G Protein-Coupled Inwardly Rectifying K⁺ Channels (GIRKs) Mediate Postsynaptic but Not Presynaptic Transmitter Actions in Hippocampal Neurons

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Summary

To study the role of G protein-coupled, inwardly rectifying K⁺ (GIRK) channels in mediating neurotransmitter actions in hippocampal neurons, we have examined slices from transgenic mice lacking the *GIRK2* gene. The outward currents evoked by agonists for GABA_B receptors, 5HT1A receptors, and adenosine A1 receptors were essentially absent in mutant mice, while the inward current evoked by muscarinic receptor activation was unaltered. In contrast, the presynaptic inhibitory action of a number of presynaptic receptors on excitatory and inhibitory terminals was unaltered in mutant mice. These included GABA_B, adenosine, muscarinic, metabotropic glutamate, and NPY receptors on excitatory synapses and GABA_B and opioid receptors on inhibitory synapses. These findings suggest that a number of G protein-coupled receptors activate the same class of postsynaptic K⁺ channel, which contains GIRK2. In addition, the GIRK2 channels play no role in the inhibition mediated by presynaptic G protein-coupled receptors, suggesting that the same receptor can couple to different effector systems according to its subcellular location in the neuron.

Introduction

Neurotransmitters activate one or both of two classes of receptor: ligand-gated ion channels and G protein-coupled receptors. A large number of G protein-coupled receptors have been shown to hyperpolarize cells by activating inwardly rectifying K⁺ channels (GIRKs) in postsynaptic neurons of the CNS as well as to inhibit the release of neurotransmitter presynaptically by an unknown mechanism (Nicoll, 1988; North, 1989; Thompson et al., 1993; Wu and Saggau, 1997). Four different mammalian GIRK channel subunits have been cloned. GIRK1–3 are expressed in high levels by CA1 and CA3 pyramidal cells as well as by dentate granule cells

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(Karschin et al., 1996; Liao et al., 1996; Ponce et al., 1996; Drake et al., 1997). It is thought that native channels consist of tetramers and that they are likely to contain at least two different subunits (Duprat et al., 1995; Kofuji et al., 1995; Krapivinsky et al., 1995; Lesage et al., 1995; Hedin et al., 1996; Spauschus et al., 1996).

Despite a large amount of research on the mechanisms underlying neurotransmitter action, the role of GIRKs in pre- and postsynaptic inhibition in the CNS is unclear. For instance, it is not clear what role, if any, GIRKs play in the presynaptic inhibition induced by G protein-coupled receptors. Alternate hypotheses involve the inhibition of Ca²⁺ channels (Dittman and Regehr, 1996; Takahashi et al., 1996; Wu and Saggau, 1997) or a direct interaction with the release machinery downstream of Ca²⁺ entry (Scanziani et al., 1992; Thompson et al., 1993).

On the postsynaptic side, there is evidence that K⁺ channels with different properties can be activated by different G protein-coupled receptors in the hippocampus (Ogata et al., 1987; Müller and Misgeld, 1989; Premkumar and Gage, 1994; Jarolimek et al., 1994).

Decisive studies are still missing, mainly due to the lack of specific pharmacological antagonists for GIRKs. We have taken advantage of the recent development of mutant mice that lack the GIRK2 protein (Signorini et al., 1997), which also show a marked posttranscriptional and probably posttranslational down-regulation of the GIRK1 protein. These mice exhibit spontaneous seizure activity, suggesting impaired inhibition, but otherwise appear normal.

Results

Elimination of Hyperpolarizing Responses to Activation of GABA_B, 5HT1A, and Adenosine A1 Receptors in GIRK2⁻/⁻ Hippocampal Principal Cells

In the first series of experiments, we examined the actions of a number of G protein-coupled receptors on the holding current recorded in CA1 and CA3 pyramidal cells and dentate gyrus granule cells. A typical experiment is shown in Figure 1A for the selective GABA_B agonist baclofen, which is known to activate K⁺ channels via a G protein (Andrade et al., 1986; Thompson and Gähwiler, 1992). In the GIRK2⁻/⁻ mouse, baclofen had no effect on the holding current or on the input resistance (R_in; Figure 1A1) in a CA1 pyramidal cell. Application of the same concentration of baclofen (40 μM) for the same period of time to a CA1 pyramidal cell from a wild-type animal (+/+) elicited an outward current and a decrease in the input resistance (Figure 1A2). This experiment was repeated a number of times for baclofen, not only in CA1 pyramidal cells but also in CA3 pyramidal cells and dentate granule cells (Figure 1B). The outward current was greatly (>85%) reduced in the CA1 pyramidal cells, although a small outward current remained. The size of the outward current induced by the same concentration of baclofen was progressively

