Agonist-Promoted Homologous Desensitization of Human Airway Smooth Muscle Bitter Taste Receptors

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Bitter taste receptors (TAS2Rs) were shown to be expressed in human airway smooth muscle (ASM). They couple to specialized $[Ca^{2+}]_i$ release, leading to membrane hyperpolarization, the relaxation of ASM, and marked bronchodilation. TAS2Rs are G-protein-coupled receptors, known to undergo rapid agonist-promoted desensitization that can limit therapeutic efficacy. Because TAS2Rs represent a new drug target for treating obstructive lung disease, we investigated their capacity for rapid desensitization, and assessed their potential mechanisms. The pretreatment of human ASM cells with the prototypic TAS2R agonist quinine resulted in a $31\% \pm 5.1\%$ desensitization of the $[Ca^{2+}]_i$ response from a subsequent exposure to quinine. No significant change in the endothelin-stimulated $[Ca^{2+}]_i$ response was attributed to the short-term use of quinine, indicating a homologous form of desensitization. The TAS2R agonist saccharin also evoked desensitization, and cross-compound desensitization with quinine was evident. Desensitization of the $[Ca^{2+}]_i$ response was attenuated by a dynamin inhibitor, suggesting that receptor internalization (a G-protein coupled receptor kinase [GRK]-mediated, β -arrestin-mediated process) plays an integral role in the desensitization of TAS2R. Desensitization was insensitive to antagonists of the second messenger kinases protein kinase A and protein kinase C. Using intact airways, short-term, agonist-promoted TAS2R desensitization of the relaxation response was also observed. Thus these receptors, which represent a potential novel target for direct bronchodilators, undergo a modest degree of agonist-promoted desensitization that may affect clinical efficacy. Collectively, the results of these mechanistic studies, along with the multiple serines and threonines in intracellular loop 3 and the cytoplasmic tail of TAS2Rs, suggest a GRK-mediated mode of desensitization.

Keywords: airway smooth muscle relaxation; taste receptors; tachyphylaxis; phosphorylation; G-protein-coupled receptor kinases

Airway smooth muscle (ASM) expresses a large repertoire of G-protein-coupled receptors (GPCRs) that act to constrict (primarily G_q -coupled) or relax (primarily G_s -coupled) ASM and thereby regulate airway caliber (1). Responding to locally generated agonists such as acetylcholine and leukotrienes, the bronchoconstrictive GPCRs have been targets for therapeutic antagonists for the treatment of asthma and chronic obstructive pulmonary disease. Direct bronchodilating agonists acting at G_s -coupled receptors currently used for therapy are restricted to β -agonists, acting at ASM β_2 -adrenergic receptors (β_2ARs) via a cyclic adenosine monophosphate (cAMP)/protein kinase A-mediated mechanism. We recently undertook studies to identify novel GPCR pathways that evoke the relaxation of

CLINICAL RELEVANCE

Bitter taste receptors were recently found on airway smooth muscle, and when activated caused relaxation through a calcium-dependent mechanism. Agonists to these receptors may comprise a new class of useful direct bronchodilators for treating obstructive lung disease, but because they are members of the G-protein-coupled receptor superfamily, they may undergo desensitization. We show a modest amount of desensitization of the calcium response that appears to be mediated by the family of G-protein-coupled receptor kinases, and this desensitization translates into physiologic desensitization in intact airways.

ASM and that could also be used for therapeutic purposes, thereby providing additional means for direct bronchodilation (2, 3). We found the expression on human ASM of multiple bitter taste receptors (TAS2Rs) (3), a family of GPCRs composed of 25 members. Human ASM was found to express six dominant TAS2Rs (subtypes 10, 14, 31, 5, 4, and 3), with substantially lower expression of an additional 11 subtypes. ASM TAS2Rs appear to signal by gustducin-associated $\beta\gamma$ -activating phospholipase-C β (PLC β), generating inositol 1,4,5 tri-phosphate that stimulates the release of calcium within a spatially restricted and temporally distinct compartment (3). This signal leads to the activation of the large conductance Ca^{2+} -activated K^+ channel, causing membrane hyperpolarization and the marked relaxation of ASM *in vivo* and *ex vivo*. The relaxation of constricted ASM from bitter tastants acting at TAS2Rs was greater in magnitude than we found for any other direct bronchodilator, including the full β -agonist isoproterenol (3). These studies gave rise to the idea that TAS2Rs may constitute a new therapeutic target for the treatment of obstructive lung disease.

