Altered Regulation of Potassium and Calcium Channels by GABA<sub>B</sub> and Adenosine Receptors in Hippocampal Neurons From Mice Lacking Ga<sub>o</sub>

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INTRODUCTION

Many effects of neurotransmitters are mediated by heterotrimeric G proteins, composed of Ga and Gβγ subunits. Ga is the most abundant type of G protein in the brain (Huff et al. 1985; Neer et al. 1984; Sternweis and Robishaw 1984). However, the range of effects mediated by Ga is still unclear. Considering the abundance of the protein, the effects of inactivating the Ga<sub>o</sub> gene in mice are surprisingly modest. Mice lacking Ga<sub>o</sub> are viable and have no gross abnormalities of the brain, although they develop tremors and occasional seizures and have impaired motor control (Jiang et al. 1998; Valenzuela et al. 1997).

One role of G proteins is to mediate neurotransmitter control of excitability by modulating the activity of ion channels (Hille 1994). G protein activation of inwardly rectifying potassium-selective (GIRK) channels is one such pathway, mediated by direct binding of Gβγ subunits to the channels (see Clapham and Neer 1997). A second well-studied case of G protein-mediated modulation is transmitter inhibition of voltage-dependent Ca<sup>2+</sup> channels, which can proceed by multiple pathways (Beech et al. 1992; Diverse-Pierluissi and Dunlap 1993; Diverse-Pierluissi et al. 1995; Dolphin 1995; Shapiro and Hille 1993). One pathway prominent in many neurons is rapid, membrane-delimited inhibition mediated by direct binding of Gβγ subunits to Ca<sup>2+</sup> channels (De Waard et al. 1997; Herlitze et al. 1996; Ikeda 1996; Zamponi et al. 1997). Although Gβγ subunits directly control GIRK channels and Ca<sup>2+</sup> channels, the type of the Ga subunit from which the Gβγ is released can determine specificity and kinetics of transmitter action. In addition, there is evidence for more direct involvement of Ga subunits in at least some pathways (Furukawa et al. 1998a,b).

Based on sensitivity to pertussis toxin (PTX), all transmitters known to activate GIRK channels appear to act through Gβγ released from members of the G<sub>i</sub>/G<sub>o</sub> family, as do most, but not all, transmitters that inhibit Ca<sup>2+</sup> channels by rapid, membrane-delimited action (Dolphin 1995; Hille 1994; Wickman and Clapham 1995; Zhu and Ikeda 1994). For Ca<sup>2+</sup> channel inhibition, G<sub>i</sub> has been specifically implicated by studies in a variety of neurons and neuroendocrine cells using antibodies or antisense oligonucleotides against specific G protein subunits (Campbell et al. 1993; Caulfield et al. 1994; Ewald et al. 1988; Hescheler et al. 1987; Kleuss et al. 1991; Lledo et al. 1992; Moises et al. 1994; Taussig et al. 1992).

Mice lacking both isoforms of Ga<sub>o</sub> present a useful model to study the role of Ga<sub>o</sub> in G protein-mediated ion channel modulation. Several alterations of channel modulation in Ga<sub>o</sub><sup>−/−</sup> mice are already known. In cardiac myocytes, acetylcholine activation of GIRK channels appears normal but there is disruption of a pathway by which muscarinic acetylcholine receptors regulate L-type Ca<sup>2+</sup> channels (Valenzuela et al. 1997). In neurons, a recent study found that inhibition by opioid receptors of Ca<sup>2+</sup> channel in sensory neurons was diminished in Ga<sub>o</sub><sup>−/−</sup> mice (Jiang et al. 1998).

To further study the role of Ga<sub>o</sub> in control of neuronal GIRK channels and Ca<sup>2+</sup> channels, we examined transmitter modulation in hippocampal CA3 neurons where a number of trans-
mitters can both inhibit $\text{Ca}^{2+}$ channels and activate GIRK channels. We find that modulation of $\text{Ca}^{2+}$ channels and GIRK channels by GABA$_\text{A}$ and adenosine receptor agonists is unchanged in magnitude in neurons from $\text{G}o_{\text{o}}^{-/-}$ mice but that kinetics of transmitter modulation are dramatically different compared with neurons from wild-type mice.

