Functional Specificity of Gα_q and Gα_{11} in the Cholinergic and Glutamatergic Modulation of Potassium Currents and Excitability in Hippocampal Neurons

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In hippocampal and other cortical neurons, action potentials are followed by a slow afterhyperpolarization (sAHP) generated by the activation of small-conductance Ca^{2+}-activated K$^+$ channels and controlling spike frequency adaptation. The corresponding current, the apamin-insensitive sI_{AHP}, is a well known target of modulation by different neurotransmitters, including acetylcholine (via M$_3$ receptors) and glutamate (via metabotropic glutamate receptor 5, mGluR$_5$), in CA1 pyramidal neurons. The actions of muscarinic and mGluR agonists on sI_{AHP} involve the activation of pertussis toxin-insensitive G-proteins. However, the pharmacological tools available so far did not permit the identification of the specific G-protein subtypes transducing the effects of M$_3$ and mGluR$_5$ on sI_{AHP}. In the present study, we used mice deficient in the Gα_q and Gα_{11} genes to investigate the specific role of these G-protein α subunits in the cholinergic and glutamatergic modulation of sI_{AHP} in CA1 neurons. In mice lacking Gα_q, the effects of muscarinic and glutamatergic agonists on sI_{AHP} were nearly abolished, whereas β-adrenergic agonists acting via Gα_s were still fully effective. Modulation of sI_{AHP} by any of these agonists was instead unchanged in mice lacking Gα_{11}. The additional depolarizing effects of muscarinic and glutamatergic agonists on CA1 neurons were preserved in mice lacking Gα_q or Gα_{11}. Thus, Gα_q, but not Gα_{11}, mediates specifically the action of cholinergic and glutamatergic agonists on sI_{AHP}, without affecting the modulation of other currents. These results provide to our knowledge one of the first examples of the functional specificity of Gα_q and Gα_{11} in central neurons.

Key words: G-protein; muscarinic; metabotropic glutamate; calcium-activated potassium current; afterhyperpolarization; CA1 pyramidal neurons

In the hippocampus, glutamatergic and cholinergic regulation of neuronal excitability is thought to play a pivotal role in learning and memory processes (Pin and Bockaert, 1995; Riedel, 1996; Segal and Auerbach, 1997; Holscher et al., 1999; Perry et al., 1999). Acetylcholine and glutamate, beside mediating fast excitatory synaptic transmission in the CNS, modulate neuronal metabolic responses by acting on metabotropic receptors (muscarinic and metabotropic glutamate receptors, mGluRs). Previous studies have focussed on the molecular and cellular mechanisms by which muscarinic and mGluR agonists regulate membrane excitability in central and peripheral neurons and have revealed their effects on several ionic currents, such as the following: (1) the M current (I_M), a voltage-dependent K$^+$ current (Brown and Adams, 1980; Halliwell and Adams, 1982); (2) a voltage-independent "leak" K$^+$ current [$I_{K(leak)}$] (Madison et al., 1987; McCormick and von Krosigk, 1992; Guerineau et al., 1994); (3) the slowly inactivating K$^+$ current I_D (Wu and Barish, 1999); (4) the delayed rectifier K$^+$ current I_K (Zhang et al., 1992); (5) the unspecific cationic pacemaker current (I_h) (Colino and Halliwell, 1993); (6) a Ca^{2+}-dependent, cation-nonspecific current (Crepel et al., 1994; Greene et al., 1994; Guerineau et al., 1995; Haj-Dahmane and Andrade, 1997, 1999); and (7) the Ca^{2+}-activated K$^+$ current responsible for the slow afterhyperpolarization (sI_{AHP}) (Benardo and Prince, 1982; Cole and Nicoll, 1983, 1984; Charpak et al., 1990).

The signal transduction pathways mediating these muscarinic and mGluR effects are only partly understood in hippocampal as in other central neurons. In particular, the involvement and molecular identification of G-proteins has been carefully investigated in some peripheral (Hille, 1994; Brown et al., 1997), but not in central neurons. Pertussis toxin-insensitive G-proteins have been proposed to be involved in the suppression of I_M, sI_{AHP}, and $I_{K(leak)}$ by muscarinic agonists in CA1 neurons (Dutar and Nicoll, 1988). However, the lack of selective pharmacological tools and the difficulty of successfully applying antisense or antibody techniques, as in dissociated or cultured cells (McFadzean et al., 1994; Ikeda, 1997; Buckley et al., 2000), has so far prevented the identification of specific G-protein subtypes mediating cholinergic and glutamatergic actions in central neurons *in situ*. Their identification is important, because the diversity of G-protein forms is likely to be matched by a corresponding range of cellular targets and functions.

The high structural homology, overlapping distribution pat-

terns, and similar coupling properties to downstream effectors of $G\alpha_q$ and $G\alpha_{11}$ subunits (Wu et al., 1992; Offermanns et al., 1994; Exton, 1996; Offermanns, 1999) have raised the question of their functional specificity in physiological systems. In this study, we unequivocally identified the G-protein subtypes that selectively transduce the signals of muscarinic M_3 and glutamatergic $mGluR_5$ receptors on sI_{AHP} in CA1 pyramidal neurons in mouse hippocampal slices. Using knock-out mice lacking $G\alpha_q$ or $G\alpha_{11}$ subunits, we showed that $G\alpha_q$, but not $G\alpha_{11}$, mediates the down-regulation of sI_{AHP} by both muscarinic and mGluR agonists. Neither $G\alpha_q$ nor $G\alpha_{11}$ seem instead to be required for the slow depolarization induced by these agonists. This study provides, to our knowledge, the first evidence of precise and distinct roles of these two G-protein α subunit isoforms in physiologically relevant signaling pathways in central neurons.

MATERIALS AND METHODS

$G\alpha_q$- and $G\alpha_{11}$-deficient mice. Mice deficient in the $G\alpha_q$ gene ($G\alpha_q$ −/−) or the $G\alpha_{11}$ gene ($G\alpha_{11}$ −/−) were generated by targeted disruption with a neomycin gene as described previously (Offermanns et al., 1997, 1998). $G\alpha_q$ −/− and $G\alpha_{11}$ −/− mice used in the experiments were obtained by mating heterozygous males and females to obtain wild-type ($G\alpha_q$ +/+; $G\alpha_{11}$ +/+) and knock-out ($G\alpha_q$ −/−; $G\alpha_{11}$ −/−) littermates. Mice were kept on a C57BL/6 × 129/Sv background, and genotypes were confirmed by PCR on genomic DNA from tail biopsies of each mouse. All experiments were performed in a double-blind manner, and the code was broken only after completion of the experimental work and data analysis.

