Bimodal Regulation of the Human H1 Histamine Receptor by G Protein-coupled Receptor Kinase 2*

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The H1 histamine receptor (H1HR) is a member of the G protein-coupled receptor superfamily and regulates numerous cellular functions through its activation of the Gq/11 subfamily of heterotrimeric G proteins. Although the H1HR has been shown to undergo desensitization in multiple cell types, the mechanisms underlying the regulation of H1HR signaling are poorly defined. To address this issue, we examined the effects of wild type and mutant G protein-coupled receptor kinases (GRKs) on the phosphorylation and signaling of human H1HR in HEK293 cells. Overexpression of GRK2 promoted H1HR phosphorylation in intact HEK293 cells and completely inhibited inositol phosphate production stimulated by H1HR, whereas GRK5 and GRK6 had lesser effects on H1HR phosphorylation and signaling. Interestingly, catalytically inactive GRK2 (GRK2-K220R) also significantly attenuated H1HR-mediated inositol phosphate production, as did an N-terminal fragment of GRK2 previously characterized as a regulator of G protein signaling (RGS) protein for Gq/11. Disruption of this RGS function in holo-GRK2 by mutation (GRK2-D110A) partially reversed the quenching effect of GRK2, whereas deletion of both the kinase activity and RGS function (GRK2-D110A/K220R) effectively relieved the inhibition of inositol phosphate generation. To evaluate the role of endogenous GRKs on H1HR regulation, we used small interfering RNAs to selectively target GRK2 and GRK5, two of the primary GRKs expressed in HEK293 cells. A GRK2-specific small interfering RNA effectively reduced GRK2 expression and resulted in a significant increase in histamine-promoted calcium flux. In contrast, knockdown of GRK5 expression was without effect on H1HR signaling. These findings demonstrate that GRK2 is the principal kinase mediating H1 histamine receptor desensitization in HEK293 cells and suggest that rapid termination of H1HR signaling is mediated by both the kinase activity and RGS function of GRK2.

G protein-coupled receptors (GPCRs)† comprise a superfamily of seven transmembrane-spanning receptors that transduce extracellular signals into discrete intracellular signals to regulate cell functions. GPCR signaling is regulated not only by ligand availability but also by complex mechanisms that regulate receptor responsiveness to their cognate stimuli. The regulatory process of desensitization utilizes a wide variety of regulatory proteins that interact with a given GPCR to render it hyporesponsive to agonists. For the majority of GPCRs, desensitization caused by agonist exposure is mediated by one or more members of a family of GPCR kinases (GRKs) that phosphorylate the agonist-occupied receptor and promote the subsequent binding of arrestin molecules (1–3). Arrestin binding to GPCRs disrupts receptor activation of heterotrimeric G proteins and can also initiate the process of receptor internalization, which can lead to either GPCR recycling or degradation (1–3). However, studies to date clearly demonstrate that the propensity for a particular GPCR to be regulated by second messenger-dependent kinases, GRKs, or arrestins is receptor-specific.

The H1 histamine receptor (H1HR) mediates the functional effects of histamine in multiple cell types through activation of the Gq/11 heterotrimeric G protein and its downstream effector phospholipase C (PLC). Stimulation of the H1HR-Gq/11-PLC pathway results in the synthesis of inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, which in turn stimulate an increase in intracellular Ca2+ and the activation of protein kinase C (PKC). These histamine-induced intracellular messengers promote diverse functions in multiple cell types, including smooth muscle and nonsmooth muscle contraction (4–9) and exocytotic release of neurotransmitters and various autocrine/paracrine factors (5, 10), both of which can contribute to inflammation and inflammatory disease processes (reviewed in Refs. 4, 5, and 10–12).