A1

-/-

A2

+/+

B
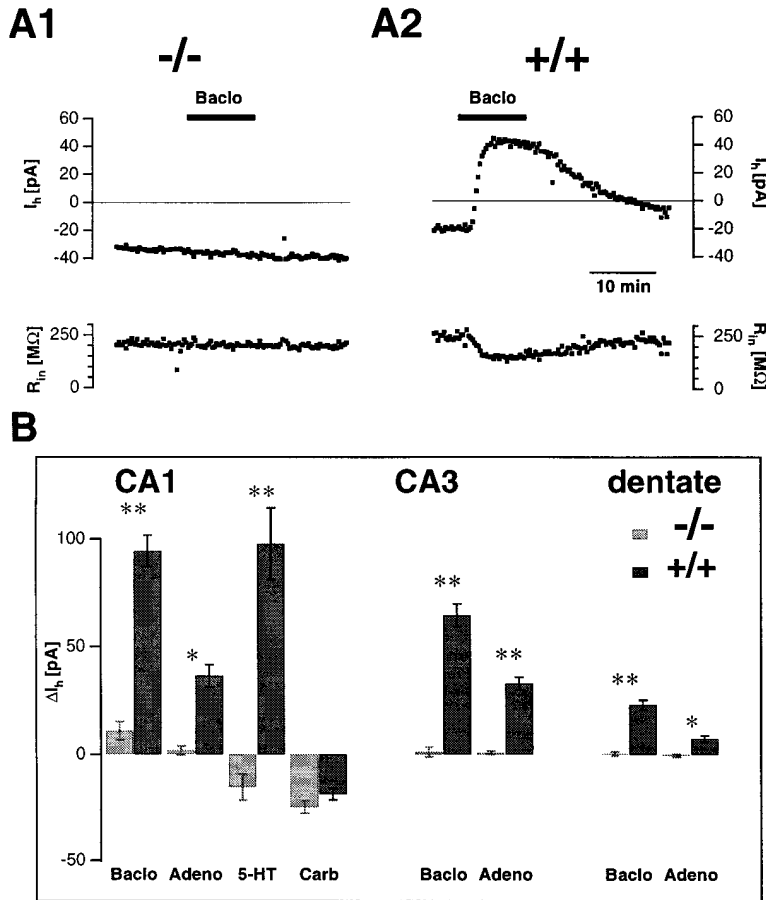
Figure 1. Reduction of G Protein-Coupled Receptor-Mediated Outward Currents in the GIRK2$^{-/-}$ Mouse

(A1) Holding current (upper trace) and input resistance (lower trace) of a representative CA1 pyramidal cell in voltage clamp ($V_h = -79$ mV) in a GIRK2$^{-/-}$ mouse. Neither parameter changed when baclofen (40 μM) was applied to the bath.

(A2) In a GIRK2$^{+/+}$ cell, bath application of baclofen led to an outward current (upper trace, $V_h = -79$ mV), which was associated with a decrease of the input resistance (lower trace), suggesting the opening of a K$^+$ conductance. The holding current at rest is smaller for the wild-type compared to the GIRK2$^{-/-}$ mouse, suggesting a difference in resting membrane potential.

(B) Summary graph of all agonists tested (mean ± SEM). The amplitude of the outward current (ΔI_h) caused by the application of baclofen and adenosine was significantly reduced in CA1 and CA3 neurons (**, $p < 0.001$; *, $p < 0.01$). In CA1, baclofen induced a residual outward current of 10.9 ± 4.1 pA ($p < 0.05$ for a hypothesized mean of zero; see text for discussion). 5-HT elicited an outward current in the GIRK2$^{+/+}$ mouse and an inward current in the GIRK2$^{-/-}$ mouse, most probably due to uncovering the effects of a 5-HT4 receptor. The amplitude of a carbachol-induced inward current was not different in mutant and control mice. Concentrations applied in CA1 were (in μM) 40 baclofen (Baclo; n = 7, $-/-$; n = 5, $+/+$), 100 adenosine (Adeno; n = 5, $-/-$; n = 4, $+/+$), 60 serotonin (5-HT; n = 3, $-/-$; n = 3, $+/+$), and 10 carbachol (Carb; n = 3, $-/-$; n = 3, $+/+$). In CA3 and dentate gyrus, n = 3 for each drug and genotype.

smaller in CA3 pyramidal cells and granule cells of wild-type mice but was completely absent in the mutant mice.

We next examined the action of a number of additional neurotransmitter receptor agonists that are known to modulate postsynaptic K$^+$ channel activity. The postsynaptic action of adenosine, which activates K$^+$ channels via an A1 receptor (Trussell and Jackson, 1987; Nicoll, 1988; Thompson et al., 1992), was abolished in the mutant mice in all three types of hippocampal neurons (Figure 1B). Serotonin (5-HT), which also activates K$^+$ channels via 5-HT1A receptors (Andrade and Nicoll, 1987; Colino and Halliwell, 1987), actually induced an inward current and an increase of input resistance (data not shown) in CA1 pyramidal cells in the mutant mice instead of the outward current and decrease in input resistance (data not shown) observed in wild-type mice (Figure 1B). In addition to 5-HT1A receptors, CA1 pyramidal cells possess 5-HT4 receptors, which close K$^+$ channels (Andrade and Nicoll, 1987; Colino and Halliwell, 1987; Andrade and Chaput, 1991). Given the increase of the input resistance, it is presumably this latter current that is unmasked in the mutant mice, strongly suggesting that the channels closed by serotonin are distinct from those that are activated by serotonin. At high concentrations, the muscarinic agonist carbachol (>10 μM) closes two types of K$^+$ channels, M channels and leak channels (Madison et al., 1987; Benson et al., 1988;

Storm, 1990). At lower concentrations (1–10 μM), carbachol selectively closes the leak channels (Madison et al., 1987). This latter action of carbachol was unaltered in the mutant mice (Figure 1B), suggesting that the muscarinic receptor-sensitive leak channels are not GIRKs.

To characterize the baclofen-activated current in more detail, voltage ramps were carried out in the absence and presence of baclofen (Figure 2A). The baclofen-induced current was then obtained by calculating the difference between the two curves (Figure 2B). The slope conductance of the current in the mutant mouse is much smaller than in the wild-type mouse, but it still shows rectification. The rectification seen with these ramps, however, must be treated cautiously, because other K$^+$ channels are activated at depolarized membrane potentials, and this could shunt the baclofen-activated current. However, in acutely isolated cells where recording conditions are better controlled, the baclofen-activated current has been shown to have all the properties of inwardly rectifying channels (Sodickson and Bean, 1996).