Slice preparation. Acute slices were obtained from Wistar rats (18–28 d old), $G\alpha_q$ −/− mice (11–24 weeks old), $G\alpha_{11}$ −/− mice (14–25 weeks old), or wild-type mice. Wild-type mice were NMRI albino mice (4–8 weeks old), C57BL/6J (8–12 weeks old) mice, or littermates of $G\alpha_q$ −/− or $G\alpha_{11}$ −/− (11–25 weeks old). Throughout the text, it is specified to which group we refer according to the experiment performed. Transversal hippocampal slices (300–400 μm) were cut with either a tissue chopper (Mickle Laboratory, Gomshall, Surrey, UK) or a vibratome (Leica, Nussloch, Germany) and subsequently incubated in a humidified interface chamber at room temperature for ≥1 hr.

Electrophysiology. Tight-seal whole-cell voltage-clamp recordings were obtained using the "blind method" (Blanton et al., 1989). Patch electrodes (4–7 MΩ) were filled with an intracellular solution containing (in mM): 140 potassium methylsulfate, 10 HEPES, 2 Na_2-ATP, 0.4 Na_3-GTP, and 3 $MgCl_2$ (osmolarity, 280–300 mOsm), pH 7.2–7.3 with KOH. Recordings were performed in a submerged recording chamber with a constant flow of artificial CSF (ACSF) (2 ml/min) at room temperature (22–24°C). Drugs were applied in the bath solution. ACSF contained (in mM): 125 NaCl, 1.25 KCl, 2.5 $CaCl_2$, 1.5 $MgCl_2$, 1.25 KH_2PO_4, 25 $NaHCO_3$, and 16 D-glucose [bubbled with carbogen (95% O_2–5% CO_2)]. In all recordings, tetrodotoxin (TTX) (0.5 μM), tetraethylammonium (TEA) (5 mM), and picrotoxin (5 μM) were added to the superfusing ACSF. Whole-cell patch-clamp recordings were obtained from 90 neurons from mice and 13 from rats in acute hippocampal slices. Neurons were voltage clamped at −60 mV, and 100- to 200-msec-long depolarizing pulses to 0–30 mV were delivered every 30 sec. These pulses led to unclamped Ca^{2+} action currents sufficient to activate AHP currents. Slow depolarizing currents were measured as changes in the holding current under the same experimental conditions (same intracellular and extracellular solutions; holding potential of −60 mV). Series resistance was regularly monitored, and only recordings with stable series resistances ≤35 MΩ were included in this study. No series resistance compensation and no corrections for liquid junction potentials were made. Data were acquired using a patch-clamp EPC9 amplifier (Heka Elektronik, Lambrecht/Pfalz, Germany), filtered with a −3 dB cutoff frequency at 250 Hz, sampled at 1 KHz, and stored on a Macintosh Power personal computer (Apple Computers, Cupertino, CA). Analysis was made using the programs Pulse and Pulsefit (Heka), Igor Pro 3.01 (WaveMetrics Inc., Lake Oswego, OR), and Excel (Microsoft, Seattle, WA). We analyzed the amplitude, charge transfer (area enclosed by I_{AHP} and sI_{AHP}), and time course of AHP currents decay. No discrepancies in the variations of these parameters on pharmacological manipulations were observed; therefore, in Results and the figures, we reported only the values concerning I_{AHP} and sI_{AHP} amplitudes. The amplitude of I_{AHP} was determined at the peak of the current, whereas that of sI_{AHP} was
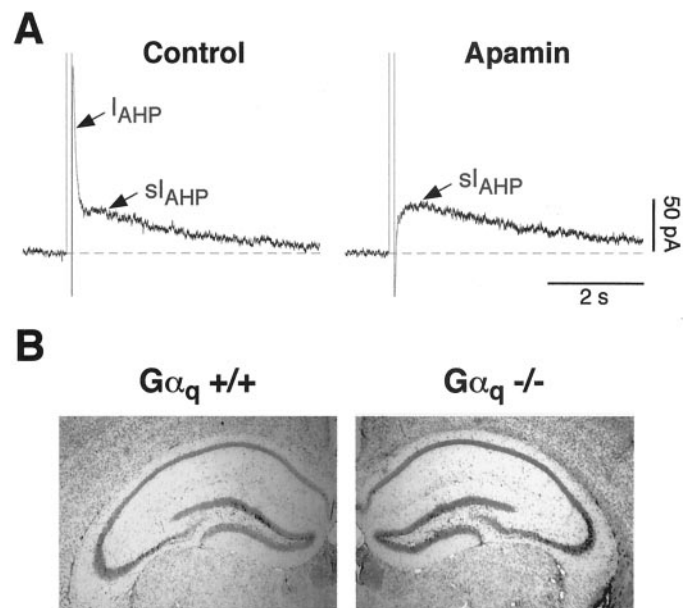
Figure 1. *A*, Mouse CA1 pyramidal neurons present a Ca^{2+}-activated, apamin-sensitive afterhyperpolarizing current (I_{AHP}) that deactivates in the hundreds of milliseconds range, followed by a Ca^{2+}-activated, apamin-insensitive AHP current (sI_{AHP}), lasting seconds. I_{AHP} is partially overlapping with the rise time of sI_{AHP}. These currents were elicited by Ca^{2+} influx induced by a 100 msec depolarizing pulse to +10 mV. Traces shown were recorded from CA1 neurons of a $G\alpha_{11}$ −/− mouse. $G\alpha_{11}$ +/+ littermates, as well as $G\alpha_q$ +/+ and $G\alpha_q$ −/− mice, displayed similar currents (see Results). In the *right panel*, I_{AHP} was blocked by 50 nM apamin, leaving sI_{AHP} amplitude and kinetics unaffected. *B*, Nissl stains of the hippocampal formation from $G\alpha_q$ +/+ and $G\alpha_q$ −/− mice. No changes in the gross morphology of the hippocampus were observed in the knock-out animals when compared with their wild-type littermates.

determined at a point 700–800 msec after the end of the command pulse, in which a possible contamination by the partially overlapping I_{AHP} was negligible (Stocker et al., 1999). Values are presented as mean ± SEM. For statistical analysis, the unpaired, two-tailed Student's *t* test was used, and differences were considered statistically significant if $p \leq 0.05$.