Numerous studies have demonstrated that both endogenously expressed as well as heterologously expressed H1HRs exhibit hyporesponsiveness/desensitization when exposed to either PKC-activating agents or histamine (7, 13–19) and that agonist-specific desensitization can be associated with H1HR internalization or down-regulation (14, 18). However, beyond a basic appreciation that the H1HR desensitizes and internalizes, little is known regarding the mechanisms by which these processes are mediated. In the present study, we examined the roles of PKC and GRKs in agonist-specific H1HR desensitization.

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1 The abbreviations used are: GPCR, G protein-coupled receptor; GRK, G protein-coupled receptor kinase; HA, hemagglutinin; H1HR, H1 histamine receptor; IP, inositol phosphate; PKC, protein kinase C; PLC, phospholipase C; RGS, regulator of G protein signaling; siRNA, small interfering RNA; DMEM, Dulbecco’s modified Eagle’s medium; SC, scrambled.
tion, and assessed the relative contribution of distinct functional domains within GRK2 responsible for terminating H1HR signaling.

**EXPERIMENTAL PROCEDURES**

**Materials**—A pcDNA3-H1HR expression construct encoding the human H1 histamine receptor was provided by Dr. R. Leurs (Vrije Universiteit, Amsterdam, The Netherlands). Gs-signaling-defective GRK2 mutants (GRK2-D110A, GRK2-R106A) were provided by Dr. R. Sterne-Marr (Siena College, Loudonville, NY). Human embryonic kidney (HEK293) cells were purchased from the American Type Culture Collection (Manassas, VA). FuGENE-6 was from Roche Applied Science. Monoclonal and polyclonal antibodies for the hemagglutinin (HA) epitope were purchased from Covance Research Products (Berkeley, CA). Alexa 594 conjugate anti-HA clonal antibodies for the hemagglutinin (HA) epitope were purchased from PBL Bioreagents (Edison, NJ). Human embryonic kidney (HEK293) cells grown in 100-mm dishes were transfected with 10 μg of pcDNA3 vector (Mock) or 5 μg of pcDNA3-HA-H1HR and 5 μg of pcDNA3 vector (Mock) or pcDNA3-GRK2, GRK5, or GRK6. The cells were metabolically labeled with [35S]orthophosphate and stimulated with 100 μM histamine for 10 min. After the cells were solubilized, HA-H1HR was immunoprecipitated as described under "Experimental Procedures." Immunoprecipitated receptor was subjected to SDS-PAGE followed by autoradiography. Expression levels of receptor were comparable, as determined by radioimmunobinding. This result is representative of two independent experiments.

**Plasmid Construction**—To eliminate the promoter sequence, the 5′-terminal region of H1HR in pcDNA3-H1HR was amplified by PCR using two oligos, 5′-CGGGGTACCACCAGGCGAACATGGCCTTCCCAATTCTTC-3′ and 5′-TAACTATGATCCTCTGGATTCTCGC-3′, and cloned back into the KpnI sites of the original pcDNA3-H1HR construct. The open reading frame of pcDNA3-H1HR was also subcloned in-frame with the hemagglutinin (HA) epitope to generate pcDNA3-H1HR. pcDNA3-HA-H1HR was digested with EcoRI and HindIII, and the open reading frame of pcDNA3-H1HR was ligated by T4 DNA ligase, followed by transformation into competent E. coli. The correct orientation of the open reading frame was confirmed by DNA sequencing. pcDNA3-H1HR was then sequenced to confirm the correct orientation and to ensure that no spurious mutations were introduced. pcDNA3-H1HR was transfected into HEK293 cells grown in 100-mm dishes with 10 μg of pcDNA3-H1HR, or 5 μg of pcDNA3-GRK2, GRK5, or GRK6 vector control. The following day, the cells were seeded into two 10-cm dishes for phosphorylation analysis and one 6-cm dish for radioimmunobinding (to confirm equivalent expression).