A frequently encountered property of GPCR signaling involves desensitization, defined as a loss of signaling during persistent or repetitive agonist exposure (4). This loss of function may limit the therapeutic efficacy of agonists by rapidly attenuating the early signal, or by evoking tachyphylaxis during prolonged treatment. The earliest event in GPCR desensitization is phosphorylation of the receptor by kinases such as G-protein-coupled receptor kinases (GRKs) or "feedback" kinases such as protein kinase A and C (PKA and PKC, respectively). To assess the potential therapeutic liability of desensitization, we characterized the short-term desensitization properties of TAS2R expressed on human ASM.

MATERIALS AND METHODS

Cell Culture

Human ASM cells were obtained from Clonetics (Lonza, Walkersville, MD) and grown as previously described (3, 5) in 10% FCS at 37°C in a 95% air, 5% CO₂ environment. The day before the experiments, cells were detached and plated in 96-well plates (Corning, Inc., Corning,

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NY) coated with Type 1 collagen, at a density of 100,000 cells/well. Twenty-four hours later, cells were more than 95% confluent, and were used for the $[Ca^{2+}]_i$ experiments.

$[Ca^{2+}]_i$ Measurements

Cells in 96-well plates were loaded with the fluorescence calcium indicator Fluo-4AM (BD Biosciences, Franklin Lakes, NJ) in Hanks' balanced salt solution (HBSS) without phenol red and containing 1.5 mM $CaCl_2$ and 1.15 mg/ml probenecid (loading buffer) for 1 hour. The addition of agonists, excitation at 485 nm, and detection at 525 nm were performed by robotic pipetting, excitation, and a signal acquisition system (FlexStation II; Molecular Devices, Downingtown, PA). Data were acquired every 1.52 seconds/well for 60 seconds, and are expressed as relative fluorescence units (RFUs). Unless otherwise indicated, these data are shown after the subtraction of background fluorescence.

ASM $[Ca^{2+}]_i$ Desensitization

For agonist-promoted desensitization studies, cells were loaded with Fluo-4AM as already described, except that during the final 15 minutes (or other time periods), the agonist was added. Cells were then washed three times with 37°C HBSS for 10 seconds, the wells were filled with 200 μ l loading buffer, and the $[Ca^{2+}]_i$ assays were immediately performed with the indicated agonists, as already described. Some experiments included various receptor agonists, enzyme inhibitors, or enzyme activators. For clarity when presenting the data, these agents are denoted as a "pretreatment" (indicating a treatment before the addition of a second agent) and as " $[Ca^{2+}]_i$ stimulus" (indicating the agent added in the FlexStation after cells were washed). Desensitization was calculated as $[(R_{max1} - R_{max2}) \div R_{max1}] \times 100\%$, where R_{max1} is the net maximal RFU response with only carrier (typically HBSS) as the pretreatment, and R_{max2} is the net maximal RFU response under conditions of pretreatment with the indicated agent. Unless otherwise noted, the concentrations of agents were 1.0 mM for quinine, 1.0 mM for saccharin, 10 μ M for bradykinin, 100 μ M for histamine, 100 nM for endothelin, 10 μ M for isoproterenol, 10 μ M for dibutyryl cAMP, 100 nM for H89, 100 nM for phorbol 12-myristate 13-acetate (PMA), 10 μ M for dynasore, and 10 μ M for Bis1.

Intact Airway Desensitization

These studies were approved by the Institutional Animal Care and Use Committee of the University of Maryland (Baltimore, MD). Intact, third-order bronchi were rapidly dissected from the right upper lobe of a euthanized rhesus macaque. Five-millimeter rings were prepared, and *ex vivo* studies were performed using a myograph (AD Instruments, Colorado Springs, CO), exactly as previously described for murine tracheas (3). Airway rings were passively stretched to 5 mN, and contracted with 100 μ M methacholine. After stabilization, 300 μ M of quinine were added to the bath (with methacholine still present), and the relaxation response was determined. Subsequently, the rings

were washed three times and then incubated with the desensitizing dose (1.0 mM) of quinine for 15 minutes at 37°C. After incubation, the rings were washed three times and contracted with 100 μ M methacholine, and 300 μ M of quinine were added to the bath as before, to determine the relaxation response after exposure to the desensitizing dose. The percent desensitization was calculated in the same manner as for the $[Ca^{2+}]_i$ experiments.