**METHODS**

**Cell preparation**

$\text{G}o_{\text{o}}^{-/-}$, $\text{G}o_{\text{o}}^{+/+}$, and $\text{G}o_{\text{o}}^{+/+}$ mice were obtained from +/- matings as described by Valenzuela et al. (1997). Hippocampal CA3 pyramidal neurons were isolated enzymatically from the brains of mice (aged 9–15 d). Mice were anesthetized with methoxyflurane before decapitation and hippocampi were dissected out in oxygenated ice-cold dissociation solution containing (in mM) $82 \text{Na}_2\text{SO}_4$, 30 K$_2\text{SO}_4$, 5 MgCl$_2$, 10 HEPES, 10 glucose, and 0.01% phenol red indicator (pH 7.4, adjusted with NaOH) and cut into 400 $\mu$m thick slices. After incubation with 3 mg/ml protease XXIII in dissociation solution (37°C for 9 min), the enzyme solution was replaced with dissociation solution containing 1 mg/ml trypsin inhibitor and 1 mg/ml bovine serum albumin and the slices were stored at room temperature with oxygen blown over the surface of the fluid. As cells were needed, the CA3 region was dissected out of individual slices and triturated mechanically with fire-polished glass pipettes to liberate individual cells. Neurons were allowed to settle in the recording chamber for a few minutes and were superfused with Tyrode’s solution containing (in mM) 150 NaCl, 4 KCl, 2 CaCl$_2$, 2 MgCl$_2$, 10 glucose, and 10 HEPES (pH 7.4 with NaOH). CA3 pyramidal neurons were identified morphologically (Sodickson and Bean 1996).

**Electrophysiological methods**

All recordings were done at room temperature using whole cell voltage clamp techniques (Hamill et al. 1981). Patch pipettes were pulled from 100 $\mu$l Boralex micropipettes (Dynalab, Rochester, NY). Pipette resistance ranged 2–6 M$\Omega$ when pipettes were filled with internal solution. Pipette capacitance was reduced by wrapping the tip with thin strips of Parafilm. After the whole cell configuration was obtained in Tyrode’s solution, the cell was lifted from the bottom of the chamber and control and agonist-containing external solutions were applied from different reservoirs through gravity-driven micro-capillary perfusion pipes (internal diameter of 250 $\mu$m), positioned directly in front of the cell. Solutions were exchanged (<1 s) by moving the pipes. Baclofen, 2-chloro-adenosine, and somatostatin were from RBI (Natick, MA) and N-ethyl-maleimide (NEM) was from Sigma. Agonists were stored as concentrated aliquots at −70°C and a fresh aliquot was diluted into external recording solution on the day of the experiment.

**Data acquisition and analysis**

Currents were recorded with an Axopatch 200 amplifier, filtered at 2 kHz, digitized at 20–50 kHz, and stored on a computer using a Digidata interface and pClamp6 software (Axon Instruments, Foster City, CA). Series resistance (~2–2.5 times higher than the pipette resistance) was compensated by 70–90%. Membrane potentials were corrected for liquid junction potential.

**Solutions and voltage protocol**

We used ionic solutions and a voltage protocol that allowed simultaneous recording of $\text{Ca}^{2+}$ and inwardly rectifying K$^+$ currents. The internal solution contained (in mM) 189 Cs$_2\text{HPO}_4$, 9 CsCl, 9 HEPES, 9 EGTA, 14 Tris-creatineP$_{0.3}$, 4 Mg-ATP, and 0.3 Tris-GTP (pH 7.4 with CsOH). Tris-creatineP$_{0.3}$, Mg-ATP, and Tris-GTP were added freshly each day to pipette solutions from aliquots stored at −70°C. External recording solution consisted of modified Tyrode’s solution with 16 mM KCl, with KCl substituted for an equimolar amount of NaCl. 1 $\mu$M Tetrodotoxin (TTX) was included in the external solutions to block sodium currents. With these solutions, transmitter-activated inwardly rectifying K$^+$ current (carried by 16 mM [K$^{+}]_o$) could be recorded as a transmitter-sensitive inward current at voltages negative to −60 mV, whereas voltage-activated calcium current could be recorded in response to a depolarizing voltage steps to −12 mV (where outward K$^+$ current is blocked by the internal Cs$^+$).

To determine the time course of transmitter action, the voltage protocol was delivered every 2 s. Time-course plots show current measured at the end of the steps to −142 and −12 mV. Kinetics of K$^+$ currents were analyzed only for cells in which there was a stable baseline before and after application of transmitters and where transmitter-activated current was ≥70 pA.