**Receptor Phosphorylation**—HEK293 cells grown in 100-mm dishes were transfected with 5 μg of pcDNA3-HA-H1HR and 5 μg of pcDNA3-GRK2, GRK5, or GRK6 vector control. The following day, the cells were washed twice in serum-free and sodium phosphate-free DMEM followed by serum-free DMEM and sodium phosphate-free DMEM were from Life Technologies, Inc. GRK-specific and scrambled (SC) siRNAs were purchased from Dharmacon. The siRNA duplexes (600 pmol of siRNA in a 10-cm dish, final concentration of 40 nM) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) following the manufacturer's instructions and were analyzed for GRK expression and calcium flux 3 days after transfection. In some experiments, cells were transfected with siRNA, split after 6 h, then transfected a second time 24 h after, and then analyzed 4 days after the initial transfection. All siRNAs were 21-nucleotide duplexes and had the following sequences: siRNA-GRK2, 5′-GAUUGCGUCAUCCUAUCUAGCaU GdTdT-3′; siRNA-GRK5, 5′-GAUCUCUGCUGCUAGAAdTdT-3′; and siRNA-SC, 5′-GGCGCGGUGA GGA UCC GdTdTdT-3′. Receptor Phosphorylation—HEK293 cells grown in 100-mm dishes were transfected with 5 μg of pcDNA3-HA-H1HR and 5 μg of pcDNA3-GRK2, GRK5, or GRK6 vector control. The following day, the cells were seeded into two 10-cm dishes for phosphorylation analysis and one 6-cm dish for radioimmunobinding (to confirm equivalent expression). Forty-eight h after transfection, the cells were washed twice in serum-free and sodium phosphate-free DMEM followed by incubation in the same medium for 1 h. The cells were subsequently labeled with 0.5 μCi of [32P]orthophosphate for 2 h and then incubated with or without 100 μM histamine for 10 min. The medium was removed, and the cells were washed three times with buffer (25 mM Tris-HCl, pH 7.5, 137 mM NaCl, 5 mM KCl, 0.9 mM CaCl2, 0.5 mM MgCl2, 0.075 mM Na3HPO4, 1 mM EGTA, and 10% calf serum). A 0.8 ml of protein A-agarose beads were added to the precleared supernatant, and the beads were resuspended in 50 μl of 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% (v/v) Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 1 mM phenylmethylsulfonyl fluoride, 5 mM benzamidine, 10 μg/ml leupeptin, and 2 μg/ml aprotinin. All subsequent steps were performed at 4 °C. The lysate was solubilized for 1 h on a rocker and then centrifuged at 100,000 × g for 20 min. The resultant supernatant was isolated and precleared by the addition of 50 μl of an ~50% slurry of protein A-agarose beads with gentle rocking for 50 min. Sixteen μl of anti-HA polyclonal antibody was then added to the precleared supernatant and incubated for 1 h; 50 μl of protein A-agarose beads were added, and the suspension was incubated on a rocker overnight. Immune complexes were collected by centrifugation the following day and washed 4 times with ice-cold lysis buffer. The beads were resuspended in 50 μl of SDS
sample buffer, and the immunoprecipitates were resolved on 10% polyacrylamide gels. The gels were stained with Coomassie Blue, destained, dried, and subjected to autoradiography at \( ^{32} \text{P} \) at \( 80 \, ^\circ\text{C} \).

**Inositol Phosphate Production**—Measurement of inositol phosphate (IP) production in cells was as described previously (21). Briefly, subconfluent HEK293 cells grown in 100-mm dishes were transfected with pcDNA3-HA-H1HR and GRK constructs using FuGENE-6. The total amount of transfected plasmids was adjusted to 10 \( \mu \)g by the addition of pcDNA3 vector. The following day, the cells were seeded onto a 24-well dish and labeled with \( ^{32} \text{P} \)-myo-[\( ^3\text{H} \)]inositol for 17–22 h in 0.5% bovine serum albumin in DMEM. The cells were washed two times and incubated with inositol-free DMEM containing 5 mM LiCl for 30 min at 37 \(^\circ\text{C} \). The cells were washed with 1 ml of 20 mM formic acid for 30 min at 4 \(^\circ\text{C} \) and then neutralized with 130 \( \mu \)l of 3% ammonium hydroxide. The inositol fractions were separated using Dowex AGX (100–200 mesh) columns, counted, and data reported as described under “Experimental Procedures.” Data are presented as the percent of mock-transfected cells stimulated with 100 \( \mu \)M histamine (% maximal control response) and represent the mean \( \pm \) S.E. of four independent experiments, each done in triplicate. In C and D, expression levels of GRK2 were analyzed by immunoblotting.