Statistical Analysis

Comparisons were performed according to two-way paired or unpaired *t* tests, with significance set at $P < 0.05$. When *P* values are assigned to desensitization, they represent comparisons of raw data between cells or airways in the presence or absence of treatment. In comparable studies under multiple conditions, data were subjected to ANOVA with *post hoc t* tests. Results are presented as mean $\pm$ SE.

RESULTS

For most of the present experiments, we used the prototypic TAS2R agonist quinine because it activates four of the highest-expressing TAS2Rs in human ASM (i.e., TAS2Rs 10, 14, 31, and 4) (3, 6). Because we previously showed a direct correlation between the whole-cell, TAS2R-mediated $[Ca^{2+}]_i$ response and the relaxation response (3), we used real-time measurements of $[Ca^{2+}]_i$ as the functional readout for desensitization. ASM cells were pretreated with quinine (or HBSS alone) at 37°C for 15 minutes, washed for 10 seconds, and then re-exposed to quinine with the $[Ca^{2+}]_i$ transients measured for the next 60 seconds. In addition, the effect of quinine pretreatment on endothelin-mediated $[Ca^{2+}]_i$ stimulation was also determined, to monitor the potential nonreceptor (heterologous) components of desensitization, such as from the depletion of sarcoplasmic reticulum Ca^{2+} stores. Finally, some cells were pretreated with bradykinin, washed, and rechallenge with bradykinin, which acted as a positive control because the B2-bradykinin receptor is known to undergo rapid agonist-promoted desensitization (7). Figure 1 shows the results of such experiments. Quinine pretreatment evoked a decrease in subsequent peak quinine-stimulated $[Ca^{2+}]_i$ (Figures 1A and 1B), equivalent to a $31\% \pm 5.1\%$ desensitization. (When the $[Ca^{2+}]_i$ signals from these same experiments were quantitated by the area under the curve, the extent of desensitization amounted to $28\% \pm 6.3\%$, which is not different from the calculated desensitization obtained from peak $[Ca^{2+}]_i$ signals.) With quinine pretreatment, the $[Ca^{2+}]_i$ response to endothelin was not desensitized (Figures 1A and 1B), suggesting that heterologous processes common to both signaling pathways were not perturbed under these conditions. Bradykinin-mediated desensitization of the

Figure 1. Agonist-promoted desensitization of bitter taste receptors (TAS2Rs) in human airway smooth muscle (ASM). Fluo-4AM-loaded cells were pretreated with buffer alone (control) or 1 mM quinine for 15 minutes, washed, and exposed to 1 mM quinine or 100 nM endothelin with an immediate acquisition of $[Ca^{2+}]_i$ during the next 60 seconds. Similar experiments were performed with pretreatment using 10 μ M bradykinin and subsequent re-exposure to bradykinin. (A) Representative $[Ca^{2+}]_i$ tracings show a desensitization of the quinine response, but not the endothelin response, to pretreatment

with quinine. (B) Mean results of the maximal $[Ca^{2+}]_i$ response under the indicated conditions. Columns 1 and 2 represent bradykinin-promoted desensitization of the bradykinin B2 receptor, which acted as positive control. Columns 3 and 4 show the quinine-promoted desensitization of TAS2Rs, which amounted to $31\% \pm 5.1\%$. Columns 5 and 6 show no desensitization of the endothelin-mediated $[Ca^{2+}]_i$ response from pretreatment with quinine, indicative of a lack of heterologous loss of $[Ca^{2+}]_i$ signaling during quinine-promoted TAS2R desensitization. Results are from 4–7 experiments. ctl, control; bra, bradykinin; qui, quinine; end, endothelin. $*P < 0.01$ versus control condition. RFU = relative fluorescence units.

B2-bradykinin receptor revealed $94\% \pm 2.0\%$ desensitization (Figure 1B), in agreement with previous reports on this receptor (7). Additional studies were performed with the structurally distinct TAS2R agonist saccharin, which activates TAS2R31 in human ASM (3). Pretreatment with saccharin evoked a $45\% \pm 3.4\%$ desensitization of subsequent saccharin-promoted $[Ca^{2+}]_i$ ($P < 0.01$, $n = 7$). Furthermore, cross-compound desensitization was evident, insofar as pretreatment with quinine evoked desensitization of the subsequent saccharin response, amounting to $39\% \pm 9.7\%$ ($P < 0.01$, $n = 4$). These data indicate that the TAS2R response to agonist is acting at these receptors and undergoing desensitization, rather than some other (nonreceptor) effect of these agents. Thus, known TAS2R agonists with diverse structures would be unlikely to evoke similar nonreceptor-mediated events. The greater degree of desensitization with saccharin (which activates only one TAS2R subtype) compared with quinine is likely attributable to quinine activating four TAS2Rs and thus having a “collective receptor reserve.” Because we envision an agent that activates multiple TAS2Rs in human ASM as a new therapy, quinine was used in subsequent studies. With both of these TAS2R agonists, which display low apparent affinity for these receptors (3), no desensitization was evident at 100-fold lower concentrations (10 μ M) of the challenge dose (data not shown).

