A New Class of Noninactivating K+ Channels from Aplysia Capable of Contributing to the Resting Potential and Firing Patterns of Neurons

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Summary

From the nervous system of Aplysia, we have cloned a new class of noninactivating K+ channels (αKv5.1) that are activated at low voltage and are capable of contributing to the resting potential and firing patterns of neurons. Expression of αKv5.1 in Aplysia neuron R15 revealed that αKv5.1 exerts an unusual control over cell excitability; it increased the resting potential by more than 20 mV and abolished the spontaneous bursting activity of the cell. In its ability to suppress the endogenous rhythm of R15, αKv5.1 differs in its actions from transient, inactivating K+ channels such as αKv1.1a, an Aplysia homolog of Shaker. αKv1.1a shortens the duration of the spike and increases the afterpotential, but does not suppress bursting. Thus, by expressing different classes of K+ channels, it is possible to redesign, in specific ways, the signaling capabilities of specific, identified neurons.

Introduction

K+ channels differ from one another in their voltages of activation and in their kinetic properties. These differences underlie the distinctive and varied contributions of the different K+ channels to neuronal signaling, including contributions to the resting potential, the duration of the action potential, its capability to regulate transmitter release, the pattern of neuronal firing, and membrane excitability (Hille, 1992; Rudy, 1988). By expressing appropriately or deleting various K+ channels, it should be possible to control each of these aspects of neuronal signaling. Such selective control could, in principle, be therapeutically important for diseases, such as focal epilepsy, that affect neuronal excitability.

Following the initial breakthrough in the cloning of Shaker K+ channels (Kv1.1) from Drosophila (Kamb et al., 1987; Papazian et al., 1987; Pongs et al., 1988), three additional K+ channels were cloned from Drosophila by low stringency homology screening: Shab (Kv2.1), Shaw (Kv3.1), and Shal (Kv4.1) (Butler et al., 1989; Wei et al., 1990). Homologs of each of these four Drosophila classes of K+ channels were soon found in other species, including Aplysia (for a list of the clones, see Chandy, 1991; Perney and Kaczmarek, 1991; Pfaffinger et al., 1991).

However, earlier biophysical studies have revealed a greater degree of K+ channel diversity than even these four classes would imply. This suggests that additional classes of K+ channels remain to be delineated on the molecular level. In particular, no class of noninactivating K+ channels has been cloned as yet. All classes of K+ channels that have so far been cloned show varying degrees of inactivation in response to membrane depolarizations lasting many seconds. The one noninactivating channel that has been cloned, Drosophila Shaw, seems an exception rather than an example of a class, since, in other species, all known homologs of Shaw encode inactivating K+ channels (Yokoyama et al., 1989; McCormack et al., 1990; Lu-neau et al., 1991; Schroter et al., 1991; Rettig et al., 1992). This suggests that Shaw may represent a variant in a class of inactivating channels and that the major classes of low-voltage-activated, noninactivating K+ channels remain to be characterized on the molecular level.

One major group of noninactivating K+ channels that is particularly interesting consists of channels that are activated at low voltages. These channels are voltage dependent, yet they can contribute to the resting potential of the neuron. In the initial formulation of the ionic hypothesis, Hodgkin and Huxley (1952) distinguished between two classes of channels: non-gated leakage channels (permeable to Na+, Cl, as well as to K+) that are voltage independent are always open and contribute to the resting potential, and gated channels that are closed at rest are opened by voltage and contribute to the action potential. Subsequent work has shown that some K+ channels that contribute to the resting potential are also voltage dependent to varying degrees. These include the S channel, the M channel, and the inward rectifiers (for review, see Rudy, 1988). Despite their interest, however, there is little direct molecular information about voltage-gated K+ channels that contribute to the resting potential.