**Measurement of \( \text{Ca}^{2+}\) Flux**—HEK293 cells transfected with SC, GRK2, or GRK5 siRNAs were harvested with Cellstripper (Mediatech, Herndon, VA), washed twice with phosphate-buffered saline, and resuspended at \( 5 \times 10^6 \) cells/ml in Hanks’ balanced salt solution (140 mM NaCl, 5 mM KCl, 10 mM HEPES, pH 7.4, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), 1 mg/ml glucose) containing 0.025% bovine serum albumin. The cells were then loaded with 3 \( \mu \)M Fura-2 acetoxymethyl ester derivative (Fura-2/AM) (Molecular Probes, Eugene, OR) for 30 min at 37 \(^\circ\text{C} \). The cells were washed once in Hanks’ solution, resuspended in Hanks’, incubated at room temperature for 15 min, washed twice in Hanks’ solution, and then resuspended in Hanks’ at a concentration of \( \sim 3 \times 10^7 \) cells/ml. A typical experiment contained 1.5 \( \times 10^6 \) cells/1.6 ml in a quartz cuvette and stimulation with 1–1000 \( \mu \)M histamine. Receptor specificity studies were done by measuring the calcium flux stimulated by 100 \( \mu \)M histamine in the presence of H1-, H2-, or H3-specific antagonists. Calcium flux was measured using excitation at 340 and 380 nm in a fluorescence spectrometer (LS55, PerkinElmer Life Sciences). Calibration was performed using 0.1% Triton X-100 for total fluorophore release and 10 mM EGTA to chelate free \( \text{Ca}^{2+} \). Intracellular \( \text{Ca}^{2+} \) concentrations were calculated using a fluorescence spectrometer measurement program as described previously (26).

**RESULTS AND DISCUSSION**

**Pharmacological and Functional Properties of HA-tagged H1HRs**—Our initial series of studies focused on characterizing...
H1HRs transiently expressed in HEK293 cells. [3H]Pyrilamine binding assays demonstrated high affinity binding of recombinant H1HRs expressed in HEK293 cells (Kd = 1.5 ± 0.8 nM, Bmax = 4.0 ± 1.3 pmol/mg protein; n = 3), similar to previously reported values (27). Kd and Bmax values obtained for HA-H1HR (Kd = 0.8 ± 0.1 nM, Bmax = 5.5 ± 0.5 pmol/mg protein; n = 3) were similar to those for untagged H1HR. Histamine-stimulated IP production in HEK293 cells expressing H1HR (EC50 = 69 ± 13 nM, fold basal increase = 5.2 ± 1.3) and HA-H1HR (EC50 = 78 ± 16 nM, fold basal increase = 3.9 ± 0.3) was also comparable. Thus, the HA tag at the N terminus of the H1HR does not appear to affect H1HR expression, ligand binding, or signaling properties.

Role of GRKs in H1HR Phosphorylation—To assess the ability of the H1HR to undergo agonist-promoted phosphorylation, HEK293 cells were transfected with or without HA-H1HR and loaded with [32P]orthophosphate as described under “Experimental Procedures.” The HA-H1HR migrated as a broad band from HA-H1HR transfected cells (Fig. 1A, left panel). The H1HR also appeared as a diffuse H1HR band observed in the absence of GRK co-expression (Fig. 1B). Interestingly, all three GRKs also led to a mobility shift in the diffuse H1HR band observed in the absence of GRK co-expression to a much sharper band of ~90 kDa (Fig. 1B). Similar agonist-promoted mobility shifts have been observed with other GPCRs (28–30), and this likely reflects enhanced phosphorylation of specific residues on the H1HR.