In some experiments, Ba$^{2+}$ currents through Ca$^{2+}$ channels were measured. These experiments used an external solution containing (in mM) 2 BaCl$_2$, 160 NaCl, and 10 HEPES (pH 7.4 with NaOH), along with the standard Cs-based intracellular solution. Data are expressed as mean ± SE.

**RESULTS**

**Simultaneous recording of baclofen effects on Ca$^{2+}$ and K$^+$ currents**

We wished to examine modulation of both voltage-dependent Ca$^{2+}$ currents and GIRK currents, both of which are modulated by a variety of transmitters in hippocampal CA3 neurons. Typically, these currents are examined in isolation using appropriate ionic substitutions (for example, blocking both inward and outward K$^+$ currents when recording Ca$^{2+}$ channel currents). To facilitate efficient collection of data from the minimal necessary number of animals, we used ionic solutions and a voltage protocol that allowed simultaneous recording of Ca$^{2+}$ and K$^+$ currents. The internal solution used Cs$^+$ as the main cation, and the external solution was Tyrode’s solution with increased (16 mM) KCl and containing 1 $\mu$M tetrodotoxin (TTX) to block sodium currents.

With these solutions, transmitter-activated inwardly rectifying K$^+$ current (carried by 16 mM [K$^{+}]_o$) can be recorded as a transmitter-sensitive inward current at voltages negative to −60 mV, because the internal Cs$^+$ eliminates outward K$^+$ currents but not inward K$^+$ currents. Voltage-activated calcium current can be recorded in response to depolarizing voltage steps positive to −50 mV or so, and the calcium currents are well-isolated because outward K$^+$ current that would otherwise overlap with them is eliminated by the internal Cs$^+$. Currents were thus recorded using a voltage protocol consisting of a strongly hyperpolarizing step (to −142 mV for 5 ms) and a depolarizing step to −12 mV (10 ms), which gave maximal Ca$^{2+}$ current. The currents elicited by this protocol, and the effects of baclofen on them, are shown in Fig. IA in a recording from a neuron from a wild-type animal. The voltage steps were delivered from a steady holding potential of −92 mV. The hyperpolarizing step was given first because there was little time dependence of the current before or after this step, whereas the calcium current during the depolarizing step was followed by a slowly-decaying tail current (mainly from inward K$^+$ current through voltage-activated K$^+$ channels opened during the depolarization). The experiments shown in Fig. 1, B and C, tested the ability of this protocol to separate the
The step is too short to activateelicits increased inward current with little time-dependence

\[ \text{BaCl}_2 \].

inward and outward current through GABAB-activated potas-

The inward current elicited by the depolarizing step is reduced

by baclofen, as expected for calcium channel inhibition. In the

experiment shown in Fig. 1

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FIG. 1. Simultaneous monitoring of baclofen modulation of inwardly rec-
tifying K⁺ current and voltage-activated Ca²⁺ current. Illustrated voltage
protocol was applied every 2 s. Dashed lines: zero current. A: currents before
and 4 s after application of 100 uM baclofen. B: currents with and without
baclofen (in the same cell as in A) but in external solution containing 300 µM
BaCl₂. C: currents with and without baclofen recorded in external solution in
which 2 mM CoCl₂ replaced 2 mM CaCl₂. Same cell as in A and B.

effects of baclofen on GIRK current and Ca²⁺ current in a single application of transmitter. Resting current is inward at the holding potential of −92 mV, and the step to −142 mV elicits increased inward current with little time-dependence (the step is too short to activate \( I_h \), if present). Baclofen increases the inward current at both −92 mV and −142 mV, as expected for activation of GIRK channels. In the absence of baclofen, the step to −12 mV activates a time-dependent inward current that appears fully activated at the end of the 10-ms step, consistent with a voltage-activated Ca²⁺ current. The inward current elicited by the depolarizing step is reduced by baclofen, as expected for calcium channel inhibition. In the experiment shown in Fig. 1B, the effect of baclofen was tested in the presence of 300 µM external barium, which blocks both inward and outward current through GABA_B-activated potassium channels in rat neurons (Sodickson and Bean 1996). With GIRK current blocked, baclofen had no effect on the currents at −92 mV and −142 mV but baclofen still effectively inhibited the depolarization-activated inward current. To test whether the current activated by depolarization to −12 mV was purely Ca²⁺ current, we repeated the baclofen application in an external solution in which 2 mM Co²⁺ replaced the 2 mM Ca²⁺ present in normal external solution. In Co²⁺-containing solution, the current at −12 mV was near zero both in the

absence and presence of baclofen. Thus in control conditions the current at −12 mV appears to be almost purely voltage-activated Ca²⁺ current, and baclofen does not activate or influence any other current at this voltage.