Here, we report the molecular cloning of a class of low-voltage-activated, noninactivating K+ channel from the nervous system of Aplysia. In this paper, we describe the properties of αKv5.1, a channel that can contribute to the resting potential and firing pattern of the Aplysia neuron.
Figure 1. Deduced Amino Acid Sequence of aKv5.1

Transmembrane segments are assigned by hydropathy analysis. Putative phosphorylation sites by protein kinase A are marked by a triangle. A putative phosphorylation site by cyclic AMP-dependent protein kinase A is marked by a star. Putative N-glycosylation sites are marked by a pound symbol. The shown amino acid sequence is encoded by cDNA clone H-3. Screening of another cDNA library of the Aplysia nervous system by using clone H-3 as a probe at standard high stringency conditions leads to ten more positive clones. The entire coding region of a full-length clone (DH3B5) was sequenced, and we found that a Thr in this clone replaced a Cys in the H-3 clone at position 99. However, further sequencing of five additional clones in this region all confirmed the presence of Glu-99, which suggests that Thr-99 in DH3B5 is a cloning artifact and the sequence of clone H-3 we present here is correct.

**Results**

**aKv.5.1 Represents a New Class of K⁺ Channels**

To delineate new classes of low voltage-activated, noninactivating K⁺ channels, we used an improved variation of homology screening. We focused our search for new classes of K⁺ channels by using the H-5 region as a guide, because this is the most conserved region in most cloned KC channels and is thought to contribute to K⁺ ion permeation and selectivity (Hartmann et al., 1991; Yellen et al., 1991; Yool and Schwartz, 1991).

Our initial examination of the H-5 region revealed that most amino acid residues in this region are encoded by highly degenerate codons, a situation that would make it difficult to design oligonucleotide probes for cDNA library screening. Upon careful examination of the specific codon usage for the H-5 region in the four cloned Aplysia K⁺ channels (aKv1.1a, aKv2.1, aKv3.1, and aKv5.2), we found that in many of the positions, there was surprisingly little degeneracy. This conservation of codon at specific positions allowed us to design oligonucleotide probes for cDNA library screening that were only 4-fold degenerate.

Using the 4-fold degenerate oligonucleotide probes, we screened 5 × 10⁵ independent recombinants from a cDNA library under conditions that allowed for only a few mismatches. We obtained two cDNA clones (aKv5.1, aKv6.1), each of which represents a new class of K⁺ channel. Here, we report our studies on aKv5.1.

The cDNA clone for aKv5.1 has an open reading frame of 1731 bp that predicts a protein of 577 amino acids (Figure 1). We assigned as the initiation codon the first ATG downstream from an in-frame stop codon. Based on hydropathy analysis, aKv5.1 showed seven potential transmembrane segments, as is the case with other K⁺ channels. There were two putative N-glycosylation sites: one putative phosphorylation site for the cyclic AMP (cAMP)-dependent protein kinase near the carboxyl terminus and five putative phosphorylation sites for protein kinase C near the amino and carboxyl termini of the molecule.

Pairwise comparison of the amino acid sequence of aKv5.1 with those of others was done on the core region of the molecules, from about 150 amino acid residues before the S1 to a few amino acids residues after the S6. The comparison revealed the identity among aKv5.1 and Drosophila Shaker, Shab, Shaw, Shal (Butler et al., 1989; Wei et al., 1990) to be 33%, 37%, 36%, and 35%, respectively. aKv5.1 is 33% and 28% identical to lK8 and K13, respectively (Drewe et al., 1992). The identity between these and eag and slo is less than 20% (Warmke et al., 1991; Atkinson et al., 1991). An identity of less than 40%, based on pairwise amino acid comparison, distinguishes different voltage-gated K⁺ channel classes from one another, even across species. This criterion has been used to classify the voltage-gated K⁺ channels that have been cloned and expressed functionally (Chandy, 1991; Perney and Kaczmarek, 1991). For example, Aplysia homologs of Shaker, Shab, Shaw and Shal have all been cloned (Pfaffinger et al., 1991; unpublished data). Each of them is less than 40% identical to aKv5.1; thus, aKv5.1 constitutes a new class of voltage-gated K⁺ channel.

