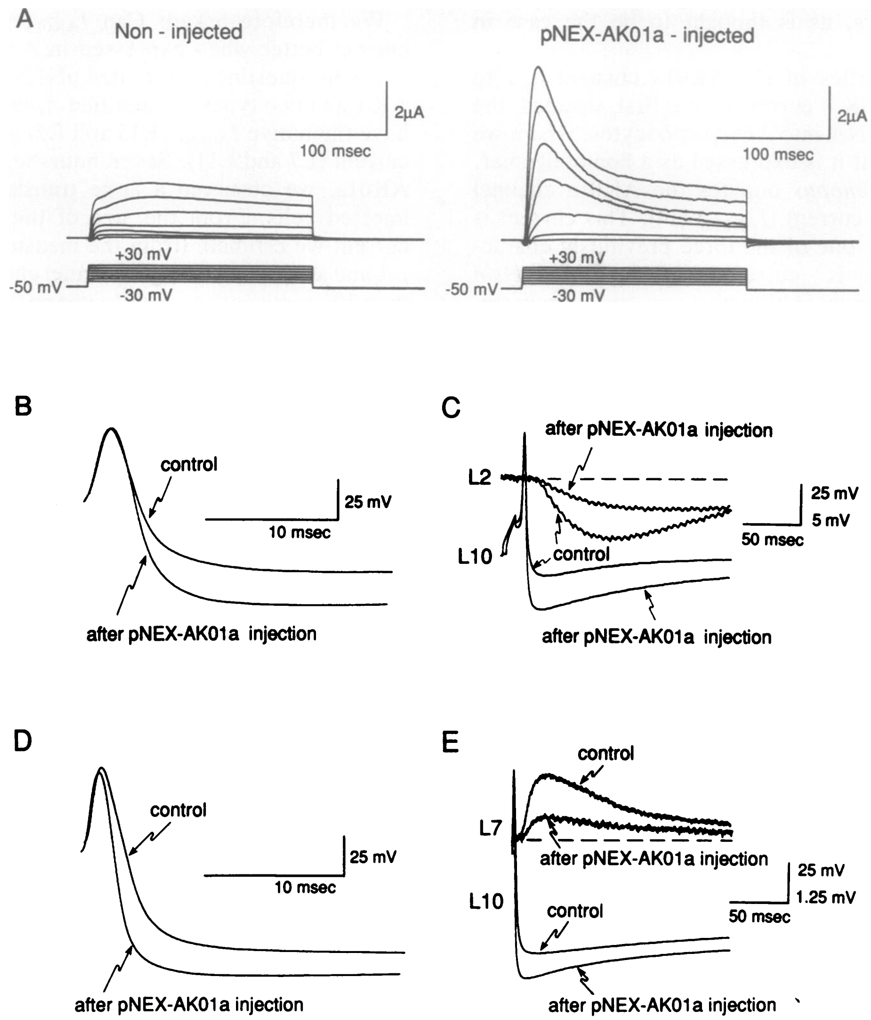

FIG. 3. Effect of the expression of AK01a channels on the action potential and transmitter release. (*A*) The membrane current of noninjected L10 and pNEX-AK01a-injected L10. Pulse protocols are shown underneath. (*B*) Action potentials of L10 before and after the expression of AK01a channels. (*C*) Comparison of monosynaptic inhibitory synaptic potentials in L2 produced by a spike of L10 before and after the expression of AK01a channels in L10. The resting potentials of L10 and L2 were −44 and −50 mV, respectively. *B* and *C* are from the same preparation. The synaptic potentials before and after the expression of AK01a were −7.1 ± 0.5 mV and −4.2 ± 0.4 mV, respectively. In this and *D*, the broken line shows the resting level of postsynaptic cells as well as the reference level (0 mV) of L10. (*D*) Action potentials of L10 before and after the expression of AK01a channels. (*E*) Comparison of monosynaptic excitatory synaptic potentials in L7 produced by a spike of L10 before and after the expression of AK01a channels in L10. The resting potentials of L10 and L7 were −49 and −60 mV, respectively. *D* and *E* are from the same preparation. The synaptic potentials before and after the expression of AK01a were 1.7 ± 0.3 mV and 0.5 ± 0.1 mV, respectively.

sion of larger amounts of AK01a protein (unpublished observation). We therefore restricted ourselves here to the early period of expression in which cells showed an exponentially decaying I_{AK01a}.