The separation of baclofen effects on GIRK current and voltage-activated Ca²⁺ current by the different voltage-sensitivity of the currents is illustrated in Fig. 2, showing the current-voltage relationship (determined with 5-ms steps from −92 mV) in the absence and presence of baclofen. Baclofen induces inward current negative to −60 mV, and this baclofen-activated current is completely blocked by 300 µM external barium (Fig. 2A). Baclofen reduces inward current activated by steps positive to −50 mV, and both this current and the effect of baclofen are blocked by substitution of Co²⁺ for Ca²⁺ (Fig. 2B). Voltage-activated Ca²⁺ current is maximal at −12 mV, at which voltage the leak current remaining in 2 mM Co²⁺ is near zero. Thus inward current at −12 mV in Ca²⁺-containing solution provides an accurate measurement of Ca²⁺ current without requiring significant correction for leak current. In each cell studied, we first performed the voltage protocol with the 2 mM Co²⁺-containing solution before testing transmitters in the 2 mM Ca²⁺ solution, and the Ca²⁺ current at −12 mV was corrected for any current (always small) remaining in Co²⁺-containing solution.

The ability of 300 µM external barium to block the current activated by baclofen negative to −50 mV is consistent with this being an inwardly rectifying potassium current. To test further the identity of this current, we characterized its reversal

FIG. 2. Current voltage relationships for baclofen (100 µM) application for various ionic conditions. Pulses (5 ms) were applied every 100 ms from a steady holding potential of −90 mV. Current at end of 5-ms pulse is plotted vs. membrane potential. (Different cell than in Fig. 1.) A: 300 µM Ba²⁺ blocks baclofen activation of inward current negative to −60 mV but has no effect on inhibition of inward current positive to −50 mV. B: substitution of Co²⁺ for Ca²⁺ blocks inward current elicited by depolarization positive to −50 mV. Net current is near zero at −12 mV and baclofen has no effect on current at this voltage.
potential and current-voltage relationship in experiments using intracellular solution containing K\(^+\) in place of Cs\(^+\) ions (Fig. 3). The current reversed at \(-62\) mV, near the calculated equilibrium potential for potassium (\(-58\) mV) and had an inwardly rectifying current-voltage relationship. It appeared identical in all respects to the baclofen-activated GIRK current previously characterized in rat hippocampal neurons using the same solutions (Sodickson and Bean 1996).

### Modulation of Ca\(^{2+}\) and K\(^+\) currents in hippocampal CA3 neurons

We compared Ca\(^{2+}\) and K\(^+\) current modulation by 2-chloro-adenosine and the GABA\(_B\) receptor agonist baclofen in freshly dissociated pyramidal CA3 neurons of hippocampi from G\(_{\alpha_o}^{-/-}\) mice and from G\(_{\alpha_o}^{+/+}\) littersmates. The genotypes of the animals were confirmed after the experiment by PCR.

In CA3 neurons from G\(_{\alpha_o}^{+/+}\) mice, baclofen reliably activated inwardly rectifying K\(^+\) currents and inhibited Ca\(^{2+}\) currents (Fig. 4A). Both baseline currents and effects of baclofen were similar in cells from G\(_{\alpha_o}^{-/-}\) mice (Fig. 4B).

Baclofen inhibition of Ca\(^{2+}\) current was identical in magnitude (25 \pm 1\%; \(n = 34\)) in cells from G\(_{\alpha_o}^{-/-}\) mice as in cells from G\(_{\alpha_o}^{+/+}\) mice (25 \pm 1\%; \(n = 30\)) (Fig. 4E). Baclofen activation of inwardly rectifying K\(^+\) currents was also little different: baclofen-activated current (measured at \(-142\) mV) was 6.6 \pm 0.5 pA/pF (\(n = 34\)) in cells from G\(_{\alpha_o}^{-/-}\) mice compared with 7.9 \pm 0.7 pA/pF (\(n = 30\)) in cells from G\(_{\alpha_o}^{+/+}\) mice (Fig. 4F).