As might be expected of a new class, aKv5.1 has features in its S4 region that distinguish it from Shaker, Shab, Shaw, and Shal (Butler et al., 1989; Wei et al., 1990) to be 33%, 37%, 36%, and 35%, respectively. aKv5.1 is 33% and 28% identical to lK8 and K13, respectively (Drewe et al., 1992). The identity between these and eag and slo is less than 20% (Warmke et al., 1991; Atkinson et al., 1991). An identity of less than 40%, based on pairwise amino acid comparison, distinguishes different voltage-gated K⁺ channel classes from one another, even across species. This criterion has been used to classify the voltage-gated K⁺ channels that have been cloned and expressed functionally (Chandy, 1991; Perney and Kaczmarek, 1991). For example, Aplysia homologs of Shaker, Shab, Shaw and Shal have all been cloned (Pfaffinger et al., 1991; unpublished data). Each of them is less than 40% identical to aKv5.1; thus, aKv5.1 constitutes a new class of voltage-gated K⁺ channel.

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Noninactivating K⁺ Channels in Aplysia

651 bp (akv 5.1)

534 bp (calreticulin)

Figure 2. PCR Analysis of aKv5.1 Expression in Aplysia Nervous System and Ovotestis

After 30 cycles of reaction, a 651 bp aKv5.1 band is seen only on the nervous system mRNA that has been reverse transcribed (lane N). The Aplysia calreticulin (Kennedy et al., 1992) was used as a positive control, and a 534 bp calreticulin band is seen in both the nervous system and ovotestis mRNA that have been reverse transcribed (lanes N and O). No PCR products were detected from nonreverse transcribed mRNA (lanes N' and O'). N, nervous system mRNA reverse transcribed; N', nervous system mRNA not reverse transcribed; O, ovotestis mRNA reverse transcribed; O', ovotestis mRNA not reverse transcribed.

residues are thought to cause a significant bend in a alphahelical structure (Branden and Tooze, 1991), the replacement of the proline by threonine could have a major impact on the conformation of the channel proteins.

To confirm that aKv5.1 was expressed in Aplysia nervous system, we performed polymerase chain reaction (PCR) analysis on oligo(dT)- and random-primed cDNAs, and we found that aKv5.1 was present in the nervous system but absent from the ovotestis (Figure 2). We were unable to amplify aKv5.1 from only oligo(dT)-primed cDNAs, which suggests that, as is true for other Aplysia K⁺ channels, aKv5.1 is probably encoded by a very large mRNA.

aKv5.1 Is Expressed in the Xenopus Oocytes

Since the S4 region has been implicated in voltage-dependent gating and the S6 region in inactivation, one might expect that aKv5.1 shows novel gating properties. To begin to explore these properties, we first expressed in vitro transcribed RNA in Xenopus oocytes (Figure 3). We found that the channel activated very slowly; the current started to activate around −50 mV, and the conductance saturated around 20 mV (Figures 3A1 and 3A2). We examined the inactivation properties of aKv5.1 by measuring the response to a 20 mV depolarizing pulse preceded by 5 s prepulses of various depolarizing levels from −80 mV to +20 mV. The activated channel clearly did not inactivate, even during 5 s prepulses (Figures 3B1 and 3B2).