Drugs and solutions. TEA, carbamylcholine (carbachol, CCh), Na_2-ATP, and Na_3-GTP were obtained from Sigma (St. Louis, MO). TTX was from Alomone Labs (Jerusalem, Israel). Picrotoxin and isoproterenol were from Research Biochemicals (Natick, MA). *trans*-1-Amino-cyclopentyl-1,3-dicarboxylate (*trans*-ACPD) and (*S*)-3,5-dihydroxyphenyl-glycine (DHPG) were from Tocris Cookson (Bristol, UK). All drugs were dissolved in water, stored at +4°C or −20°C, and bath applied in the perfusing ACSF.

RESULTS

AHP currents in the mouse hippocampus

Rat CA1 pyramidal neurons have been shown to present two distinct Ca^{2+}-dependent afterhyperpolarizing currents, contributing to the early and late spike frequency adaptation: I_{AHP}, deactivating in hundreds of milliseconds and sensitive to the toxin apamin; and sI_{AHP}, characterized by a slow deactivation (in the range of seconds), insensitive to apamin and sensitive to the neuromodulatory effects of a number of transmitters (Stocker et al., 1999). In mouse hippocampal pyramidal neurons, the types of AHP currents and their key features are still unexplored. In CA1 pyramidal neurons from mouse slices, short depolarizing pulses inducing Ca^{2+} influx elicited an apamin-sensitive I_{AHP} in 94.9% of the cells, followed by an apamin-insensitive sI_{AHP} in 77.8% (Fig. 1*A*). In two different strains of wild-type mice tested, NMRI and C57BL/6, sI_{AHP} presented an amplitude of 19.6 ± 3.5 (*n* =

11) and 25.6 ± 3.4 (*n* = 31) pA, respectively, whereas in Wistar rats, the sI_{AHP} amplitude was substantially larger (109.8 ± 13.5 pA; *n* = 13) under the same experimental conditions. The time courses of decay of the two AHP currents were clearly different: the apamin-sensitive I_{AHP} presented an average decay time constant of 88.3 ± 4.9 msec, and the apamin-insensitive sI_{AHP} presented an average decay time constant of 3.5 ± 0.2 sec. In most cells measured, I_{AHP} coexisted and partially overlapped with the rising phase of sI_{AHP}, as shown in Figure 1*A* (*left panel*). The pharmacological separation by apamin (Fig. 1*A*) suggests that I_{AHP} and sI_{AHP} are distinct currents, as reported previously in rat CA1 pyramidal neurons (Stocker et al., 1999). In all CA1 pyramidal neurons from mouse, as in rat, the Ca^{2+} spikes and sI_{AHP} remained stable for the duration of the recording (usually ~2–3 hr). Additionally, sI_{AHP} displayed a normal sensitivity to a number of neurotransmitters (see below), as observed previously in rat neurons.

## Cholinergic and glutamatergic modulation of sI_{AHP} is inhibited in Gα_q-deficient mice

Activation of muscarinic (M_3; Rouse et al., 2000; M. Krause and P. Pedarzani, unpublished observation) or metabotropic (mGluR$_5$; Gereau and Conn, 1995) glutamate receptors leads to the suppression of the apamin-insensitive sI_{AHP} in CA1 pyramidal neurons. The actions of both muscarinic and mGluR agonists on sI_{AHP} have been shown to involve activation of G-proteins of the pertussis toxin-insensitive type (Dutar and Nicoll, 1988; Gerber et al., 1992; Abdul-Ghani et al., 1996a; Krause and Pedarzani, 2000). However, pertussis toxin and GDP-β-S used in those studies blocked a wide subset or all G-proteins, respectively. Therefore, this approach did not allow the identification of specific G-protein subtypes transducing the effects of M_3 muscarinic and mGluR$_5$ receptor stimulation on sI_{AHP}. We used mice deficient in the Gα_q gene (Gα_q −/−) to investigate the specific role of this G-protein in the cholinergic and glutamatergic modulation of sI_{AHP}. The hippocampus of Gα_q-deficient mice presented an overall structure and gross morphology indistinguishable from those of wild-type animals (Fig. 1*B*). Additionally, sI_{AHP} amplitude, time course, and passive membrane properties (resting membrane potential, V_m; input resistance, R_i) were not significantly different in Gα_q −/− (V_m of −71.0 ± 1.4 mV; R_i of 186 ± 9 MΩ) when compared with Gα_q +/+ mice (V_m of −72.4 ± 1.2 mV; R_i of 190 ± 11 MΩ) or mice of a different strain (NMRI; V_m of −68.3 ± 1.8 mV; R_i of 173 ± 13 MΩ). The only observed difference was an intrinsically smaller amplitude of the apamin-sensitive I_{AHP} in the absence of neuromodulatory agents in Gα_q −/− mice (87.6 ± 15.9 pA in Gα_q +/+, *n* = 25; 44.1 ± 5.0 pA in Gα_q −/−, *n* = 19; *p* = 0.01).

In wild-type mice, the cholinergic agonist carbachol (5 μM) (Fig. 2*A*,*E*) and the mGluR agonists DHPG (2–3 μM) (Fig. 3*A*,*E*) and *trans*-ACPD (10–20 μM; data not shown) all produced a robust inhibition of sI_{AHP} but did not significantly affect the apamin-sensitive I_{AHP}. In mice lacking Gα_q, the effects of carbachol and DHPG on sI_{AHP} were strongly reduced (Fig. 2*C*,*E* for CCh; Fig. 3*C*,*E* for DHPG), arguing in favor of a substantial contribution of Gα_q in mediating the suppression of sI_{AHP}. The apparent small decrease in I_{AHP} amplitude, observed in wild-type as in knock-out Gα_q mice during application of these neuromodulatory agents, was most likely attributable to the partial overlap between I_{AHP} and sI_{AHP} and the concomitant suppression of sI_{AHP} (Stocker et al., 1999).