GRK Function in Quenching H1HR-mediated Inositol Phosphate Production—We next examined the capacity and functional properties of GRKs in quenching H1HR signaling. Co-expression of GRK2 with HA-H1HR completely inhibited H1HR-mediated IP production (Fig. 2A), consistent with previous studies demonstrating the ability of GRK2 to effect rapid agonist-specific desensitization of numerous other GPCRs (1–3). Interestingly, catalytically inactive GRK2 (GRK2-K220R) (31), previously shown as being capable of acting in a dominant negative fashion to reverse desensitization of some GPCRs (1–3), also significantly attenuated histamine-stimulated IP production, suggesting that the kinase activity of GRK2 is partially dispensable in its ability to quench H1HR signaling.
catalytically inactive GRK2 quenches H1HR signaling and to clarify the role of discrete GRK2 functional domains in regulating H1HR signaling. Carman et al. (21) previously demonstrated that an N-terminal polypeptide containing residues 45–178 of GRK2 (GRK2-RGS) associates strongly with both the transition and activated states of Goα and functions as an RGS protein to inhibit Goα-stimulated PLC activity. We therefore tested the capacity of this N-terminal functional domain to inhibit H1HR-mediated IP production. Co-expression of GRK2-RGS and H1HR resulted in ~60% inhibition of histamine-stimulated IP production (Fig. 2B), demonstrating that the RGS function of GRK2 can effectively inhibit H1HR signaling. Conversely, co-expression of GRK2-CT, a C-terminal pleckstrin homology domain construct of GRK2 known to sequester Gβγ subunits necessary for activation of endogenous GRK2 (32, 33), significantly increased histamine-stimulated IP production. This result is similar to recent findings on the metabotropic glutamate 1 receptor (34) and supports a role for endogenous GRK2 in the regulation of H1HR signaling in HEK293 cells.

The involvement of the RGS domain of GRK2 in H1HR regulation was subsequently examined by employing a GRK2 mutant (GRK2-D110A) that lacks the ability to interact with Goα (22). Co-expression of GRK2-D110A significantly inhibited H1HR-mediated IP production (~80%; Fig. 2C) to an extent even greater than that observed for GRK2-RGS (Fig. 2B), suggesting that the RGS function of GRK is at least partially dispensable in the ability of GRK2 to inhibit H1HR signaling. Co-expression of GRK2-R106A, which also lacks the ability to interact with Goα (22), inhibited H1HR-mediated IP production in a manner identical to that affected by GRK2-D110A (data not shown).

To assess the requirement for kinase and RGS function in GRK2-mediated desensitization of the H1HR, we introduced the D110A mutation into catalytically inactive GRK2 to generate a construct lacking both kinase and RGS function (GRK2-K220R/D110A). Co-expression of GRK2-K220R/D110A with H1HR had a relatively weak inhibitory effect on IP production at low histamine concentrations but approached 40–50% at saturating concentrations (Fig. 2C). A possible explanation for this residual efficacy of GRK2-K220R/D110A is the steric hindrance of receptor-Goα coupling caused by GRK2-K220R/D110A binding to H1HR. By binding to the activated receptor, exogenous GRK mutants not only block access of endogenous GRK to the receptor, but potentially block receptor-Goα interactions. Because this latter function might be considered experimentally artifactual, we reduced the levels of co-expressed GRK constructs by reducing the concentration of their respective plasmid DNAs in our transfection protocol. Under these conditions, both GRK2 and GRK2-K220R largely retained their ability to inhibit H1HR-mediated IP production (Fig. 2D). However, lower levels of GRK2-K220R/D110A expression reduced the inhibitory effect of this construct to ~20% (Fig. 2D). These data suggest that steric hindrance of H1HR-Goα coupling probably contributes to inhibition of H1HR signaling under experimental conditions, although under physiologic conditions, GRK2 likely regulates H1HR signaling via its kinase and RGS activities.