In a solution containing 2 mM K⁺, the reversal potential of the expressed currents determined by the reversal of the tail current was −90.2 ± 4.1 mV (n = 5). When the K⁺ concentration was increased by replacing Na⁺ with K⁺, the reversal potential became more positive. In the solutions containing 7 mM, 19.6 mM, and 38.8 mM K⁺, the values of the reversal potentials were −75.6 ± 5.4 mV, −50.1 ± 6.4 mV, and −31.1 ± 2.9 mV (n = 5), respectively. The relationship between the reversal potential and the external K⁺ concentration could be approximated by the Goldman–Hodgkin–Katz equation. Our analysis indicated that the channel was 30 times more selective to K⁺ than to Na⁺. The expressed current was blocked by external tetraethyl ammonium (TEA; Kₐ = 0.36 mM) but not affected by 10 mM 4-aminopyridine (4-AP; Figures 4A and 4B). This preceded by 5 s prepulses from −80 mV to various depolarizing potentials in 20 mV increments. (A2) shows the inactivation curve of aKv5.1. The conductance was calculated as described in (A) except that I₉₀ was taken at the end of 400 ms pulses preceded by inactivating prepulses described in (B) in 10 mV increments.
Figure 4. Effects of TEA and 4-AP on aKv5.1 Currents in Xenopus Oocytes

(A) Effects of TEA. Currents were evoked by the same depolarization step from −80 to +20 mV in the presence of different TEA concentrations (left). The semilogarithmic plot of TEA concentration versus the normalized current was fit by \( I_{norm} = I_0/K_d + I_{ss} \), where \( I_{norm} \) is normalized current and \( I_s \) is the steady-state current that cannot be blocked by TEA. The fit gives a \( K_d \) of 0.36 mM by TEA.

(B) Currents were evoked by the same depolarization step from −80 to −10 mV with or without the presence of 10 mM 4-AP.

was in marked contrast to the Drosophila Shaw K+ channel, which was highly sensitive to 4-AP blockade (80% block by 1–2 mM 4-AP) and insensitive to TEA block (8% block by 10 mM TEA; Wei et al., 1990).

**aKv5.1 Can Control the Firing Pattern and Contribute to the Resting Potential in Neuron R15**

How does aKv5.1 contribute to membrane excitability of neurons? To address this question directly, we expressed aKv5.1 in identified Aplysia neurons of the intact abdominal ganglion. Since aKv5.1 does not inactivate and starts to activate at −50 mV, aKv5.1 may be able to contribute to the resting potential in Aplysia neurons, some of which have a resting potential between −40 mV and −60 mV. Moreover, because aKv5.1 can be activated readily near the resting potential, it should be able to counteract excitatory inputs and suppress firing, even in neurons with more negative resting potential. Indeed, we found that aKv5.1 had a profound effect on both the membrane potential and the firing pattern of neurons.

We focused on neuron R15, a well-characterized spontaneously bursting cell in the abdominal ganglion of Aplysia (Strumwasser, 1967; Frazier et al., 1967), whose activity is driven by ion conductances that change rhythmically below the firing threshold (Benson and Adams, 1987; Canavier et al., 1991). This bursting rhythm resembles the paroxysmal depolarization of focal epilepsy. As a result, this cell has been used as a model for studying focal epileptogenic processes (Lewis et al., 1986).

We subcloned aKv5.1 into a neuronal expression

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**Table 1. A Comparison of Steady State Currents in the Neuron and in the Oocyte**

<table>
<thead>
<tr>
<th></th>
<th>Activation Properties</th>
<th>% of Current Remaining After</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( V_m ) (mV)</td>
<td>( k ) (mV)</td>
</tr>
<tr>
<td>Native R15</td>
<td>( (n = 7) )</td>
<td>( (n = 7) )</td>
</tr>
<tr>
<td>Current</td>
<td>1.3 ± 3.5</td>
<td>21.9 ± 3.3</td>
</tr>
<tr>
<td>aKv5.1 in R15</td>
<td>−21.4 ± 5.3</td>
<td>17.3 ± 2.2</td>
</tr>
<tr>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
</tr>
<tr>
<td>aKv5.1 in Oocyte</td>
<td>−21.6 ± 4.5</td>
<td>9.0 ± 1.0</td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
</tbody>
</table>

a Activation properties: \( V_m \) (midpoint of activation) and \( k \) (slope factor) values are derived from the Boltzmann fit, as described in Figure 3A legend, to the activation data. The current amplitude of all R15 cells was measured at the end of a 4 s pulse, but that of oocytes was measured at the end of 1 s pulses.