Absence of Monosynaptic Slow Inhibitory Postsynaptic Currents (IPSCs) in GIRK2$^{-/-}$ CA1 Neurons

It is well established in the hippocampus that synaptically released GABA generates a chloride-dependent
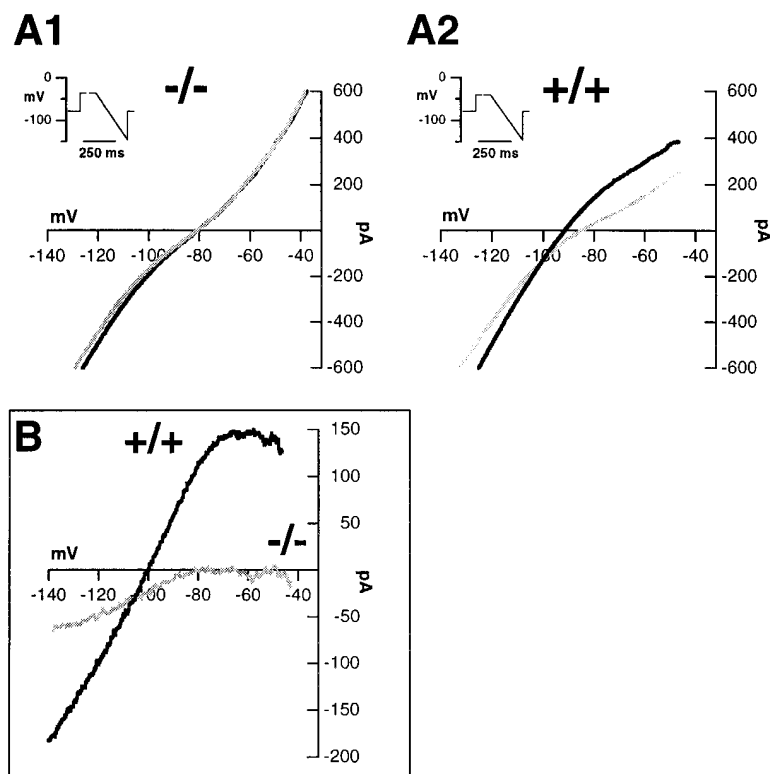
Figure 2. Current–Voltage (I–V) Relationship of a Residual Baclofen-Induced Current in the GIRK2$^{-/-}$ Mouse

(A1) I–V curve recorded in a GIRK2$^{-/-}$ mouse in the presence (dark trace) and absence (light trace) of 40 μM baclofen at voltages from -40 mV to -140 mV, using a ramp of 250 ms duration (insets). Traces are averages of five ramp protocols.

(A2) Identical experiment in GIRK2$^{+/+}$ mouse.

(B) Baclofen-induced current obtained by calculating the difference between the I–V curves before and after addition of baclofen for mutant and wild-type mice (A1 and A2) plotted on the same graph. Compared to control, the baclofen-induced current in the mutant mouse is markedly reduced but not zero (n = 4 for each genotype). The residual current is still inwardly rectifying and has a slightly higher reversal potential. This current could be mediated by remaining GIRK subunits, forming either heteromultimers or homomultimers (see text for discussion).

fast inhibitory postsynaptic potential/current (IPSP/C) mediated by GABA$_A$ receptors and a K$^+$-dependent slow IPSP/C mediated by GABA$_B$ receptors (Nicoll, 1988; Misgeld et al., 1995). We therefore tested for the presence of this synaptic current in the mutant mice. Monosynaptic slow IPSCs were isolated by blocking glutamate receptors and GABA$_A$ receptors (see Experimental Procedures). While slow IPSCs were routinely recorded in the wild-type mice, they were not seen in the mutant mice (Figure 3A). To ensure that our stimulating electrode was actually activating inhibitory interneurons, we monitored the fast IPSC in the absence of GABA$_A$ receptor antagonists (Figure 3B). In this experiment, the membrane was held between E$_{Cl}$ (-47 mV, [Cl$-$]$_{internal}$ = 20 mM for this series of experiments) and E$_K$ (-103 mV), and thus the fast IPSC is inward and the slow IPSC is outward. Despite the large fast IPSC recorded in the mutant mouse, no slow IPSC was recorded. We also varied the stimulus strength and plotted the size of the fast IPSC (GABA$_A$) against the size of the slow IPSC (GABA$_B$; Figure 3B3). In the wild-type mouse, low stimulus strengths selectively activated the fast IPSC, but as the stimulus strength was increased, a slow IPSC appeared, which quickly reached a saturating level. On the other hand, in the mutant mouse, even very high stimulus strengths only activated the fast IPSC.