Human ASM TAS2R desensitization was evident as early as 5 minutes after exposure to quinine, and became progressively greater with increasing incubation times (Figure 2A). However, with quinine exposures of 30 and 60 minutes, endothelin-

Figure 2. Time dependence of homologous desensitization of TAS2R in human ASM. Cells were treated for the indicated times with quinine, washed, and stimulated with either quinine or endothelin. (A) Desensitization of the quinine response, uncorrected for heterologous loss of endothelin signaling. (B) Desensitization of the endothelin response after pretreatment with quinine for the indicated times. Results are from 3–5 experiments.

mediated responses were also reduced at these time points (Figure 2B). This effect was also evident at longer time points, when bradykinin was used after pretreatment with quinine (data not shown). These results suggest that a nontrivial heterologous mechanism is in play at these time points, and thus to assess quinine-specific desensitization for longer periods of quinine pretreatment would be difficult. The rapid homologous desensitization of TAS2Rs in human ASM suggests a phosphorylation-dependent mechanism, such as those described for other GPCRs. The potential for the second messenger-dependent kinases PKA and PKC to evoke the desensitization of TAS2R was explored via pretreatment with the phorbol ester PMA and the cell-permeable cAMP analogue dibutyryl cAMP. To assess potential non-PKA-mediated crosstalk between β_2 ARs and TAS2Rs, cells were also pretreated with the β -agonist isoproterenol. None of these agents evoked desensitization of the quinine response, suggesting that PKA and PKC are not involved in the desensitization of TAS2Rs; nor do we have evidence of β_2 AR–TAS2R crosstalk (Figure 3). Because TAS2Rs couple to PLC β , which activates PKC, we were particularly concerned about a negative feedback loop via this kinase during desensitization. To explore this further, we used the PKC inhibitor Bis1. The H1-histamine receptor is known to be desensitized by phorbol esters (8), so the concentrations of these agents and the conditions in human ASM cells were optimized through this positive control. As shown in Figure 4, histamine-mediated increases in $[Ca^{2+}]_i$ were decreased by more than 80% with a 15-minute exposure to 0.1 μ M PMA, and this response was completely blocked by 10 μ M Bis1. These concentrations of kinase activators and inhibitors are comparable to what we previously used for these types of experiments (9–11). Under these conditions, the quinine-promoted desensitization of TAS2Rs was then studied to explore further the potential role of PKC. Bis1 exerted no effect on quinine-promoted desensitization (Figure 4). Moreover, complementing the experiments in Figure 3, the PKA inhibitor H89 exerted no effect on the desensitization of TAS2R.

These results suggest that TAS2Rs may undergo desensitization by GRK-mediated phosphorylation, leading to an uncoupling of the receptor to the G-protein and a loss of cell-surface

Figure 3. Activators of protein kinases A and C (PKA and PKC, respectively) do not mimic the quinine-promoted desensitization of human ASM TAS2Rs. Cells were exposed to buffer control (ctl), quinine (qui), the phorbol-ester phorbol myristate acetate (PMA), the cell-permeable cyclic adenosine monophosphate (cAMP) analogue dibutyryl cAMP (dbc), or the β -agonist isoproterenol (iso) for 15 minutes, washed, and stimulated with quinine, followed by an immediate acquisition of $[Ca^{2+}]_i$. None of these pretreatments desensitized quinine-mediated $[Ca^{2+}]_i$ responses (columns 1–5). The concentration of PMA used (0.1 μ M) for these experiments was sufficient to desensitize the histamine (his) H1 receptor, and 10 μ M Bis1 (bis) blocked the effect of PMA (columns 6–8). Results are from four or more experiments. * $P < 0.001$ versus indicated control samples.