b Effect of 10 mM 4-AP was studied by comparing the current amplitudes at the end of stimulus pulses before and after 4-AP application in response to membrane depolarizations from −80 mV to 0 mV.

c Effect of 10 mM TEA was studied by comparing the current amplitudes at the end of stimulus pulses before and after TEA application in response to membrane depolarizations from −80 mV to +20 mV.
vector pNEX (Kaang et al., 1992). Plasmid DNA was then prepared and injected into neuron R15 in the abdominal ganglion carried in organ culture. A few hours after injection, a new current developed that increased over time and was distinct from the native ones. At 5 hr after injection, the amplitude of the expressed steady-state current was usually around 0.7 μA when tested at the end of a 4 s depolarizing pulse from -80 mV to +20 mV. At 9-18 hr after injection, the steady-state current increased to about 4 μA. This was about 10 times the size of the endogenous current (4.20 ± 0.13 μA [n = 4] in aKv5.1 injected cells versus 0.39 ± 0.13 μA [n = 7] in control cells; see Table 2).

As is evident from Table 1, the expressed aKv5.1 current was similar in R15 to that in oocytes. In both cases, there was a negative voltage range of activation, an insensitivity to 4-AP, and a sensitivity to TEA. The endogenous current in R15 activated at a much more positive voltage range than did aKv5.1 (V1/2 = -21.1 ± 5.3 mV [n = 4] for aKv5.1 in R15 versus V1/2 = 1.3 ± 3.5 mV [n = 7] for native R15 current). However, the current expressed in R15 activated faster (Figure 5C1) and had a larger slope factor (k; Table 1). These differences may have been due to a number of factors, such as differences in posttranslational modification, membrane environment, and intracellular and extracellular salt concentration. There was also a slight decay during the 4 s pulse for aKv5.1 expressed in R15, mainly at voltages above -20 mV. The decay could be due to the contamination of endogenous inactivating currents or to accumulation of K+ ions in the extracellular space. Alternatively, some aKv5.1 subunits might have coassembled with endogenous subunits and thereby changed the kinetic properties of the expressed channels.

aKv5.1 had a powerful effect on the resting potential and on the bursting rhythm of R15. Even at a low level expression, aKv5.1 suppressed completely the spontaneous bursting activity and caused R15 to become silent with a resting membrane potential of -46.2 ± 2.9 mV (n = 5; Figure 5B2; Table 2). In R15 cells that had a higher level of expression, the membrane potential became 20 mV more negative still, reaching -65.9 ± 2.0 mV (n = 4; Figure 5C2; Table 2).

To assure that the effect of suppressing bursting in R15 was not due to the impairment of mechanisms generating the action potential, we injected various amounts of current (from 20 to 100 nA) into the silent R15 neurons expressing aKv5.1, and we found the cells were indeed able to fire action potentials as long as
a sufficient amount of current was injected (50 ± 11 nA [n = 5] for low level expression and 84 ± 26 nA [n = 4] for high level expression; Figures 5B, and 5C; Table 2). Therefore, the silencing effect on R15 by expressing aKv5.1 seems to be due to a decrease in excitability of the cell, which prevents the endogenous depolarizing currents from driving the membrane potential above the threshold for firing action potentials. In fact, we did not observe in silent cells the noticeable fluctuations in the membrane potential characteristic of control bursting cells (Figures 5A, 5B, and 5C).