The activation and the steady-state inactivation of the exogenous I_{AK01a} and the native I_{Adepol} were quite similar (Fig. 2*B*). The mean midpoint ($V_{1/2}$) and the slope factor (k) for the activation of I_{AK01a} expressed in *Aplysia* neurons (n = 4) were 0.02 mV and −11.9 mV, respectively. The values are somewhat intermediate between those of I_{Adepol} in R15 and I_{AK01a} in *Xenopus* oocytes: the mean $V_{1/2}$ and k for the activation of I_{Adepol} in R15 are −12.0 mV and −7.5 mV (31), while those of I_{AK01a} in oocytes are 5.4 mV and −9 mV (14). The mean $V_{1/2}$ and k for the steady-state inactivation of I_{AK01a} in *Aplysia* neurons (n = 12) were −29.1 and 3.6 mV, respectively. The values were more similar to those of I_{Adepol} in R15 than I_{AK01a} in *Xenopus* oocytes: The mean $V_{1/2}$ and k of I_{Adepol} in R15 are −30.9 and 4.1 mV, while those of I_{AK01a} in oocytes are −20.9 and 3.3 mV (14, 31). Moreover, the decay time constants for the exogenous and the native

Table 1. Effect of AK01a channel or the β-galactosidase expression on the L10 spike and the amplitude of postsynaptic potentials (PSPs) in its follower cells

	Change in parameters of the L10 spike, %			
Plasmid	Overshoot	Afterhyperpolarization	Duration*	PSP, %
pNEX-AK01a	71.3 ± 31.2	120.4 ± 11.5	54.9 ± 4.9	42.1 ± 11.5
pNEX-lacZ	106.6 ± 21.1	95.9 ± 9.0	130.4 ± 46.4	93.3 ± 43.8

The change of parameters of the L10 spike and PSPs is expressed as the percentage of control. The values are shown as mean ± SD of five preparations in both cases.

*The duration of an action potential was defined as the time required to repolarize to −50 mV from the spike peak.

currents, measured at depolarized membrane potentials (more positive than 20 mV), were independent of voltage and were between 20 and 30 msec in duration. Finally, the I_{AK01a} in *Aplysia* neurons resembles the native I_{Adepol} in having a similar, high and dose-dependent, sensitivity to 4-AP (Fig. 2 *C* and *D*).

These overall similarities in the biophysical and pharmacological characteristics of I_{AK01a} expressed in *Aplysia* neurons to those of I_{Adepol} suggest that I_{Adepol} is carried through K^+ channels assembled from AK01a channel proteins. Although we cannot exclude the participation of an additional subunit, the fact that the exogenous channel is expressed at such a high level in relation to the native channel strongly suggests that the exogenous channel is likely to be assembled as a homooligomer. Since the exogenous channel, in turn, phenocopies the native I_{Adepol} fairly accurately, it is likely that the native channel is also a homooligomer of AK01a. The difference between I_{AK01a} in *Aplysia* neurons and in *Xenopus* oocytes therefore is most likely due either to different ionic or lipid environments or to different post-translational modifications.

Given the finding that the AK01a channel phenocopies one of the native channels, we next asked: How does this current contribute to neuronal signaling? In particular, does it contribute to the repolarization of the action potential? If so, can it regulate the ability of the action potential to release transmitter (17, 26, 27)? To address these questions, we injected pNEX-AK01a into the cholinergic cell L10, a well-studied presynaptic neuron that makes both excitatory and inhibitory synaptic connections with a large number of follower cells in the abdominal ganglion of *Aplysia* (28–30). After the injection of pNEX-AK01a and the expression of I_{AK01a}, the transient outward current was dramatically increased without noticeable change in other currents (Fig. 3*A*). The expression of I_{AK01a} made the undershoot more negative by an average of 20% (13 mV) above control, and it led to a 45% decrease in the duration of the action potential (Fig. 3 *B* and *D*). Concomitant with this change in the wave form, the ability of the action potential to release transmitter was reduced dramatically. Both the excitatory and inhibitory synaptic potentials measured in the follower cells were decreased by as much as 70%, and on average by 60% compared with control (Fig. 3 *C* and *E* and Table 1). By contrast, expression of β-galactosidase (Table 1) had no systematic effect on the spike shape and the synaptic potentials. Because I_{AK01a} is so similar to I_{Adepol} it appears likely that the native transient K^+ current, I_{Adepol}, contributes to the repolarization of the action potential and that the modulation of I_{Adepol} can strongly regulate synaptic efficacy. Indeed, in a cell that normally expresses a relatively large amount of I_{Adepol}, such as cell R2, the application of 1 mM 4-AP, which blocks specifically only I_{Adepol}, causes marked spike broadening (unpublished observation).

These experiments show that it is readily possible to express cloned K^+ channels in differentiated neurons of the intact nervous system of *Aplysia* and that a native K^+ channel can be assembled as a homooligomer. In addition, the transfer of a gene encoding a specific K^+ channel illustrates the ability to redesign the electrical properties and synaptic effectiveness of cells in which the cloned gene is expressed. Since this gene transfer method should be applicable to any cloned gene, it opens for molecular exploration a new range of problems in the large identified nerve cells of *Aplysia* and in the nerve cells of other invertebrates. It therefore may now be possible to explore, in these invertebrates, the role of specific genes in neuronal integration, in the mechanisms of synaptic transmission, and in various forms of neuronal plasticity related to learning.