Beside downregulating sI_{AHP}, activation of M_3 receptors and
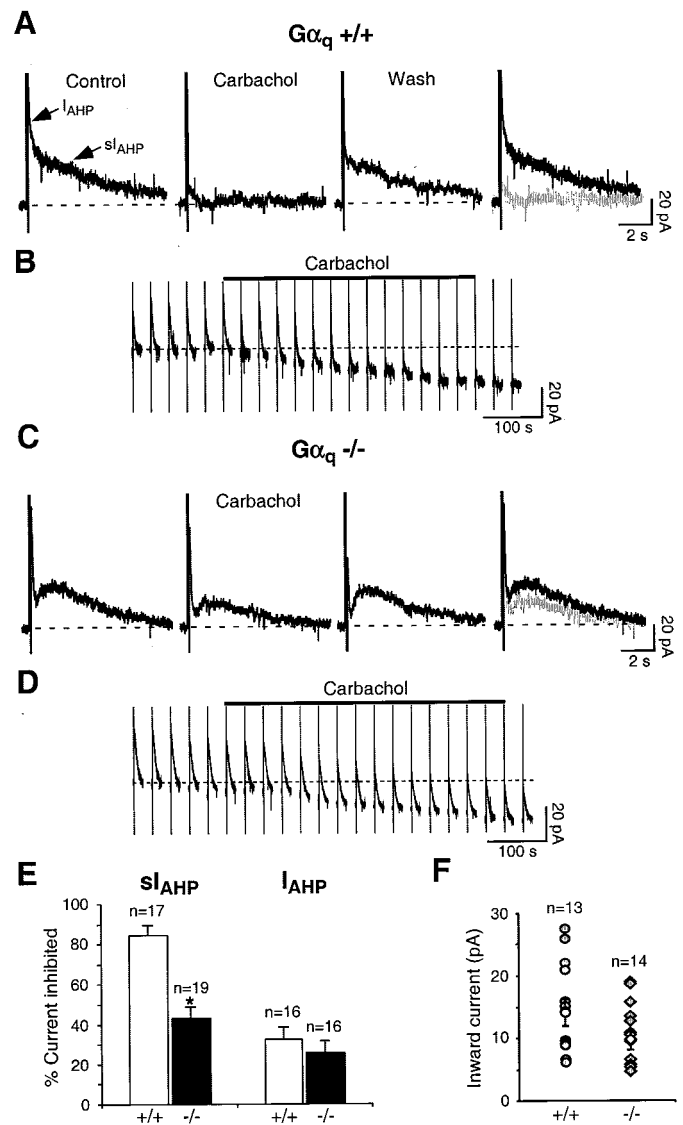
Figure 2. A, In Gα_q +/+ mice, the cholinergic agonist carbachol (5 μM) strongly and reversibly suppressed sI_{AHP}. *B*, Voltage-clamp recording of the inward current underlying the depolarizing response to carbachol (5 μM) in CA1 neurons from Gα_q +/+ mice. The *vertical lines* correspond to depolarizing pulses used to elicit the AHP currents once every 30 sec. The *dotted line* represents the baseline holding current before the application of carbachol. The carbachol application lasted 7 min (*solid bar*). *C*, In Gα_q −/− mice, 5 μM carbachol induced a significantly smaller inhibition of sI_{AHP} than in *A*. In *A* and *C*, the *rightmost panels* show superimpositions of the *traces* before and during carbachol application. *D*, Inward current underlying the depolarizing response to carbachol (5 μM) in CA1 neurons from Gα_q −/− mice. The carbachol application lasted 7.5 min (*solid bar*). All of the rest are as in *B*. *E*, Summary of the results obtained with carbachol in CA1 pyramidal neurons from Gα_q +/+ and Gα_q −/− mice. In Gα_q +/+, carbachol suppressed sI_{AHP} by 84.0 ± 4.2% (*n* = 17), whereas in Gα_q −/−, 42.6 ± 5.6% of sI_{AHP} was inhibited during carbachol application (*n* = 19). *represents a statistically significant difference (*p* < 0.001). In the same pools of CA1 neurons, carbachol did not significantly downregulate I_{AHP} in Gα_q +/+ mice (31.8 ± 5.9% inhibition) compared with Gα_q −/− mice (25.5 ± 5.5% inhibition; *n* = 16; *p* = 0.44). *F*, Scatter plot summarizing the effect of 5 μM carbachol on the membrane potential of CA1 pyramidal neurons from Gα_q +/+ (*filled circles*) and Gα_q −/− (*filled diamonds*) animals. Carbachol elicited a mean inward shift in the holding current of 14.6 ± 2.0 pA in Gα_q +/+ (*open circle*; *n* = 13) and 10.3 ± 1.4 pA in Gα_q −/− mice (*open diamond*; *n* = 14; *p* = 0.10).
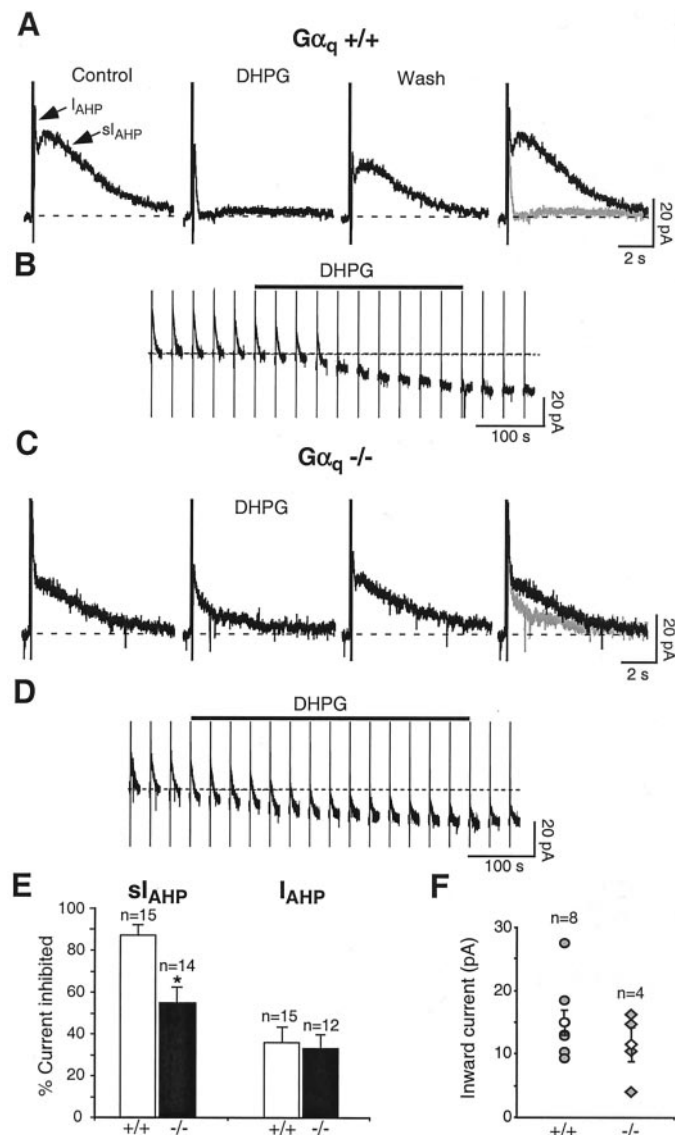
Figure 3. A, The group I mGluR receptor agonist DHPG (3 μM) suppressed sI_{AHP} in a reversible manner in Gα_q +/+ mice. *B,* Inward shift in the holding current underlying the depolarizing response to DHPG (3 μM) in CA1 neurons from Gα_q +/+ mice. The *vertical lines* correspond to depolarizing pulses used to elicit the AHP currents once every 30 sec. The *dotted line* represents the baseline holding current before the application of DHPG. The DHPG application lasted 5 min (*solid bar*). *C,* In Gα_q −/− mice, 3 μM DHPG produced a significantly smaller inhibition of sI_{AHP}. In *A* and *C,* the *rightmost panels* show superimpositions of the *traces* before and during DHPG application. *D,* Depolarizing current elicited in response to DHPG (3 μM) in CA1 neurons from Gα_q −/− mice. The DHPG application lasted 7 min (*solid bar*). All of the rest are as in *B. E,* Bar diagram summarizing the results obtained with DHPG in CA1 pyramidal neurons from Gα_q +/+ and Gα_q −/−. In Gα_q +/+, 86.9 $\pm$ 4.4% of sI_{AHP} was inhibited after DHPG application ($n = 15$), whereas in Gα_q −/−, DHPG suppressed sI_{AHP} by 54.7 $\pm$ 7.3% ($n = 14$). *represents a statistically significant difference ($p = 0.001$). In the same pools of CA1 neurons, the small inhibitory effect of DHPG on I_{AHP} was similar in Gα_q +/+ (35.9 $\pm$ 7.4% inhibition; $n = 15$) and Gα_q −/− (32.5 $\pm$ 6.2% inhibition; $n = 12$; $p = 0.73$) mice. *F,* Summary of the effect of DHPG (2–3 μM) on the holding current in Gα_q +/+ (*filled circles*) and Gα_q −/− (*filled diamonds*) neurons. DHPG elicited a mean inward depolarizing current of 15.2 $\pm$ 2.0 pA in Gα_q +/+ (*open circle*; $n = 8$) and 11.8 $\pm$ 2.7 pA in Gα_q −/− (*open diamond*; $n = 4$; $p = 0.33$).