To compare the effectiveness in silencing the bursting rhythm of aKv5.1, a nonactivating current, with that of a transient current, we next expressed in R15 the Aplysia homolog of Shaker (aKv1.1a), aKv1.1a is activated at suprathreshold voltage range and is inactivated soon after activation (Kuang et al., 1992). A few hours after the injection of aKv1.1a construct DNA into R15, a few microamperes of aKv1.1a current developed when tested by depolarizing the membrane from −50 to +20 mV (4.24 ± 1.7 μA [n = 3]; Figure 6; Table 2). This expressed current decreased the duration of the action potential form 5.8 ± 0.9 ms in control cells (n = 7) to 3.1 ± 0.9 ms (n = 3) and increased the hyperpolarizing afterpotential from −61.1 ± 3.2 mV (n = 7) to −88.0 ± 4.9 mV (n = 3). However, despite the expression of a substantial current by aKv1.1a, R15 continued to burst spontaneously (Figure 6; see Table 2). Therefore, the ability to increase the resting potential and silence the bursting rhythm of R15 occurs as a result of injection of aKv5.1.

**Discussion**

**aKv5.1 Represents a New Class of Low Voltage–Activated, Noninactivating K+ Channels**

aKv5.1 represents a new class of K+ channels in parallel with Shaker, Shab, Shaw and Shal. aKv5.1 is characterized by a noninactivating K+ current that is activated at low voltages. Nonactivating K+ channels are particularly important in controlling neuronal firing, as exemplified in the studies of the mammalian M channels (for review, see Rudy, 1988). Because they are voltage dependent, low voltage, nonactivating channels such as aKv5.1 are particularly effective in counteracting depolarizing inputs, because as the membrane starts to depolarize, more channels become activated.

The only other inactivating channel so far cloned is D-Shaw, but this channel differs significantly from aKv5.1. D-Shaw is very sensitive to 4-AP inhibition but not to TEA. By contrast, aKv5.1 is sensitive to TEA but not to 4-AP. Since the majority of nontransient K+ currents in the neuron are not sensitive to 4-AP but are sensitive to TEA, it seems likely that aKv5.1 represents a more widespread class than does D-Shaw. Consistent with this view, homologs of D-Shaw in all other species examined encode inactivating K+ channels (Yokoyama et al., 1989; McCormack et al., 1990; Lu-neau et al., 1991; Schroter et al., 1991; Retting et al., 1992). By contrast, aKv5.2, a channel in Aplysia that shares 72% identity with aKv5.1, encodes a nonactivating K+ currents (unpublished data). This suggests that aKv5.1 represents a whole class of nonactivating channels, a class that may be widespread. Since homologs of each of the Drosophila K+ channels have been cloned from other species, homology screening of aKv5.1 in other species should uncover other low voltage–activated, nonactivating K+ channels such as this and the M channels. Indeed, the finding that aKv5.1 can modulate the firing of the neuron and contribute to the resting potential is reminiscent of the S channel (Siegelbaum et al., 1982). Both channels have a low voltage for activation and both are noninac-

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**Table 2. Effects of K+ Channel Overexpression on the Activity of Neuron R15**

<table>
<thead>
<tr>
<th>Expression</th>
<th>Current (nA)</th>
<th>AP/Burst</th>
<th>AHP (mV)</th>
<th>AP Width (ms)</th>
<th>Membrane Potential (mV)</th>
<th>Current Injected (nA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control R15</td>
<td>0.39 ± 0.13</td>
<td>6.8 ± 2.2</td>
<td>−61.1 ± 3.2</td>
<td>5.8 ± 0.9</td>
<td>Bursting</td>
<td>−</td>
</tr>
<tr>
<td>Low-Level aKv5.1</td>
<td>0.69 ± 0.20</td>
<td>Silent</td>
<td>Silent</td>
<td>Silent</td>
<td>−46.2 ± 2.9</td>
<td>50.0 ± 11</td>
</tr>
<tr>
<td>High-Level aKv5.1</td>
<td>4.28 ± 0.13</td>
<td>Silent</td>
<td>Silent</td>
<td>Silent</td>
<td>−65.9 ± 2.0</td>
<td>84.0 ± 26</td>
</tr>
<tr>
<td>aKv1.1a</td>
<td>4.24 ± 1.7</td>
<td>7.0 ± 1</td>
<td>−88.0 ± 4.9</td>
<td>3.1 ± 0.9</td>
<td>Bursting</td>
<td>−</td>
</tr>
</tbody>
</table>

a For cells expressing aKv5.1 and getting control values for aKv5.1, current amplitude was measured at the end of 4 pulses stepped from −80 mV to +20 mV. For cells expressing aKv1.1a, current amplitude was obtained by measuring peak current size in response to depolarizations from −50 mV to +20 mV in the presence of 30 mM TEA.