We next examined the capacity of other kinases to effect H1HR desensitization. We first examined the effects of wild type GRK5 and GRK6, both shown to weakly phosphorylate H1HR (Fig. 1). Co-expression of GRK6 with H1HR had a modest effect on histamine-stimulated IP production, whereas expression of GRK5 attenuated IP production by ~50% at saturating concentrations of histamine (Fig. 3A). However, catalytically inactive GRK5 (GRK5-K215R) was ineffective in inhibiting H1HR signaling. This observation is consistent with a requirement for kinase activity in GRK5-mediated GPCR desensitization and the reported inability of GRK5 to associate with activated Goα (21).

Because the H1HR has been reported to be subject to heterogeneous desensitization by phorbol esters in a PKC-dependent manner (13), we also examined the role of PKC in agonist-stimulated IP production. Pretreatment of HEK293 cells with bisindolylmaleimide I had little effect on histamine-stimulated IP production, whereas expression of GRK5 attenuated IP production by ~50% at saturating concentrations of histamine (Fig. 3A). However, catalytically inactive GRK5 (GRK5-K215R) was ineffective in inhibiting H1HR signaling. This observation is consistent with a requirement for kinase activity in GRK5-mediated GPCR desensitization and the reported inability of GRK5 to associate with activated Goα (21).

Role of Endogenous GRKs in Regulating H1HR Signaling—While our overexpression studies suggest that GRK2 can promote H1HR phosphorylation and desensitization, we were also interested in evaluating the role of endogenous GRKs in H1HR regulation. To address this issue, we first analyzed GRK expression in HEK293 cells using monoclonal antibodies that are selective for either GRK2 and -3 or GRK4, -5, and -6 (35). HEK293 cells have readily detectable levels of GRK2 with little if any GRK3 (Fig. 4, upper panel), a finding previously shown by Schulz et al. (36) and confirmed using a GRK2-specific monoclonal (data not shown). These cells also have two primary bands that are detected by the GRK4–6 monoclonal, which we
believe are GRK5 and GRK6 (Fig. 4, middle panel). In an effort to reduce expression of endogenous GRKs, we generated siRNAs to specifically target GRK2 and GRK5. These GRKs were targeted because they had the ability to inhibit H1HR-stimulated inositol phosphate production when overexpressed (Figs. 2 and 3A). The siRNAs were transfected into HEK293 cells (two transfections were performed 24 h apart), and after 4 days, the cells were evaluated for GRK expression. Expression of GRK2 was effectively knocked down (>80%) by the GRK2 siRNA treatment but was unaffected in cells treated with the SC or GRK5-specific siRNA (Fig. 4, upper panel). Similarly, GRK5 (lower panel) was effectively knocked down (>80%) by treatment with the GRK5-specific siRNA but was unaffected by the SC or GRK2-specific siRNA (Fig. 4, middle panel). Immunoprecipitation of GRK5 with a subtype-specific polyclonal antibody and subsequent detection with the GRK4–6 monoclonal confirmed that GRK5 expression was effectively and specifically reduced by the siRNA treatments (data not shown). Thus, siRNAs can be used to specifically reduce expression of GRK2 and GRK5 in HEK293 cells.