Figure 4. The inhibition of PKA and PKC fails to block the quinine-promoted desensitization of TAS2Rs in human ASM cells. Cells were pretreated with the PKC inhibitor Bis1 (bis), or bis with quinine (qui), for 15 minutes. Cells were washed and then exposed to quinine with an immediate acquisition of $[Ca^{2+}]_i$ (columns 3 and 4). In a similar manner, cells were treated with the PKA inhibitor H89 (columns 5 and 6). No difference was evident in the extent of quinine-promoted desensitization under these conditions, compared with conditions in the absence of kinase inhibitors (columns 1 and 2). Results are from four or more experiments. * $P < 0.001$ versus indicated control (ctl).

receptor expression by internalization. To date, the recombinant expression of a TAS2R to the level necessary for measuring phosphorylation has not been achieved. However, the internalization of GPCRs was shown to be dynamin-dependent (12). We reasoned that if dynamin function was inhibited, leading to a lack of internalization, then the agonist-promoted desensitization of TAS2Rs would be attenuated if this process was involved. Cells were pretreated with the cell-permeable dynamin inhibitor dynasore (13, 14) and exposed to HBSS alone (control) or quinine for 15 minutes, washed, and challenged with quinine, with the immediate acquisition of $[Ca^{2+}]_i$ signals. Under control conditions, quinine-promoted desensitization amounted to $32\% \pm 4.5\%$, whereas exposure to dynasore reduced quinine-promoted desensitization to $13\% \pm 4.2\%$ ($P < 0.02$ versus control, $n = 4$).

To ascertain the physiologic relevance of TAS2R desensitization, airway rings from monkey lungs were studied *ex vivo* in a serial fashion (for details, see Materials and Methods). Rings were contracted with methacholine and then exposed to quinine to ascertain the baseline relaxation response, washed, and treated

Figure 5. Agonist-promoted desensitization of TAS2R-mediated relaxation of intact airways. Bronchial rings were contracted with methacholine (mch), and the extent of relaxation by quinine was determined before, and after, a 15-minute pretreatment and washout with quinine (see MATERIALS AND METHODS). * $P < 0.01$, $n = 7$.

with quinine for 15 minutes at 37°C (the same desensitizing conditions used for the $[Ca^{2+}]_i$ experiments). After washing, the rings were contracted with methacholine, and the relaxation response to quinine was again determined to ascertain potential loss of function. Under these conditions, quinine-promoted desensitization was evident (Figure 5). In the absence of the 15-minute pretreatment with quinine, quinine-promoted relaxation amounted to $53\% \pm 5.1\%$, whereas the quinine-promoted relaxation amounted to $36\% \pm 4.7\%$ ($P < 0.01$) with pretreatment.

DISCUSSION

We recently identified (3) TAS2Rs in human ASM, and found that they signal via $[Ca^{2+}]_i$ to a pathway that leads to marked relaxation, suggesting a novel pathway for a new class of direct bronchodilators to treat obstructive lung diseases. Here, we show that TAS2Rs undergo homologous desensitization of the $[Ca^{2+}]_i$ response in ASM cells, as well as a smooth muscle relaxation response in intact airways. Desensitization was proposed as a necessary integrative and homeostatic cellular response to the activation of GPCRs. However, desensitization can impair the efficacy of therapeutic agonists acting at GPCRs by quenching some portion of the acute response to the agonist (15) or by promoting tachyphylaxis to the long-term activation of agonists (4). The rapid events that are evoked during the initial exposure often set into motion subsequent processes leading to the chronic loss of responsiveness. For example, short-term agonist exposure to $\beta_2\text{AR}$ results in receptor phosphorylation by PKA and GRKs. The PKA-mediated phosphorylation serves to partially uncouple receptors from $G_{\alpha s}$ and to promote coupling to $G_{\alpha i}$, thereby reducing the cAMP signal. The phosphorylation by GRK of $\beta_2\text{AR}$ promotes the binding of β -arrestins, which interdict between the receptor and $G_{\alpha s}$, and also contributes to receptor uncoupling and reduced cAMP signaling. GRK phosphorylation and β -arrestin binding also initiate the internalization of $\beta_2\text{AR}$ s via a clathrin-dependent and dynamin-dependent mechanism. Internalized $\beta_2\text{AR}$ s are thus removed from signal transducing and contribute to desensitization, and begin the process of moving receptors to a degradation pathway (3). The GRK-mediated phosphorylation occurs during persistent exposure to agonists, leading to a down-regulation of the net level of receptor expression.