b AP/Burst, number of action potentials in a burst.

c AHP, after hyperpolarization.

d AP width, the width of action potentials was defined as the width when the action potential reached 50% of its height.

e Membrane potential corresponding to the resting potential of the R15 cells that overexpressed aKv5.1 and became silent.

f Minimum amount of current injected under current clamp condition to evoke an action potential in cells expressing aKv5.1.

g The data presented here are obtained from the analysis of one low-level aKv1.1a expressed cell and two high-level aKv1.1a expressed cells.
Noninactivating K⁺ Channels in Aplysia

The Functional Roles of aKv5.1 in R15

A major gap in the current studies of the molecularly cloned K⁺ channels concerns their physiological roles in those nerve cells. Do they function as homo- or hetero-oligomers? What do they contribute to the generation of the resting potential, action potential, different firing patterns, or effectiveness of synaptic transmission? To address these questions, it is necessary to express or ablate a cloned channel in neurons and to assess the resulting changes in signaling properties of the cell. However, the lack of facile gene transfer methods has limited the ability to manipulate gene expression rapidly in the intact mammalian nervous system. In addition, the complexity of the mammalian nervous system poses difficulties for analysis. By contrast, the neurons of Aplysia provide an advantage for the study of channel properties in an intact nervous system. These neurons are large, easily identified, and distinctive in their firing patterns (Frazier et al., 1967; Kandel, 1976). Moreover, the expressional vector pNEX allows higher levels of expression of cloned genes in Aplysia neurons following microinjection (Kaang et al., 1992).

We have used these advantages of the Aplysia system to study the functional roles of aKv5.1 in neuron R15. We found that aKv5.1 increases the resting potential and abolishes the spontaneous firing of the neuron, whereas aKv1.1a (a high voltage-activated, transient K⁺ channel) changes the shape of the action potential of cell R15 without affecting the firing of the same neuron. In cell L10, where aKv1.1a also shortens the duration of the action potential, it is possible to show that, in addition, it depresses the capability of the action potential to release transmitter (Kaang et al., 1992). These cases illustrate that it is possible to redesign the electrical properties of neurons, thereby changing their signaling capabilities. This capability for redesigning the electrical properties of a neuron may prove to be therapeutically important.

How does the overexpression of aKv5.1 lead to the silencing of R15? aKv5.1 seems to achieve its effect by increasing both the resting conductance and the resting potential. Since cell R15 undergoes spontaneous changes in membrane conductance and therefore does not have a resting conductance, it is not possible to directly compare the resting conductance of aKv5.1 in injected cells with that of the control cells. Nevertheless, under the condition of low level expres-
sion, the cells seem to become silent because of an increase in membrane conductance. Although silenced, the cell still has a resting potential of only −46 mV, a potential that is more positive than the trough of the interburst interval of the bursting cell (between −50 mV and −60 mV). As soon as the membrane starts to depolarize, however, the activation of more aKv5.1 causes the membrane conductance to increase, thereby driving the membrane potential back to the original level and preventing the cell from firing. By contrast, under the condition of high level expression, aKv5.1 clamps the membrane at a more negative potential (−66 mV), which presumably shuts down the activity of most other ion channels in the cell. Here, the increase in resting potential adds to the enhancement of the resting conductance in silencing the cell.