We thank Drs. S. Fink and R. H. Goodman for 4×AP-1 RSV-lacZ and VIP-lacZ; Dr. G. MacGregor for pNAssβ, SV40-lacZ, CMV-lacZ, AD-lacZ, and TK-lacZ; Dr. M. Comb for ENK-lacZ; Dr. L. Des Groseillers for R14-CAT; Dr. R. Prywes for Fos-CAT; and Dr. J. Skowronski for HBA-lacZ (β-actin). We also thank Drs. S. A. Siegelbaum, T. Kubo, and T. Jessell for critical reading of the manuscript, H. Ayers and A. Krawetz for typing the manuscript, and K. Hilten and S. Mack for preparation of the figures. This research is supported by the Howard Hughes Medical Institute.

1. Hille, B. (1984) *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA).
2. Klein, M., Camardo, J. S. & Kandel, E. R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5713–5717.
3. Kandel, E. R. & Schwartz, J. H. (1982) *Science* **218**, 433–443.
4. Siegelbaum, S. A., Camardo, J. S. & Kandel, E. R. (1982) *Nature (London)* **299**, 413–417.
5. Baxter, D. A. & Byrne, J. H. (1990) *J. Neurophysiol.* **64**, 978–990.
6. Alkon, D. L. (1983) *Sci. Am.* **249**(1), 70–84.
7. Alkon, D. L., Sakakibara, M., Fórman, R., Harrigan, J., Lederhendler, I. & Farley, J. (1985) *Behav. Neurol. Biol.* **44**, 278–300.
8. Farley, J. (1988) *Behav. Neurosci.* **102**, 784–802.
9. Collin, C., Ikeno, H., Harrigan, J. F., Lederhendler, I. & Alkon, D. L. (1988) *Biophys. J.* **54**, 955–960.
10. Papazian, D. M., Schwarz, T. L., Tempel, B. L., Jan, Y. N. & Jan, L. Y. (1987) *Science* **237**, 749–753.
11. Kamb, A., Iverson, L. E. & Tanouye, M. A. (1987) *Cell* **50**, 405–413.
12. Bauman, A., Kraj-Zentgens, I., Muller, R., Muller-Holtkamp, F., Seidel, R., Kecskemethy, N., Casal, J., Ferrus, A. & Pongs, O. (1987) *EMBO J.* **6**, 3419–3429.
13. Jan, L. Y. & Jan, Y. N. (1990) *Trends Neurosci.* **13**, 415–419.
14. Pfaffinger, P. J., Furukawa, Y., Zhao, B., Dugan, D. & Kandel, E. R. (1991) *J. Neurosci.* **11**, 918–927.
15. Schacher, S. & Proshansky, E. (1983) *J. Neurosci.* **3**, 2403–2413.
16. Gardner, D. (1971) *Science* **173**, 550–553.
17. Shapiro, E., Castellucci, V. F. & Kandel, E. R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 629–633.
18. Gorman, C. M., Merlino, G. T., Willingham, M. C., Pastan, I. & Howard, B. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6777–6781.
19. Fisch, T. M., Prywes, R. & Roeder, R. G. (1987) *Mol. Cell. Biol.* **7**, 3490–3502.
20. MacGregor, G. R. & Caskey, C. T. (1989) *Nucleic Acids Res.* **17**, 2365.
21. Leavitt, J., Gunning, P., Porreca, P., Ng, S.-Y., Ling, C. S. & Kedes, L. (1984) *Mol. Cell. Biol.* **4**, 1961–1969.
22. Tsukada, T., Horovitch, S. J., Montminy, M., Mandel, G. & Goodman, R. H. (1985) *DNA* **4**, 293–300.
23. Nguyen, T. V., Kobierski, L., Comb, M. & Hyman, S. E. (1990) *J. Neurosci.* **10**, 2825–2833.
24. Des Groseillers, L., Cowan, D., Miles, M., Sweet, A. & Scheller, R. H. (1987) *Mol. Cell. Biol.* **7**, 2762–2771.
25. Zhong, Y. & Wu, C.-F. (1991) *Science* **252**, 1562–1564.
26. Llinás, R., Sugimori, M. & Simon, S. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2415–2419.
27. Hochner, B., Klein, M., Schacher, S. & Kandel, E. R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8410–8414.
28. Kandel, E. R., Frazier, W. T., Waziri, R. & Coggeshall, R. E. (1967) *J. Neurophysiol.* **30**, 1352–1376.
29. Wachtel, H. & Kandel, E. R. (1967) *Science* **158**, 1206–1208.
30. Koester, J. & Kandel, E. R. (1977) *Brain Res.* **121**, 1–20.
31. Furukawa, Y., Kandel, E. R. & Pfaffinger, P. (1992) *J. Neurosci.*, in press.