The adenosine receptor agonist 2-chloro-adenosine also reliably activated inwardly rectifying K\(^+\) currents and inhibited Ca\(^{2+}\) currents in CA3 neurons (Fig. 4C). 2-Chloro-adenosine was just as effective in cells from G\(_{\alpha_o}^{-/-}\) mice as in those from G\(_{\alpha_o}^{+/+}\) mice (Fig. 4D–F). We also tested somatostatin (5 cells from G\(_{\alpha_o}^{-/-}\) mice and 8 cells from G\(_{\alpha_o}^{+/+}\) mice) and the metabotropic glutamate receptor agonist JRT32ACDP (3 cells from G\(_{\alpha_o}^{-/-}\) mice and 5 cells from G\(_{\alpha_o}^{+/+}\) mice). Although these series of experiments were less systematic, there was no obvious difference in the size of effects on cells from G\(_{\alpha_o}^{+/+}\) and G\(_{\alpha_o}^{-/-}\) mice (data not shown). The voltage-dependence of

![Fig. 3](http://jn.physiology.org/)

**Fig. 3.** Inwardly rectifying characteristic of baclofen-activated current. Recording was done with standard external solution containing 16 mM K\(^+\) and with a K\(^+\)-based internal solution containing (in mM) 189 K\(_2\)HPO\(_4\), 9 KCl, 9 HEPES, 9 EGTA, 14 Tris-creatinePO\(_4\), 4 Mg-ATP, and 0.3 Tris-GTP (pH 7.4 with KOH). A: current–voltage relationship was determined with a ramp voltage command (\(-162\) to \(+58\) mV in 500 ms) and repeated every second. Traces were signal-averaged from 2 such ramps in control and 4 in the presence of 100 uM baclofen. B: baclofen-sensitive current obtained by subtracting the traces in A.

![Fig. 4](http://jn.physiology.org/)

**Fig. 4.** Effects of baclofen (A and B) and 2-chloroadenosine (C and D) on ionic currents in hippocampal pyramidal neurons from G\(_{\alpha_o}^{+/+}\) (A and C) and G\(_{\alpha_o}^{-/-}\) (B and D) mice. A and B: baclofen inhibition of Ca\(^{2+}\) current (carried by 2 mM Ca\(^{2+}\) and elicited by a step to \(-12\) mV) and activation of inwardly rectifying K\(^+\) current (carried by 16 mM K\(^+\) and measured at \(-142\) mV). Solutions and protocols as in Fig. 1. Thin trace: control. Bold trace: 50 uM baclofen. C and D: response to 25 mM 2-chloroadenosine (same cells as A and B). E: collected results for inhibition of Ca\(^{2+}\) current by baclofen and 2-chloroadenosine from cells of G\(_{\alpha_o}^{+/+}\) mice (black bar) and G\(_{\alpha_o}^{-/-}\) mice (gray bar). Baclofen: 30 cells for G\(_{\alpha_o}^{+/+}\) and 34 cells for G\(_{\alpha_o}^{-/-}\). 2-Chloroadenosine: \(n = 23\) cells for G\(_{\alpha_o}^{+/+}\) and 23 cells for G\(_{\alpha_o}^{-/-}\). F: mean agonist-activated K\(^+\) current (normalized relative to cell capacitance) elicited by baclofen and 2-chloro-adenosine in cells from G\(_{\alpha_o}^{+/+}\) (black bar) and G\(_{\alpha_o}^{-/-}\) (gray bar) mice. Same cells as E.
control Ca$^{2+}$ currents and the voltage-dependence of inhibition by baclofen or 2-chloro-adenosine (which was most prominent for moderate depolarizations) were also not distinguishable between cells from $\alpha_o^{-/-}$ and $\alpha_o^{+/+}$ mice (data not shown).

**Kinetics of $K^+$ and Ca$^{2+}$ current modulation by G protein-coupled receptors**

Although the magnitude of modulation was not different between neurons from $\alpha_o^{-/-}$ and $\alpha_o^{+/+}$ mice, the kinetics of the action of agonists were different, especially the time course of recovery. Figure 5 shows the time course of action of baclofen on $K^+$ and Ca$^{2+}$ currents in $\alpha_o^{-/-}$ and $\alpha_o^{+/+}$ mice, from recordings like those in Fig. 2. In cells from $\alpha_o^{+/+}$ mice, $K^+$ current was activated rapidly on exposure to agonist and it also deactivated rapidly on return to agonist-free solution. Both activation and deactivation of $K^+$ current by baclofen were too fast to be clearly resolved with the 2-s sampling period of the experiments (Fig. 5A). Interestingly, the kinetics of agonist action on Ca$^{2+}$ current were somewhat different from agonist activation of $K^+$ current in cells from $\alpha_o^{+/+}$ mice, even when determined simultaneously during the same application of agonist. Both onset of inhibition of Ca$^{2+}$ current and recovery from inhibition were biphasic, with a predominant, rapid ($\leq 2$ s) phase followed by a slower phase.