mGluR$_5$ leads to the generation of a depolarizing inward current in CA1 pyramidal neurons (Figs. 2*B,* 3*B*) (Pitler and Alger, 1990; Gereau and Conn, 1995). The signal transduction pathway leading to this depolarizing effect is only partly known. We tested whether Gα_q was mediating the generation of the depolarizing current during application of muscarinic and mGluR agonists. The inward current elicited by carbachol and DHPG was not significantly different in Gα_q knock-out mice (Fig. 2*D,F* for CCh; Fig. 3*D,F* for DHPG) when compared with their wild-type littermates (Fig. 2*B,F* for CCh; Fig. 3*B,F* for DHPG). These results suggest that Gα_q is not required for the muscarinic- or mGluR-induced depolarizing inward current in CA1 neurons.

Gα_{11} is not required for the modulation of sI_{AHP} by muscarinic and mGluR agonists

The remaining effect of muscarinic and mGluR agonists on sI_{AHP} in Gα_q-deficient mice might be attributable to activation of Gα_{11}, because this Gα subunit is also expressed, although to a lower level, in CA1 pyramidal neurons (Mailleux et al., 1992; Milligan, 1993; Tanaka et al., 2000) and displays a similar functional role as Gα_q in other systems (Wu et al., 1992; Offermanns et al., 1994; Exton, 1996; Offermanns, 1999). To investigate the role of Gα_{11} in the cholinergic and metabotropic signal transduction cascades suppressing sI_{AHP}, we recorded from CA1 neurons of mice lacking Gα_{11} (Gα_{11} −/−). As in the case of the Gα_q knock-outs, mice lacking Gα_{11} presented a normal hippocampal gross morphology and intrinsic neuronal properties indistinguishable from those of control mice (data not shown). In Gα_{11} −/− mice and in their wild-type littermates (Gα_{11} +/+), carbachol (Fig. 4*A,C*) and DHPG (Fig. 4*B,D*) suppressed sI_{AHP} to the same extent. In the same neurons, the apamin-sensitive I_{AHP} was only marginally affected by application of muscarinic (Fig. 4*C*) and mGluR (Fig. 4*D*) agonists. These results demonstrate that Gα_{11} is not required to transduce the cholinergic and glutamatergic signals that result in the downregulation of sI_{AHP}. Therefore, it is unlikely that Gα_{11} is part of the signal cascade regulating the residual sI_{AHP} present in Gα_q −/− after cholinergic and glutamatergic receptor activation. In mice lacking Gα_{11}, the depolarizing effects of carbachol and DHPG were indistinguishable from those observed in their wild-type littermates (data not shown), suggesting that Gα_{11} is not mediating the muscarinic- or mGluR-induced depolarizing inward current in CA1 neurons.