Depolarized Resting Membrane Potential of GIRK2$^{-/-}$ Hippocampal Neurons

If inwardly rectifying K$^+$ channels contribute to the resting membrane potential (V$_m$), one would predict that V$_m$ would be depolarized in GIRK2$^{-/-}$. We therefore measured the V$_m$ of a number of cells in current clamp. The

average resting membrane potential in the mutant mice was ~7.9 mV (unpaired t test, p < 0.0001) more depolarized than in the wild-type mice (Figure 4). One explanation for this finding is that tonic activation of some G protein-coupled receptors leads to tonic activation of GIRKs, which contributes to the membrane potential. Although this scenario can not be completely ruled out, two observations make it unlikely. First, blockade of action potentials with tetrodotoxin had no effect on the membrane potential in wild-type mice, indicating that spontaneous action potential-dependent release of transmitter does not tonically activate a K$^+$ conductance (n = 3; data not shown). Second, we applied high concentrations of antagonists to block the receptors to the two most likely candidates, GABA$_B$ receptors and adenosine A1 receptors. Neither the GABA$_B$ receptor antagonist CGP 35348 (1 mM; n = 3) nor the A1 antagonist 8-cyclopentyltheophylline (25 μM; n = 3) had any effect on the resting membrane potential in wild-type mice (data not shown). These observations raise the possibility that GIRKs, in the absence of receptor activation, contribute to the resting membrane potential.

Unimpaired Presynaptic Inhibition in GIRK2$^{-/-}$ Hippocampus

Another major action of the G protein-coupled receptors, particularly those which activate K$^+$ currents postsynaptically, is to presynaptically inhibit the release of neurotransmitter (Thompson et al., 1993; Wu and Saggau, 1997). We therefore examined whether the presynaptic actions of a variety of G protein receptor agonists were normal in the mutant mouse. Typical experiments for baclofen are shown in Figure 5A, both in wild-type
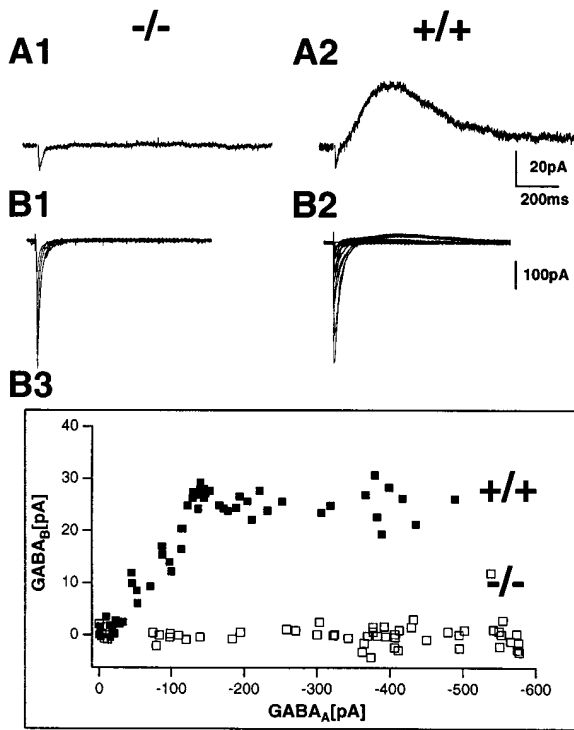
Figure 3. Synaptic Release of GABA Elicits IPSCs Mediated by GABA$_A$ but Not GABA$_B$ Receptors in GIRK2$^{-/-}$ Mice

(A1) The slow, GABA$_B$-mediated IPSC is absent in the GIRK2$^{-/-}$ mutant mouse. Currents were measured in whole-cell configuration of a CA1 pyramidal cell, and excitatory and GABA$_A$ components of the PSC were blocked by picrotoxin (100 μM), CNQX (20 μM), and APV (50 μM). The small initial inward current is a residual GABA$_A$ IPSC due to the strong stimulation intensity. Each trace is an average of five sweeps.

(A2) A control CA1 cell shows a typical slow IPSC.

(B1) Overlay of representative sweeps obtained by varying the stimulus intensity and under conditions allowing a combined GABA$_A$ and GABA$_B$ IPSC. The membrane was held between E$_{Cl}$ (−47 mV, [Cl−]$_{internal}$ = 20 mM) and E$_K$ (−103 mV), and thus the fast IPSC is inward and the slow IPSC is outward. Notice the absence of a late outward component of the current in the GIRK2$^{-/-}$ mouse.

(B2) Control experiment in a wild-type CA1 cell showing a fast inward current (GABA$_A$) and a slow outward current (GABA$_B$) which peaks at 250 ms.

(B3) Graph plotting the amplitude of the GABA$_B$ IPSC as a function of the amplitude of the GABA$_A$ IPSC obtained from the experiments in (B1) and (B2). At low stimulus intensities yielding small GABA$_A$ IPSCs, no GABA$_B$ IPSCs were measured in both groups, due to the higher threshold of GABA$_B$ IPSCs. At higher stimulus intensities, however, mutant mice still did not show any GABA$_B$ component, whereas a slow IPSC was observed in controls.

and in mutant mice. Baclofen caused the same marked depression in the field excitatory postsynaptic potential (EPSP) in both mouse strains. This depression was associated with an increase in paired-pulse facilitation (PPF), indicating a decrease in the probability of transmitter release (Manabe et al., 1993). Presynaptic adenosine A1 receptors (Proctor and Dunwiddie, 1987; Thompson et al., 1992), muscarinic receptors (Dutar and Nicoll, 1988a; Pitler and Alger, 1992), metabotropic glutamate receptors (mGluRs; Baskys and Malenka, 1991), and neuropeptide Y receptors (Colmers and Bleakman, 1994) also inhibit transmitter release from excitatory synapses in
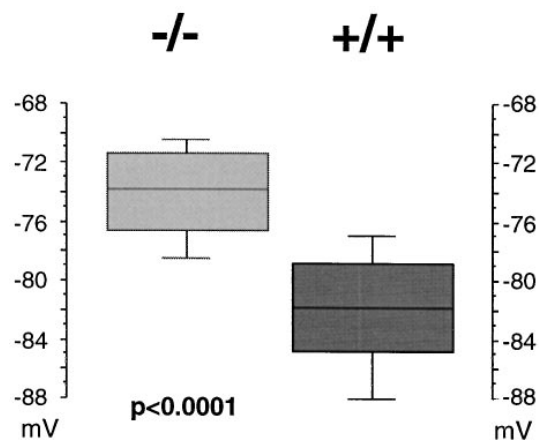
Figure 4. Depolarized Resting Membrane Potential in the GIRK2$^{-/-}$ Mouse