In comparing cells from $\alpha_o^{+/+}$ mice and $\alpha_o^{-/-}$ mice, we focused on recovery kinetics, which were dramatically different. In $\alpha_o^{+/+}$ mice, recovery from Ca$^{2+}$ current inhibition by baclofen consisted of an initial fast phase with a time constant of 2 s, where 70–90% of effect recovered, and a remaining small component with a time constant of $\sim 5$–20 s. The fast phase was poorly resolved with the 2 s sampling interval, but the magnitude ($22 \pm 4\%$) and time constant ($7.0 \pm 0.7$ s, $n = 15$) of the slower phase could be resolved reasonably well.

In cells from $\alpha_o^{-/-}$ mice, recovery from agonist was much slower than in $\alpha_o^{+/+}$ mice (Fig. 5B). In $\alpha_o^{-/-}$ mice, recovery from activation of $K^+$ current by baclofen was slow enough to be resolved and could be fit well by a single exponential, with an average time constant of $7.1 \pm 1.0$ s ($n = 12$). This is at least four- to fivefold slower than the recovery from activation of $K^+$ current in cells from normal mice (complete in $< 2$ s). Recovery from baclofen effects on Ca$^{2+}$ current was also slower in cells from $\alpha_o^{-/-}$ mice. In $\alpha_o^{-/-}$ mice, the time course of recovery from Ca$^{2+}$ current inhibition was sigmoidal, with a delay in recovery when the cell was removed from baclofen. This was followed by a main phase of recovery that was fit well with a single exponential. This had an average time constant of $14.5 \pm 1.1$ s ($n = 23$). It was notable that recovery from the effects of baclofen on Ca$^{2+}$ current was slower than decay of $K^+$ current, even when monitored simultaneously in the same cell (Fig. 5B).

The length of agonist exposure had no obvious effect on recovery kinetics. If cells were continuously exposed for 1 min to 50 $\mu$M baclofen, Ca$^{2+}$ currents recovered from inhibition with a similar time constant as for short exposure in the same cell (for cells from both $\alpha_o^{+/+}$ and $\alpha_o^{-/-}$ mice; data not shown).

Recovery from exposure to 2-chloro-adenosine was also slower in cells from $\alpha_o^{-/-}$ mice. Figure 6 shows typical examples of the kinetics of action of 2-chloro-adenosine in cells from $\alpha_o^{+/+}$ (Fig. 6A) and $\alpha_o^{-/-}$ (Fig. 6B) mice.
6A) and Gαo−/− (Fig. 6B) mice. In cells from Gαo−/− mice, recovery from 2-chloro-adenosine was rapid. Effects of 2-chloro-adenosine on K+ current recovered too fast to be resolved with the 2-s sampling interval, as for baclofen effects in wild-type cells. 2-Chloro-adenosine effects on Ca2+ currents in wild-type cells recovered more slowly than the effects on K+ currents but (unlike those of baclofen) could generally be fit well by a single exponential (Fig. 6A). The average time constant was 2.6 ± 0.2 s (n = 12). In cells from Gαo−/−, recovery from 2-chloro-adenosine effects was much slower than in Gαo+/+ mice (Fig. 6B). Recovery from activation of K+ current by 2-chloro-adenosine in Gαo−/− mice could be fit well by a single exponential, with an average time constant of 7.9 ± 0.9 s (n = 12). Recovery from 2-chloro-adenosine effects on Ca2+ current was sigmoidal, with an initial delay followed by a main phase of recovery that was fit well with a single exponential (Fig. 6B). The main phase had an average time constant of 12.0 ± 0.8 s (n = 16). As for the effects of baclofen on cells from Gαo−/− mice, recovery from effects on Ca2+ current was slower than decay of agonist-activated K+ current when monitored in the same cell (Fig. 6B).