The β-adrenergic modulation of sI_{AHP} is intact in Gα_q-deficient mice

Next, we wanted to test whether the reduction of the effects of cholinergic and glutamatergic agonists on sI_{AHP} observed in Gα_q-deficient mice is specific or whether supposedly unrelated modulatory pathways converging on sI_{AHP} might be unspecifically affected in these genetically modified animals. To this purpose, we took advantage of the fact that sI_{AHP} is strongly suppressed by monoamine transmitters (noradrenaline, serotonin, histamine, and dopamine) in rat CA1 pyramidal neurons (Nicoll, 1988; Pedarzani and Storm, 1993; Pedarzani and Storm, 1995). Among these transmitters, noradrenaline activates β-adrenergic receptors, which are coupled to Gα_s-subunits and lead to the stimulation of adenylyl cyclase, increase of cAMP level, and activation of PKA, finally resulting in a complete suppression of sI_{AHP}. We used the β-adrenergic agonist isoproterenol, which suppressed sI_{AHP} both in wild-type (Fig. 5*A,C*) and Gα_q-deficient (Fig. 5*B,C*) mice in an indistinguishable manner but had no significant effect on the apamin-sensitive I_{AHP} (Fig. 5*C*). We can therefore
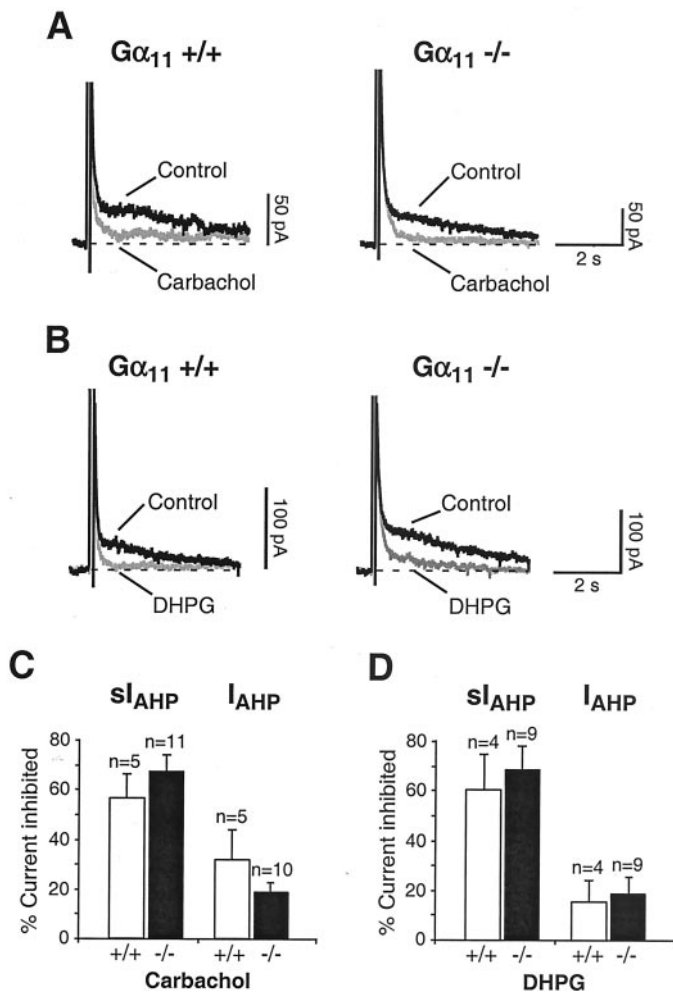
Figure 4. *A, Left,* In $G\alpha_{11}$ +/+ mice, 5 μM carbachol strongly suppressed sI_{AHP}. *Right,* In $G\alpha_{11}$ −/−, 5 μM carbachol inhibited sI_{AHP} to the same extent as observed in $G\alpha_{11}$ +/+ mice. *B, Left,* DHPG (3 μM) induced a robust suppression of sI_{AHP} in $G\alpha_{11}$ +/+ mice. *Right,* Similarly, in $G\alpha_{11}$ −/−, 3 μM DHPG produced a strong inhibition of sI_{AHP}. In *A* and *B,* *traces* before and during carbachol and DHPG applications are shown superimposed. *C,* Bar diagram summarizing the results obtained with carbachol in CA1 pyramidal neurons from $G\alpha_{11}$ +/+ and $G\alpha_{11}$ −/− mice. In $G\alpha_{11}$ +/+, carbachol suppressed sI_{AHP} by 56.2 ± 9.8% ($n = 5$), and in $G\alpha_{11}$, −/−, 67.3 ± 7.2% of sI_{AHP} was inhibited during carbachol application ($n = 11$). The difference observed between $G\alpha_{11}$ +/+ and $G\alpha_{11}$ −/− mice was not statistically significant ($p = 0.39$). In the same pools of CA1 neurons, carbachol did not significantly affect I_{AHP} in $G\alpha_{11}$ +/+ compared with $G\alpha_{11}$ −/− mice ($n = 5$ and $n = 10$, respectively). *D,* Bar diagram summarizing the results obtained with DHPG in CA1 pyramidal neurons from $G\alpha_{11}$ +/+ and $G\alpha_{11}$ −/− mice. In $G\alpha_{11}$ +/+, 60.5 ± 14.2% of sI_{AHP} was inhibited during DHPG application ($n = 4$), and in $G\alpha_{11}$ −/−, DHPG suppressed sI_{AHP} by 68.0 ± 9.6% ($n = 9$). The difference observed between $G\alpha_{11}$ +/+ and $G\alpha_{11}$ −/− mice was not statistically significant ($p = 0.68$). In the same pools of CA1 neurons, DHPG did not significantly affect I_{AHP} in $G\alpha_{11}$ +/+ compared with $G\alpha_{11}$ −/− mice ($n = 4$ and $n = 9$, respectively).

conclude that the absence of $G\alpha_q$ impairs specifically cholinergic and glutamatergic signal cascades suppressing sI_{AHP}, but it does not affect the modulation of this current by other neurotransmitters coupled to different second-messenger pathways.