The resting membrane potential of CA1 principal cells measured in current clamp (I = 0) was depolarized by 7.9 mV in the GIRK2$^{-/-}$ mice compared to controls (unpaired t test, p < 0.0001; n = 11, −/−; n = 12, +/+). The absolute values (GIRK2$^{-/-}$ V$_m$ = −73.96 ± 0.98 mV, GIRK2$^{+/+}$ V$_m$ = 81.85 ± 1.28 mV; means ± SEM) are corrected for a junction potential of 15.6 mV. Boxplots show the median (line at the center), the 25th percentile (top edge), and the 75th percentile (bottom edge), as well as the 10th and 90th percentiles (lines extending from the bottom and the top).

the CA1 region of the hippocampus. As was the case for GABA$_B$ receptors, the presynaptic inhibition induced by each one of these receptors, as well as the nonselective Ca^{2+} channel blocker Cd^{2+}, was unaltered in the mutant mice (Figure 5B). We also examined the presynaptic actions of baclofen, adenosine, and the mGluR agonist LCCG1 on mossy fiber synapses in the CA3 region. Again, the presynaptic action of these agonists was normal in the mutant mice (Figure 5B). It is possible that GIRK2 channels are present on the terminals but that their activation does not affect release. We therefore tested the effect of baclofen on the excitability of Schaffer collateral fibers of CA3 pyramidal cells by monitoring the size of the antidromic volley generated in the CA3 pyramidal cell layer by Schaffer collateral fiber stimulation. Baclofen had no effect on fiber excitability (n = 4), suggesting that K$^+$ channels were not activated on these fibers.

The release of GABA from inhibitory interneurons is also controlled by presynaptic inhibitory receptors. To test the role of GIRK2 in mediating this action, monosynaptic IPSCs were recorded in the presence of glutamate receptor antagonists. Typical experiments of the inhibitory action of baclofen on IPSCs are shown in Figure 6A. In both the wild-type and the mutant mouse, baclofen caused the same large depression of the IPSC (Figure 6B). IPSCs are also inhibited presynaptically by opioid receptors (Cohen et al., 1992; Capogna et al., 1993). The μ opioid selective agonist DAMGO was equally effective in inhibiting IPSCs in the two groups of animals (Figure 6B).

Discussion

It is well established that there is a family of inhibitory G protein-coupled receptors that can be expressed on
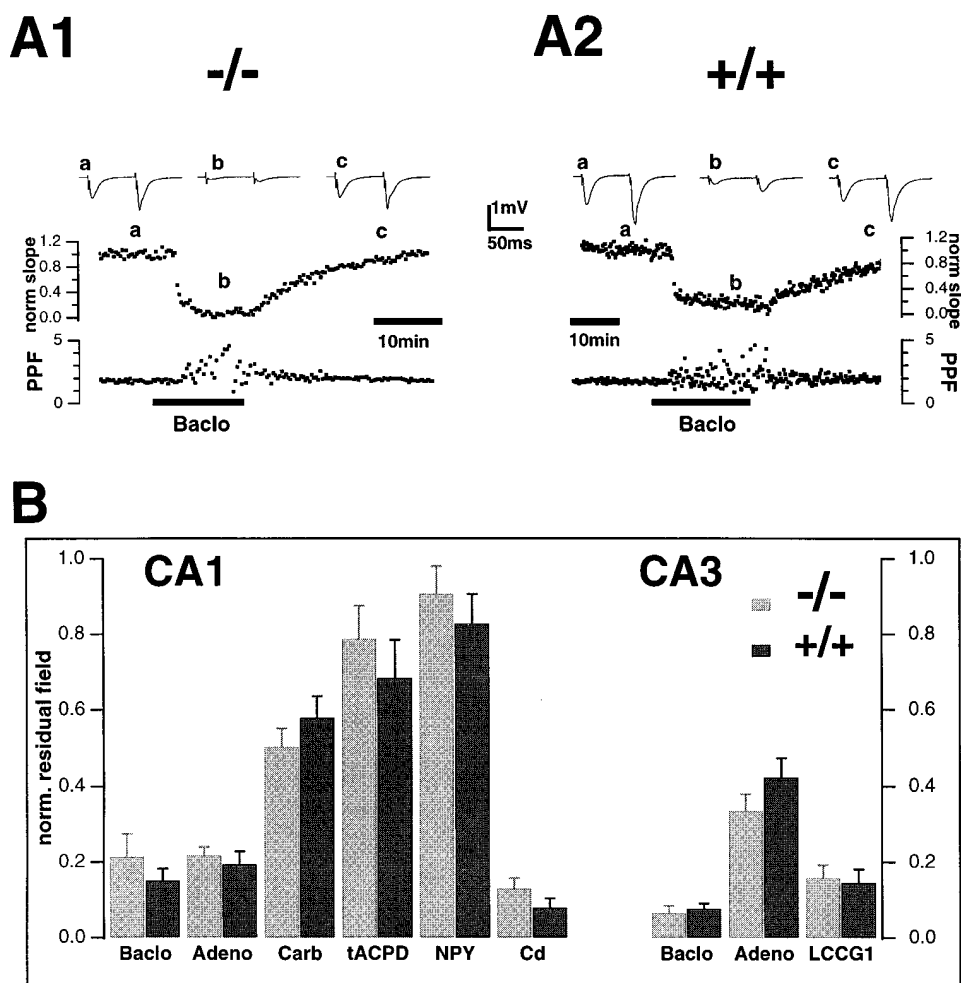
A1 -/-