The much slower recovery rates from agonist effects in cells from Gαo−/− animals compared with Gαo+/+ littermates were also seen in less systematic experiments with somatostatin and ADPA, both of which exhibited biphasic responses (Fig. 7). Effects of NEM on K+ and Ca2+ current modulation by 2-chloro-adenosine in hippocampal neurons from Gαo+/+ and Gαo−/− mice. Protocols as in Fig. 6. Gray bars: exposure to 25 μM 2-chloro-adenosine.

NEM-sensitivity of K+ and Ca2+ current modulation in Gαo−/− and Gαo+/+ mice

The sulfhydryl alkylating agent N-ethyl-maleimide (NEM) disrupts some but not other G protein-mediated transmitter pathways when applied briefly to the outside of the cell. Among the different pathways of Ca2+ current inhibition in sympathetic neurons, NEM effects appear to be selective for pathways that are also sensitive to pertussis toxin (Jeong and Ikeda 1998; Shapiro et al. 1994; Wollmuth et al. 1995). We attempted to study the pertussis toxin sensitivity of transmitter effects in CA3 neurons, but the cells deteriorated during overnight exposure to pertussis toxin. Thus short-term NEM exposure, which can be performed quickly on freshly dissociated cells, was a useful tool for distinguishing various pathways. In rat CA3 neurons, brief (1 min) exposure to 50 μM NEM completely blocks activation of inwardly rectifying K+ current by baclofen (Sodickson and Bean 1996), suggesting that this response is mediated by G1 or G2.

Figure 7A shows the effect of NEM exposure on baclofen effects in a CA3 neuron from a Gαo+/+ mouse. Both baclofen activation of K+ current and inhibition of Ca2+ current were completely disrupted by NEM, which had no effect on basal currents. However, in neurons from Gαo−/− mice, NEM had a
mice, Gi is the predominant G protein used for coupling both a2-chloro-adenosine in both Ga and Gb channels in wild-type mice and is substituted mainly by Gαi in Gαo/1−/− mice, it is not obvious why the kinetics are slower, because the intrinsic rates of GTPase activity of Gα11, Gα12, and Gα13 are all similar to that of Gαo (Linder et al. 1990). However, the intrinsic GTPase rate for purified Gαo subunits is only about 2 min−1 (Kurachi 1995; Linder et al. 1990), an order of magnitude slower than the recovery from effects of transmitters on either GIRK channels or Ca2+ channels (0.5–2 s−1). Recently, a family of regulators of G protein signaling (RGS) proteins have been discovered which influence G protein signaling by speeding up GTPase activity of various Gα subunits (Watson et al. 1996; Zaranger and Jan 1998). RGS proteins appear to be widely distributed in the brain (Gold et al. 1997), and have been shown to speed kinetics of K+ and Ca2+ channel modulation in heterologous expression systems (Doupnik et al. 1997; Jeong and Ikeda 1998; Melliti et al. 1999; Saitoh et al. 1997). A candidate RGS protein for the responses we studied is RGS8, which is expressed in neural tissue (Gold et al. 1997) and binds to both Gαi and Gαi3 subunits (Saitoh et al. 1997). One possibility is that the relevant RGS proteins in CA3 neurons are less efficacious on Gi isoforms that substitute for Gαo in the Gαi/o−/− mice.

**Kinetics of recovery of I_K versus I_Ca**

In Gαi/o−/− mice, kinetics of recovery from agonist effects on both GIRK current and Ca2+ current were slow enough to be well-resolved. It was striking that, when monitored simultaneously in the same cell, recovery of effects on Ca2+ current were slower (by a factor of 2–4) than recovery of GIRK current. Both effects are believed to be mediated by direct binding of βγ subunits to the channels. Why then do the kinetics differ? One possibility is that the difference in recovery kinetics reflects different stoichiometry of βγ binding to GIRK channels and calcium channels. Activation of GIRK channels appears to require binding of multiple βγ subunits (Ito et al. 1992; Krapivinsky et al. 1995), whereas binding of a single βγ subunit might be enough to inhibit a calcium channel (Zamponi and Snutch 1998). If so, activation of GIRK channels would be expected to have a steeper dependence on the concentration of free βγ subunits. As the concentration of free βγ subunits declines after removal of agonist, significant inhibition of calcium channels might remain after the concentration is too low for activation of GIRK channels.