HEK293 cells appear to contain endogenous histamine receptors, as histamine promotes a significant calcium flux in cells loaded with Fura-2/AM (Fig. 5A). This response was primarily due to the H1 subtype, because calcium flux stimulated by 100 μM histamine was effectively inhibited by the H1-specific antagonist pyrilamine (Fig. 5A) with an IC50 between 0.1 and 1 μM (Fig. 5B). In contrast, the H2-selective antagonist cimetidine and the H3 antagonist clobenpropit had no significant effect on the histamine-promoted calcium flux, although clobenpropit appeared to modestly increase the sustained calcium response (Fig. 5A). Taken together, these studies suggest that HEK293 cells primarily contain endogenous H1 histamine

![Figure 6: Effect of siRNA treatment on histamine-stimulated calcium flux in HEK293 cells.](http://www.jbc.org/)
and sustained phases (Fig. 6, upper panel) that the kinase activity of GRK2, as well as its ability to receptor desensitization. However, in contrast to the metabo-
time, the sustained calcium response requires extracellular calcium (data not shown) and may involve calcium influx through voltage-operated calcium channels (37). Lower doses of histamine, such as 10 and 200 μM, produced minimal effects on enhancement of the initial transient phase in GRK2 siRNA-treated cells but continued to produce significant enhancement of the sustained phase of calcium influx (Fig. 6, lower panels). In contrast, GRK5 siRNA treatment had no effect on H1HR-stimulated calcium flux (Fig. 6). Thus, our data reveal that endogenous GRK2 plays a significant role in the desensitization of the endogenous H1 histamine receptor in HEK293 cells.

Relevance of Multiple Desensitizing Functions Possessed by GRK2—A fundamental question raised by the present study concerns the need for two different domains of GRK2 in mediating H1HR regulation. Previous studies have noted that the desensitization of other Gq-coupled receptors, including the parathyroid (38), angiotensin II (39), endothelin 1 (40), 5-hydroxytryptamine 2C (41), and metabotropic glutamate 1 (34) receptors can be effected in a kinase-independent manner. In fact, the ability of the metabotropic glutamate 1 receptor to be inhibited by GRK2 appears to be dependent on the ability of GRK2 to bind both the receptor and Goq, but independent of receptor phosphorylation (42). This appears to be due to the ability of GRK2 to antagonize metabotropic glutamate 1 receptor interaction with Goq. In the present study, we extended these observations to the H1HR and clarified the importance of distinct functional domains within holo-GRK2 in mediating receptor desensitization. However, in contrast to the metabotropic glutamate 1 receptor results (42), our results suggest that the kinase activity of GRK2, as well as its ability to interact with Goq through its RGS domain, play complementary roles in inhibiting H1HR signaling after receptor activation.

The need for two distinct mechanisms contributing to H1HR regulation could potentially arise under conditions in which signal quenching is critical, but one mechanism is dynamically impaired. For Gq-coupled receptors, this possibility could arise in cells in which receptor activation promotes reduced GRK2 kinase activity through activation of Ca2+ sensor proteins such as calmodulin (i.e. negative feedback within a negative feedback mechanism) (43, 44). An alternative explanation is that in certain cell types, GRK levels may be limiting, as a function of either simply low expression (45, 46) or as a result of competition among multiple activatedGPCRs. In such instances, the bimodal nature (kinase/RGS activities) of GRK2, as well as GRK2 specificity toward a given Gq-coupled receptor, might favor the termination of the signal of that receptor in deference to the quenching of other signals. Lastly, the possibility exists that for certain forms of Gq-coupled receptor signaling, signal termination simply requires a much more rapid and powerful quenching mechanism than that afforded by GRK2 kinase activity alone.

Another important aspect of this work involves the use of siRNAs to selectively knock down expression of specific GRKs. Here we developed siRNAs to target GRK2 and GRK5, two of the major GRK isoforms present in HEK293 cells. Knockdown of GRK2 expression resulted in the significant enhancement of histamine-promoted calcium flux, whereas knockdown of GRK5 expression was without effect. These results demonstrate the importance of GRK2 in regulating H1HR function and verify the specificity established in the overexpression studies. Future studies examining GRK actions on additional endogenous Gq-coupled receptor signaling under relevant physiologic contexts, as well as analyses assessing the role of compartmentalization on GRK function, should help clarify the specificity and significance of the multifunctional nature of GRK2 in regulating Gq-coupled receptor signaling.

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