A2 +/+

CA1   CA3   -/- +/+

Figure 5. Agonist-Induced Presynaptic Inhibition in the CA1 and CA3 Region Is Unchanged in the GIRK2$^{-/-}$ Mouse

(A1) CA1 field EPSP in response to paired pulse stimulation (interpulse interval, 50 ms) in a GIRK2$^{-/-}$ mouse.

(Top) Representative traces showing the depression of the EPSP during the application of baclofen.

(Center) Normalized initial slope measurement of the first EPSP.

(Bottom) Paired pulse facilitation (PPF) increases during the application of baclofen, indicating the presynaptic nature of the inhibition.

(A2) Same experiment from a GIRK2$^{+/+}$ mouse.

(B) Summary graph of all agonists tested on CA1 and CA3 fields. None of the differences were significant (unpaired t test, $p > 0.24$; n = 4–5 for each group and genotype). Fields in CA3 were obtained by mossy fiber stimulation. Concentrations applied were 40 μM baclofen (Baclo), 100 μM adenosine (Adeno), 1 μM carbachol (Carb), 10 μM trans-(1S,3R)-ACPD (tACPD), 100 nM neuropeptide Y (NPY), 10 μM LCCG1, and 100 μM cadmium (Cd).

the postsynaptic membrane as well as on the presynaptic terminal. On the postsynaptic membrane, these receptors inhibit by activating a K$^+$ conductance. The mechanism for the presynaptic inhibitory action is not entirely clear and could involve an increase in K$^+$ conductance and/or a more direct action on Ca^{2+} channels or the release process. The purpose of this study was to determine the type(s) of K$^+$ channels involved in the postsynaptic action and to elucidate the mechanism for the presynaptic inhibition. We have used mice lacking the GIRK2 channel (Signorini et al., 1997) to explore these issues.

Lack of Postsynaptic K$^+$ Conductances Activated by GABA$_B$, 5-HT1A, or Adenosine A1 Receptors

Four types of mammalian GIRK channel subunits have been cloned. mRNA for GIRK1–3, but not GIRK4 (Kar-

schin et al., 1996; but see Spauschus et al., 1996), as well as the GIRK1 and 2 proteins (Liao et al., 1996; Drake et al., 1997; Signorini et al., 1997) have been detected at high levels in the hippocampus. Although GIRK2 has been shown to form homomeric channels, GIRK1 does not (Hedin et al., 1996). It is thought that coexpression of GIRK1 and 2 results in the formation of both homomeric GIRK2 channels and heteromeric GIRK 1/2 channels (Kofuji et al., 1996; Slesinger et al., 1996). Interestingly, in the GIRK2$^{-/-}$ mouse, GIRK1 expression is greatly reduced (Signorini et al., 1997).

In these mutant mice, we have found that the postsynaptic K$^+$ conductance increase induced by a number of G protein-coupled receptors, including GABA$_B$, adenosine A1, and serotonin 5-HT1A receptors, is markedly reduced or absent. This indicates that all of these receptors couple to K$^+$ channels that contain GIRK2 and/or
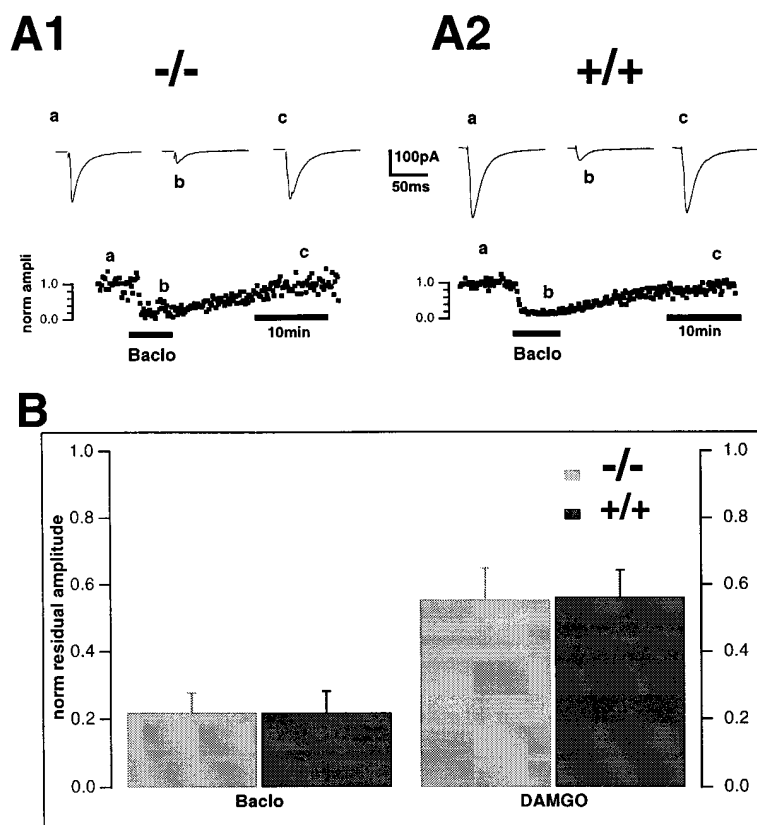
Figure 6. Presynaptic Inhibition of Monosynaptic IPSCs in CA1 Pyramidal Cells Is Unchanged in the GIRK2$^{-/-}$ Mouse

(A1) Monosynaptic IPSCs were recorded in the presence of CNQX (20 μM) and APV (50 μM). (Top) Representative traces (average of 20 sweeps) show the baclofen-induced depression in a GIRK2$^{-/-}$ mutant mouse.