**Specificity and plasticity of G proteins**

Overall, our results in hippocampal CA3 neurons are consistent with previous evidence from other neurons and cell lines suggesting that effects of many neurotransmitters on Ca2+ channels are mediated by Gi. However, the results also show that other G proteins are capable of mediating inhibition with equal efficacy. This implies that there is no special need for the predominant role of the G protein in controlling Ca2+ channel modulation in CA3 neurons.

![FIG. 9. Collected results for effects of NEM on Ca2+ current inhibition (top) and K+ current activation (bottom) by baclofen (left) and 2-chloro-adenosine (right) in Gαo+/+ and Gαo−/− mice. Black bars: before NEM. Gray bars: after NEM exposure for 1 min. Baclofen: n = 3 cells from Gαo+/+ and n = 9 cells from Gαo−/−. 2-Chloro-adenosine: n = 3 cells from Gαo+/+ and n = 7 cells from Gαo−/−.](image)
for G\textsubscript{o} in the coupling pathways. It may be that G\textsubscript{o} predominates in normal mice not because of any intrinsic selectivity for G\textsubscript{o} in the pathways but simply as a result of a large excess of G\textsubscript{o} compared with other G proteins.

Perhaps the most surprising result from our experiments is the lack of change in the magnitude of modulation of Ca\textsuperscript{2+} channels and GIRK channels, despite the likelihood that G\textsubscript{o} normally mediates modulation. An obvious possibility is that with loss of G\textsubscript{i}, there is compensatory up-regulation of expression of other G proteins. However, at least at the level of the whole brain, there is little or no change in expression of the G\textsubscript{i}/G\textsubscript{o} family proteins decreases, the local concentration of G proteins near the effector channels may not change. Perhaps the concentration of G proteins used for signaling to ion channels is tightly regulated in small subcellular areas comprising clusters of receptors, G proteins, and ion channels (and probably RGS proteins). Comparing the speed of modulation with two-dimensional diffusion, it can be calculated that receptors, G proteins, and channels are within at least 1 μ of one another (Hille 1992; Zhou et al. 1997), suggesting the existence of mechanisms by which location and stoichiometry of these proteins are coordinated. In fact, the elements of the G-protein-coupled cascade mediates phototransduction in Drosophila are known to be colocalized by means of a protein with five PDZ domains (Tsunoda et al. 1997). If an analogous mechanism exists for regulation of Ca\textsuperscript{2+} channels and K\textsuperscript{+} channels by G protein-coupled receptors, the level of expression of these proteins is probably not the limiting factor in formation of such complexes, because most G proteins are present in large excess over receptors or effectors. Thus it may be reasonable that a large reduction in total G protein expression could result in little change in local concentration near receptors and channels. Interestingly, adenylate cyclase activity is also unchanged by the dramatic reduction in G\textsubscript{i}/G\textsubscript{o} expression in G\textsubscript{o}\textsubscript{bg}−/− mice, also suggesting compartmentalization or the existence of local membrane pools (Mende et al. 1998).

Consequences for brain function

The slowed kinetics of recovery from agonist effects in G\textsubscript{o}\textsubscript{bg}−/− mice could contribute to changes in neurogenic function. It seems likely that GABA\textsubscript{M}-mediated inhibitory synaptic currents might also decay more slowly in G\textsubscript{o}\textsubscript{bg}−/− mice, because synaptic currents measured in brain slices (Otis et al. 1993) have similar kinetics as GIRK currents in isolated cells (Sodickson and Bean 1996). GABA\textsubscript{B} receptors can also act presynaptically to inhibit transmission (e.g., Dittman and Regehr 1997; Thompson and Göhwyler 1992), with kinetics very similar to that of inhibition of Ca\textsuperscript{2+} channels (Dittman and Regehr 1997; Pfrieger et al. 1994). Thus presynaptic effects of GABA are also likely prolonged in G\textsubscript{o}\textsubscript{bg}−/− mice. Prolonging GABA\textsubscript{B} effects at both presynaptic and postsynaptic sites may change the dynamic control of synaptic strength at many synapses and contribute to the neurological changes seen in G\textsubscript{o}\textsubscript{bg}−/− mice. Other changes, including subtle changes in development, are also possible.

This work was supported by National Institutes of Health Grants HL-35034 to B. P. Bean and HL-52320 and GM-36359 to E. J. Neer.

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Received 16 August 1999; accepted in final form 19 October 1999.

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