DISCUSSION

This study presents the first demonstration and characterization of apamin-sensitive and -insensitive AHP currents in the mouse
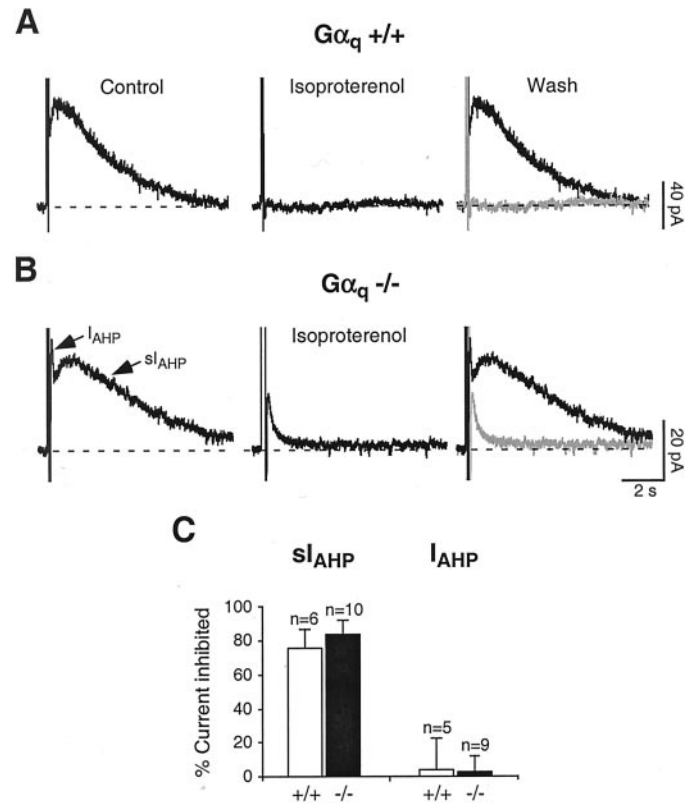
Figure 5. *A,* In $G\alpha_q$ +/+, the β-adrenergic agonist isoproterenol (1 μM) strongly suppressed sI_{AHP}. *B,* In $G\alpha_q$ −/−, 1 μM isoproterenol suppressed sI_{AHP} to a similar extent as in *A.* In *A* and *B,* the *rightmost panels* show superimpositions of the *traces* before and during isoproterenol application. *C,* Summary of the results obtained with isoproterenol in CA1 pyramidal neurons from $G\alpha_q$ +/+ and $G\alpha_q$ −/− mice. In $G\alpha_q$ +/+, isoproterenol suppressed sI_{AHP} by 75.0 ± 11.4% ($n = 6$), and in $G\alpha_q$ −/−, 83.6 ± 7.8% of sI_{AHP} was inhibited during isoproterenol application ($n = 10$). The difference observed between $G\alpha_q$ +/+ and $G\alpha_q$ −/− mice was not statistically significant ($p = 0.54$). In the same pools of CA1 neurons, isoproterenol did not affect I_{AHP} in $G\alpha_q$ +/+ differently from $G\alpha_q$ −/− mice ($n = 5$ and $n = 9$, respectively).

hippocampus. Based on this, knock-out animals could be used to analyze specific transducing elements of signal cascades regulating sI_{AHP}. The primary purpose of the present experiments was to elucidate which heterotrimeric G-proteins transduce muscarinic- and mGluR-mediated signals leading to a downregulation of sI_{AHP} and to an enhanced excitability of hippocampal pyramidal neurons. To this purpose, we used mice lacking the $G\alpha_q$ or $G\alpha_{11}$ gene. These mice did not present gross morphological abnormalities of the CNS and displayed an overall normal hippocampal morphology. In addition, intrinsic membrane properties (this study) and basic parameters of synaptic function (input–output curves and paired-pulse facilitation; Kleppisch et al., 2001) were not altered in the CA1 region. Our results demonstrate that $G\alpha_q$ is the main transducing element in these signal cascades leading to sI_{AHP} suppression, whereas $G\alpha_{11}$ does not seem to be required. Neither $G\alpha_q$ nor $G\alpha_{11}$ seem to be involved in mediating the membrane potential depolarization caused by muscarinic and mGluR agonists in CA1 pyramidal neurons.

Muscarinic and mGluR downregulation of sI_{AHP} requires $G\alpha_q$, because the effects of muscarinic and mGluR agonists were strongly reduced in neurons from $G\alpha_q$ knock-out mice. It is unlikely that this was a result of generalized secondary effects on

transduction mechanisms arising from the absence of Gα_q during development, because muscarinic and mGluR agonists were still able to elicit the inward, depolarizing current. Furthermore, transmitters acting via other G-proteins, such as the β-adrenergic agonist isoproterenol acting via Gα_s, fully suppressed sI_{AHP} in Gα_q-deficient neurons, indicating a preserved function of other signaling pathways targeting the same current.

In the absence of Gα_q, muscarinic and mGluR agonists partially suppressed sI_{AHP} (Figs. 2, 3). Our first assumption was that this could be attributable to the presence of Gα_{11}, which is expressed at lower levels in hippocampal neurons (Mailleux et al., 1992; Milligan, 1993; Tanaka et al., 2000) and presents receptor- and effector-coupling properties very similar to Gα_q (Wu et al., 1992; Offermanns et al., 1994; Exton, 1996; Offermanns, 1999). However, the results we obtained in Gα_{11} knock-out mice revealed that Gα_{11} is not required for sI_{AHP} modulation and is therefore unlikely to substitute for Gα_q in Gα_q knock-out mice. This observation is further supported by the lack of compensatory overexpression of Gα_{11} in the hippocampus of Gα_q knock-out mice, as shown recently by immunoblot experiments (Kleppisch et al., 2001). Despite this evidence, in principle the possibility remains that Gα_{11} mediates the residual effect of muscarinic and glutamatergic agonists observed in Gα_q knock-out mice. This could happen, for example, if Gα_{11} contributed in a small but significant way to the muscarinic or glutamatergic action, resulting in an invisible phenotype in the Gα_{11} knock-out mice but emerging as a sufficient contribution in the Gα_q knock-outs. A subtle, partial effect linked to Gα_{11}, unmasked in Gα_q knock-out mice, would be expected to be slower when compared with the Gα_q-mediated effect observed in their wild-type littermates, given the lower density of the Gα_{11} subtype in CA1 pyramidal neurons (Mailleux et al., 1992; Milligan, 1993; Tanaka et al., 2000). Indeed, a compensatory phenomenon with altered (slower) kinetics has been reported recently for the modulation of potassium and calcium channels by GABA$_B$ and adenosine receptors in hippocampal neurons from mice lacking Gα_o (Greif et al., 2000). This seems unlikely to occur in our case, because the time course of the residual muscarinic and glutamatergic effects on sI_{AHP} was not different in Gα_q knock-out mice when compared with their wild-type littermates [compare Figs. 2*B,D* (for carbachol, 3*B,D* (for DHPG)]. However, the possibility of a marginal Gα_{11} involvement could be conclusively ruled out only by using animal models lacking both Gα_q and Gα_{11} subunits, but unfortunately a double mutant generated by conventional gene targeting is not viable (Offermanns et al., 1998). If conditional double mutants prove to be viable, they could provide a potential future approach to unequivocally answer this question.