(Bottom) Normalized IPSC amplitude as a function of time; stimulation frequency, 0.1 Hz. (A2) Same experiment from a GIRK2$^{+/+}$ cell. (B) Summary graph comparing the residual IPSC amplitude in both groups during the application of baclofen (40 nM) or the μ opioid agonist DAMGO (100 nM). No significant difference was observed (p > 0.2) between genotypes.

GIRK1 and is consistent with previous results suggesting that these receptors share the same K$^+$ channels (Andrade et al., 1986). These findings, however, are difficult to reconcile with previous results claiming that the single K$^+$ channel conductance evoked by serotonin and baclofen differ (Premkumar and Gage, 1994). Moreover, the single channel conductances for GIRK homomeric or heteromeric channels (Kubo et al., 1993; Duprat et al., 1995) are similar to the single channel conductance reported by Premkumar and Gage for channels activated by serotonin but not baclofen. For all of these results to be consistent with our observations, one would have to postulate that while the same channels are activated by both agonists, the single channel properties depend on the receptor type that activates the channels, or that different agonists couple to GIRK2-containing channels of different subunit stoichiometry.

While it was originally proposed that baclofen and GABA, either endogenously released or exogenously applied, activate the same conductance mechanism (Nicoll, 1988; Dutar and Nicoll, 1988b), other studies have argued that different conductances are involved (Ogata et al., 1987; Müller and Misgeld, 1989; Jarolimek et al., 1994). For instance, it has been reported that intracellular Cs$^+$ blocks outward baclofen-induced currents but has no effect on the slow GABA$_B$-mediated IPSC (Jarolimek et al., 1994), and thus the authors proposed that different K$^+$ channels were activated. However, the present findings would appear to favor the conclusion that baclofen activates the same type of K$^+$ channels as activated by endogenously released GABA. There was a residual current activated by baclofen but not by

synaptic activation in the mutant mice. However, its presence was variable and, when present, accounted for a very small part of the total current. This residual current might be due to a GIRK3 channel subunit either alone or together with the GIRK1, which, although it is down-regulated in the mutant mice, is still present in small amounts (Signorini et al., 1997).

The resting membrane potential of CA1 pyramidal cells in the mutant mice was ∼7.9 mV depolarized compared to wild-type mice. The GIRK contribution to the resting membrane potential in wild-type mice was not due to the tonic activation of either GABA$_B$ or adenosine receptors. While it is still possible that the tonic activation of some unidentified receptor accounts for the GIRK contribution, the results suggest that there is a basal GIRK activity that occurs in the absence of receptor stimulation. This is consistent with the observation that GIRK channel activity can occur in a G protein-independent manner (Sui et al., 1996).

GIRK2$^{-/-}$ Mice Show Normal Inhibition of Leak K$^+$ Conductance by Muscarinic Receptors and Normal Presynaptic Inhibition of EPSCs As Well As IPSCs

Muscarinic receptor activation is known to block a leak K$^+$ conductance as well as the muscarine current (Madison et al., 1987; Benson et al., 1988). Given that GIRKs contribute to the resting membrane potential, we considered the possibility that muscarinic receptor activation might inhibit this tonic GIRK activity. However, the finding that concentrations of carbachol, which selectively reduce the leak K$^+$ conductance, had the same

postsynaptic   presynaptic

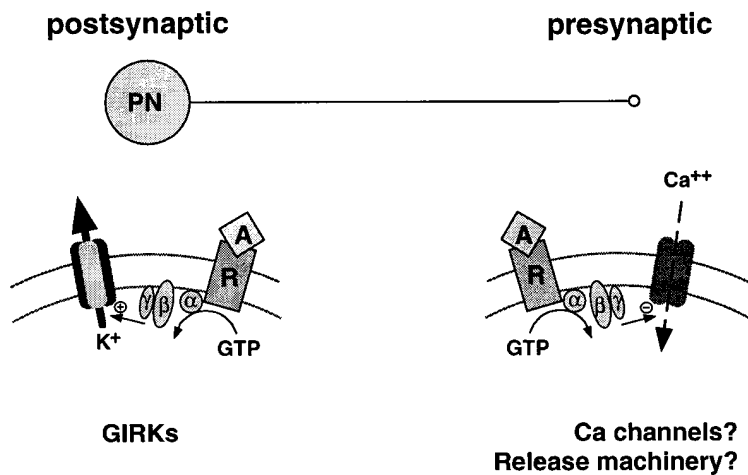**GIRKs**   **Ca channels?**
Release machinery?