Although not all GPCRs undergo rapid, agonist-promoted desensitization (16), the majority appear to do so, with phosphorylation the most common mechanism leading to uncoupling between the receptor and the G-protein. The feedback regulation of receptors caused by immediate downstream effectors is readily appreciated, prompting us to consider that if ASM TAS2Rs undergo rapid desensitization, then it may occur via the PKC phosphorylation of the receptor, because this kinase is activated by TAS2Rs in this cell type (3). For GPCRs, such phosphorylation was reported to occur on intracellular residues within the G-protein-coupling regions, which are typically the third intracellular loops and cytoplasmic tails. The alignment of these regions for the six highest-expressing TAS2Rs in human ASM is shown in Figure 6. Using the proposed consensus sequences for the phosphorylation of PKC (17, 18) and the phosphorylation prediction program Phosphorylation Site Database (PHOSIDA) (19), we found no strong *in silico* evidence for PKC phosphorylation sites in the third intracellular loops, consistent with finding a lack of PKC inhibition affecting the quinine-mediated desensitization of TAS2Rs, and, no phorbol ester-promoted TAS2R desensitization. We did not observe an increase in intracellular cAMP with TAS2R agonists (3). However, given the known desensitizing effect of cAMP-dependent PKA on several hundred distinct

Figure 6. Alignment of amino-acid sequences of the third intracellular loops and cytoplasmic tails of TAS2Rs. Sequences represent the six most highly expressed TAS2Rs in human ASM. Quinine activates TAS2Rs 10, 14, 31, and 4. The schematic provides orientation to the seven-transmembrane topology, and is based on TAS2R10. COOH, carboxy terminus; ECL, extracellular loop; ICL, intracellular loop; TM, transmembrane.

GPCRs, we were also prompted to assess this mechanism. We found no evidence for a PKA-mediated mechanism involved with TAS2R agonist-promoted desensitization. Furthermore, the lack of desensitization of TAS2R by isoproterenol suggests that TAS2R agonist therapy may not be compromised by concomitant treatment with a β -agonist.

Given the general paradigm established for the desensitization of the GPCR superfamily (4), we conclude that the homologous desensitization of ASM TAS2Rs is mediated by one or more GRKs. No consensus sequence has been established for GRK phosphorylation. Early studies with synthetic peptides suggested that Ser/Thr in close proximity to acidic residues may represent a GRK2 phosphorylation site (20). Given that phosphorylated Ser are somewhat acidic, this broadens the number of potential GRK phosphorylation sites, based on sequential phosphorylation. Moreover, we showed that all four Ser in the sequence EESSSS of adrenergic receptors are phosphorylated by GRK2 (21). However, the GRK phosphorylation sites that were found in multiple GPCRs do not necessarily fit this paradigm, indicating a complex interaction. Furthermore, the conformation of the loop or tail can affect whether a given Ser or Thr is in position to be phosphorylated by GRKs (22). This conformation is established not only by the amino-acid sequence of the intracellular segment, but also by the transmembrane regions (22). This concept is entirely consistent with agonist binding (which stabilizes the conformation of the transmembrane domains) as a requirement for GRK-mediated desensitization. Given this caveat, predicting the potential GRK-mediated phosphorylation sites within TAS2Rs is not possible. Nevertheless, we note multiple Ser/Thr within the relatively short intracellular loops and cytoplasmic tails of these receptors, which might serve as GRK phosphorylation sites (Figure 6). High levels of the recombinant expression of TAS2Rs and their purification have yet to be achieved, so these issues cannot yet be addressed. However, we found that inhibiting dynamin, a required component of the GRK-initiated internalization process, attenuated TAS2R desensitization. This result further supports the notion that TAS2R desensitization is mediated by GRKs. Interestingly, TAS2R desensitization to longer exposures of agonist resulted in a decrease in the $[Ca^{2+}]_i$ response to endothelin and bradykinin.

This heterologous event, if generalized to other G_q -coupled receptors, may be advantageous during prolonged TAS2R agonist therapy for asthma, in that bronchoconstrictive receptors may become less functional while bronchodilation via TAS2Rs is ongoing.

In conclusion, we show a homologous, agonist-promoted desensitization of TAS2R signaling to $[Ca^{2+}]_i$ in ASM cells, as well as a desensitization of the relaxation response in intact airways. This desensitization appears to be independent of the second messenger-dependent kinases PKC and PKA, and is consistent with a GRK-dependent process. Whether this degree of desensitization limits therapeutic efficacy in humans awaits clinical trials, but some loss of efficacy may be expected.

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