Both the low voltage activation and noninactivation properties are essential for the functional roles of aKv5.1. If the channel were activated by high voltages, it would not be able to influence the membrane potential and conductance at rest. On the other hand, if the channel were to inactivate near the resting level, it would not be available for activation at rest and thus would not influence the resting potential and conductance. It is the unique combination of low voltage activation and noninactivation properties that makes the aKv5.1 channel so powerful in countering depolarizing inputs at rest and thereby inhibiting cell firing.

Experimental Procedures

Molecular Cloning of aKv5.1
To look for K+ channel cDNA clones, we screened 5 x 10^5 independent recombinants from a random−primed cDNA library made from the mRNA of the Aplysia nervous tissue (Pfaffinger et al., 1991). After lifting from bacterial plates, nylon filters were hybridized to degenerate oligonucleotide probes (5'−GATCAC−CATGACGAGCCGGTGACGCAGAC at 22°C (prehybridization and hybridization solutions consisted of 5x SSPE, 5x Denhardt's, 100 µg/ml single−stranded salmon sperm DNA, and 0.2% SDS), and final wash was done in 2x SSPE, 0.5% SDS at 37°C. To look for additional cDNA clones encoding aKv5.1, we screened another 500,000 colonies from an Aplysia nervous system cDNA library (gift of Dr. D. Salomon) by using the H3 clone as a probe. The prehybridization and hybridization were done in a similar solution as above, except that formamide was added to a final concentration of 50%. The final wash was done in 0.1x SSPE, 0.1% SDS at 65°C.

PCR Analysis
mRNA was prepared from Aplysia CNS and ovotestis using the FastTrack system (Invitrogen). To remove contaminating genomic DNA, 6 µg of CNS and ovotestis mRNA were treated with 2 units RNase free DNase (in 10 mM Tris[pH 7.5], 10 mM MgCl2, 7 mM dithiothreitol) and 40 units RNasin in a 20 µl volume at 37°C for 20 min. After phenol/chloroform extraction and ethanol precipitation, half of the RNA was diluted into 20 µl water; later, 1 µl of it was used as a negative control in each PCR reaction. The other half of the DNase−treated RNA was reverse transcribed with a mixture of oligo(dT) and random primers using the cDNA Cycle Kit (Invitrogen), and 1/20 of the final product was used in each PCR reaction. The aKv5.1 sense primer corresponds to amino acid residues 18−25, and the antisense primer, to amino acid residues 188−195. The PCR reactions were performed using Boehringer−Mannheim's reagents with the following temperature cycle: 1 min at 95°C, 1 min at 55°C, and 1.5 min at 72°C. After 40 cycles, amplification products were visualized by ethidium bromide staining following resolving on 1% agarose gel.

aKv5.1 Expression in Xenopus Oocytes
All the procedures are identical to those described in Pfaffinger et al. (1991).

aKv5.1 Expression in R15
The entire coding region of aKv5.1 was amplified from H3 cDNA clone in pKS+13 with two specific primers (5’−ccggaacatcagcagcagcagcc [BamHI site is underlined]; OA, 3′−TCTTGAGCATCAGCACACTCTGcatgggg [KpnI site is underlined]) that harbor restriction enzyme sites in their 5' ends under conditions recommended by the manufacturer (Promega−Cetus).

The PCR product was then digested with BamHI−KpnI and ligated into BamHI−KpnI linearized pNEX vector. Three independent recombinants were sequenced in their entire coding region, and no mutations were found in any of them.

All other procedures for neuronal expression were performed in a manner similar to that described in Kaang et al. (1992).

Acknowledgments
We thank K. Axel and S. Siegelbaum for their comments on an earlier version of this manuscript, N. H. Kim, D. Burst, and F. Ye for help with sequencing, and P. J. Pfaffinger for communicating unpublished studies on Aplysia Shaw homolog. F. R. was supported in part by Fondation Fyssen and Institut National de la Santé et de la Recherche Medecinale fellowships.

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Received March 3, 1994; revised July 25, 1994.

References
Noninactivating K+ Channels in Aplysia


GenBank Accession Number

The accession number for the sequence shown in Figure 1 is L35766.