Figure 7. G Protein-Coupled Receptors, in Response to the Same Agonist, Exert Their Effects through Different Mechanisms According to Their Location in the Neuron

Agonists bind to G protein-coupled receptors at two locations on a pyramidal neuron (PN). Their activation leads, in the presence of GTP, to the cleavage of the α subunit of the heterotrimeric G protein complex. At the somatodendritic membrane, the $\beta\gamma$ subunit can then activate inwardly rectifying K^+ channels, yielding an outward current and a hyperpolarization of the neuron. At the presynaptic terminal, the same agonist also activates G protein-coupled receptors, which leads to presynaptic depression by a GIRK-independent signaling mechanism, such as the inhibition of Ca^{2+} channels or an interaction with the release machinery.

action in the wild-type and mutant mice indicates that the channels closed by muscarinic receptors are distinct from those opened by G protein-coupled receptors. This is also the case for the inhibition of K^+ channels by 5-HT4 receptors (Andrade and Nicoll, 1987; Andrade and Chaput, 1991), since this action remained intact in GIRK2$^{-/-}$ mice.

The finding that virtually all G protein-coupled receptors that activate K^+ channels postsynaptically can also inhibit transmitter release presynaptically raises the possibility that GIRKs might play a role in this presynaptic inhibition. For instance, it has been reported that the K^+ channel blocker Ba^{2+} can block the GABA$_B$-mediated presynaptic inhibition (Misgeld et al., 1989; Thompson and Gähwiler, 1992; but see Lambert et al., 1991). We therefore compared the presynaptic inhibitory action of a number of G protein-coupled receptors in the two groups of mice. These included GABA$_B$, adenosine A1, NPY, and mGluRs on excitatory synapses and GABA$_B$ and μ opioid receptors on inhibitory synapses. The absence of GIRK2 had no effect on the inhibitory action of any of these receptors. In particular, while the GABA$_B$-mediated postsynaptic K^+ conductance increase in CA3 pyramidal cells was abolished in the mutant mouse, the GABA$_B$-mediated presynaptic inhibition of transmitter release from the synapses made by these CA3 cells on CA1 cells was entirely normal. This indicates that GABA$_B$ receptors on the same neuron exert mechanistically distinct forms of inhibition depending on the location of these receptors (Figure 7). The lack of GIRK involvement in presynaptic inhibition could be due to the lack of GIRKs at the synapse or to the failure of GIRK activation at the synapse to alter transmitter release. The absence of any effect of baclofen on fiber excitability favors the former hypothesis, at least for the excitatory synapses in the CA1 region, and consistent with this conclusion is the finding that immunoreactivity for GIRK1 is restricted to the soma and dendrites of hippocampal neurons (Drake et al., 1997). In either case, the results of this study suggest that the presynaptic inhibitory action of G protein-coupled receptors in the hippocampus is due to a direct inhibition of Ca^{2+} entry and/or the secretory process itself (Thompson et al., 1993; Dittman and Regehr, 1996; Takahashi et al., 1996; Wu and Saggau, 1997).

In summary, all of the receptors that are known to increase postsynaptic K^+ conductance in the hippocampus require the presence of GIRK2 subunits. On the other hand, the presynaptic inhibition mediated by these same receptors is unaltered by GIRK2 deletion. Thus, the same receptor can couple to distinct effector systems in the same neuron, depending upon the subcellular location of the receptor.

Experimental Procedures

The GIRK2$^{-/-}$ mice were obtained from GIRK2$^{-/+}$ matings (derived from 129/sv–C57BL/6 hybrid crosses). Control animals were GIRK2$^{+/+}$ littermates and C57BL/6 mice. The genotype was determined by PCR amplification of genomic DNA isolated from mouse tails. Standard hippocampal slices were prepared from 2- to 8-week-old mice. The recording chamber was superfused (1–2 ml/min) with an external solution containing (in mM) 119 NaCl, 2.5 KCl, 1.3 MgSO$_4$, 2.5 CaCl$_2$, 1.0 NaH$_2$PO$_4$, 26.2 NaHCO$_3$, and 11 glucose. All experiments were carried out at room temperature. Either extracellular field potential recording or whole-cell voltage-clamp recording was used. Field potentials were made with a glass pipette filled with 2 M NaCl. To evoke synaptic potentials, stimuli (0.1 ms duration) were delivered at 0.1 Hz through bipolar stainless steel electrodes. Whole-cell recordings were made from principal cells using a "blind" whole-cell recording technique with patch pipettes (3–5 MΩ) filled with either of two internal solutions. For recording K^+ currents or GABA$_B$-mediated IPSCs, a solution containing (in mM) 140 K-Gluconate, 5 HEPES, 2 MgCl$_2$, 1.1 EGTA, 2 MgATP, and 3 Na$_3$GTP was used. For recording monosynaptic IPSCs (Figure 6), a solution containing (in mM) 117 CsOH, 117 gluconic acid, 2.5 CsCl, 8 NaCl, 10 HEPES, 10 BAPTA, 2 MgATP, and 3 Na$_3$GTP was used. Voltage measurements were corrected for the liquid junction potential (15.6 mV) of the standard internal solution (PPT Software by F. Mendez, MPI, Göttingen, Federal Republic of Germany).

Recordings were amplified with an Axopatch 1D or Axoclamp 2B, filtered at 2 kHz, digitized at 5–10 kHz (National Instruments Board MIO-16, NI-DAQ Igor 3.01 Software, Wave Metrics), and stored on a hard disk. In CA1 field recordings, initial slopes were measured, and for mossy fiber fields the amplitude of the second response of a double stimulus (20 ms interpulse interval) was calculated. Data are expressed as means $\pm$ SEM. Drugs used were CNQX (Tocris), APV (Sigma), picrotoxin, $\pm$baclofen (RBI), adenosine (Sigma), 5-HT (Sigma), trans-(1S,3R)-ACPD (RBI), carbachol (Sigma), neuropeptide Y (RBI), DAMGO (RBI), and LCCG1 (Tocris).

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