In several mammalian cells, receptors activating G$_q$ family members do not seem to discriminate between Gα_q and Gα_{11} (Wange et al., 1991; Wu et al., 1992; Offermanns et al., 1994), and, in reconstituted systems, both G-proteins are indistinguishable in their ability to regulate different phospholipase C (PLC) isoforms (Exton, 1996) and inward rectifier potassium channels (Lei et al., 2001). In the light of such apparent functional redundancy, the prominent involvement of Gα_q and the lack of effect of Gα_{11} in mediating the cholinergic and glutamatergic suppression of sI_{AHP} provide therefore a remarkable indication of functional specificity for members of the G$_q$ family in the CNS. In line with this view, Gα_q, but not Gα_{11}, has been shown recently to be critically involved in the induction of mGluR-dependent long-term depression in CA1 pyramidal neurons (Kleppisch et al., 2001).

The picture emerging from the results presented here is differ-

ent from that which has been observed for another potassium current, the voltage-dependent I_M, in the peripheral nervous system. In rat superior cervical ganglion neurons, M$_1$ muscarinic receptors inhibit I_M primarily via Gα_q (Haley et al., 1998). In mouse, inhibition is also mediated by M$_1$ receptors (Hamilton et al., 1997) but appears to involve both Gα_q and Gα_{11} and also a pertussis toxin-sensitive G-protein (Haley et al., 2000). The suppression of sI_{AHP} by muscarinic agonists in CA1 neurons is instead linked to M$_3$ receptor activation (Rouse et al., 2000) and is mediated principally by Gα_q, in a similar way to that observed for the M$_1$-mediated inhibition of the N-type calcium current in sympathetic neurons (Haley et al., 2000). The preponderant involvement of Gα_q in the M$_3$- and mGluR$_5$-mediated suppression of sI_{AHP} presented in this study is in agreement with previous works using pharmacological approaches to show that G-proteins, and in particular pertussis toxin-insensitive ones, were essential components of the muscarinic and mGluR signal cascades converging on sI_{AHP} (Dutar and Nicoll, 1988; Gerber et al., 1992; Abdul-Ghani et al., 1996a; Krause and Pedarzani, 2000).

Beside modulating sI_{AHP}, muscarinic and mGluR agonists induce a depolarization of the membrane potential in CA1 neurons. The underlying current has been ascribed to the inhibition of at least two types of K$^+$ currents: the voltage-dependent M current (Brown and Adams, 1980; Halliwell and Adams, 1982; McCormick and Williamson, 1989) and a voltage-independent leak current (Madison et al., 1987; Guerineau et al., 1994). More recently, a cation nonselective current has been proposed to be mainly responsible for the depolarization generated by muscarinic and mGluR agonists in cortical and hippocampal neurons (Crepel et al., 1994; Greene et al., 1994; Guerineau et al., 1995; Haj-Dahmane and Andrade, 1997, 1999). Interestingly, mGluR$_1$ receptors have been shown recently to elicit an inward, depolarizing current associated with a slow excitatory postsynaptic response in a G-protein-independent manner in CA3 pyramidal neurons (Heuss et al., 1999). Our experiments show that neither the lack of Gα_q nor Gα_{11} affect the depolarizing action of M$_3$ muscarinic and mGluR$_5$ receptors in CA1 pyramidal neurons. Our results are compatible with the possibility that muscarinic and mGluR agonists induce a depolarization of the membrane potential in a G-protein-independent manner in CA1 neurons, as proposed recently for CA3 neurons (Heuss et al., 1999).

The signal transduction events downstream from M$_3$ or mGluR$_5$ and G-protein activation, leading to sI_{AHP} suppression, $I_{K(leak)}$ inhibition, and activation of a depolarizing cationic conductance, are still rather unclear. Calcium/calmodulin-dependent kinase II has been shown to be involved in the muscarinic, but not in the mGluR, modulation of sI_{AHP} (Müller et al., 1992; Pedarzani and Storm, 1996), suggesting that the two receptors might be coupled to distinct pathways. In dentate gyrus granule cells, PLC, IP$_3$, and a tyrosine kinase have been reported to mediate the glutamatergic suppression of sI_{AHP} (Abdul-Ghani et al., 1996a,b), whereas in CA1 pyramidal neurons neither PLC nor IP$_3$ seem to be involved in the inhibition of this current by muscarinic or mGluR agonists (Krause and Pedarzani, 1999, 2000). Instead, we obtained evidence for the involvement of a protein phosphatase (Krause and Pedarzani, 1999, 2000), but the mechanism of coupling to G-proteins needs to be elucidated. The present study provides evidence that signaling of both M$_3$ muscarinic and mGluR$_5$ receptors converge onto the same G-protein, Gα_q, to modulate sI_{AHP}. Future experiments will show whether the residual effect on sI_{AHP} observed in Gα_q-deficient mice is attributable to the coupling of M$_3$ and mGluR$_5$ to a parallel, second

pathway, activated by $\beta\gamma$ subunits, by G-proteins not belonging to the G_q family, or by a G-protein-independent mechanism.

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