G protein-coupled receptor kinase 5 regulates airway responses induced by muscarinic receptor activation


Five muscarinic cholinergic receptor subtypes (M1–M5) have been cloned, and four subtypes can be distinguished pharmacologically (21). Radioligand binding studies demonstrate differential distribution of M1–M3 muscarinic receptor subtypes in airways (18). In brief, M1 muscarinic receptors, located at the parasympathetic ganglion, are thought to filter the transmission of the neuronal signal. M3 muscarinic receptors mediate acetylcholine-induced contraction of airway smooth muscle (36) (Fig. 1). M2 muscarinic receptors, located both pre- and postjunctionally, effect dual regulation of airway smooth muscle tone (22). In a classic negative feedback loop, prejunctional M2 muscarinic receptors limit the amount of acetylcholine released from stimulated cholinergic nerves, thereby limiting the level of bronchoconstriction (12). In contrast, postjunctional M2 muscarinic receptors, through the activation of Gq, can enhance the overall level of bronchoconstriction by opposing Gs-mediated events, including activation of adenylyl cyclase or opening of large conductance calcium-activated potassium (KCa) channels.

Characteristic of G protein-coupled receptors (GPCRs), muscarinic receptor signaling is regulated by the coordination of two critical events. GPCR kinases (GRKs) phosphorylate the agonist-activated receptor, and this chemical modification promotes binding of an arrestin protein to the receptor. These events terminate further receptor activation of downstream G protein signaling while at the same time promoting arrestin-dependent signals.

We recently generated mice lacking the GRK5 gene (GRK5−/−) and showed that behavioral responses mediated by central muscarinic receptors were enhanced and prolonged in these mice when a muscarinic receptor agonist was administered (16). Because GRK5 is a ubiquitously expressed member of the GRK family, we hypothesized that in addition to regulating central muscarinic receptors, GRK5 might also regulate responses mediated by peripheral muscarinic receptors.

Because the mechanism of muscarinic receptor “dysfunction” as it relates to airway responsiveness is not well defined, it is important to determine whether GRK5 modulates muscarinic receptor-mediated airway responses. We measured tracheal pressure in GRK5−/− and littermate wild-type (WT) control mice during bilateral vagal stimulation or intravenous injection of the muscarinic receptor agonist methacholine (MCh). Because GRK5 mRNA is robustly expressed in the

Numerous studies indicate that dysfunction of either pre- or postjunctional airway M2 muscarinic receptors is implicated in the pathophysiology of asthma. Evidence suggests that human asthmatics and animal models of asthma demonstrate prejunctional M2 receptor dysfunction (14, 15, 23). Other studies show that the activity of postjunctional M2 muscarinic receptors is exaggerated in sensitized tracheal tissue, resulting in diminished responsiveness to β2-adrenergic receptor (β2-AR) agonist-mediated relaxation (11, 19, 20, 43).

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whether GRK5 regulates heart rate.

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tors limit the amount of ACh released from stimulated cholinergic nerves, as assessed by the 

M2 muscarinic receptors mediate parasympathetic control over cardiac chronotropy, where M2 muscarinic receptors with little or no direct effect on smooth muscle, as assessed by the 

Fig. 1. M2 muscarinic receptors mediate acetylcholine (ACh)-induced contraction of airway smooth muscle (ASM). Prejunctional M2 muscarinic receptors limit the amount of ACh released from stimulated cholinergic nerves, thereby limiting the level of bronchoconstriction. In contrast, activation of postjunctional M2 muscarinic receptors can enhance the overall level of bronchoconstriction by opposing -mediated ASM relaxation, as might occur through activation of -adrenergic receptors ( -ARs), prostaglandin E2 receptors (PGE2), or through vasoactive intestinal polypeptide (VIP) recep -
tors. GRK5, G protein-coupled receptor kinase 5.

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Methods

Generation of GRK5(-/-) mice and genotyping. The murine GRK5 gene was deleted as described previously, and genotyping was performed using triplex PCR on DNA purified from tail tips (16). Heterozygous SVJ129xC57BL/6 mice were interbred to obtain the littermate WT and GRK5-deleted animals used in this study.

General. Mice were acclimated to an ambient temperature of 21-22°C and a photoperiod between 0600 and 2000. They were fed standard mouse chow and allowed free access to water. Experiments were carried out between 0800 and 1800. All experimental procedures conformed to the guidelines of the Canadian Council of Animal Care and were approved by the Queen’s University Animal Care Committee and Duke University Institutional Animal Care and Use Committee.

In vivo animal preparation and experimental protocols. Animals were surgically prepared as previously reported (42). Briefly, mice were anesthetized with pentobarbital sodium (60 mg/kg) and mechanically ventilated through a steel cannula inserted into the trachea. A catheter in the jugular vein was used for intravenous administration of anesthetic and other drugs (injection volumes of 1 ml/kg). In respiratory protocols, mice were paralyzed with doxacurium chloride (0.25 mg/kg) to prevent respiratory efforts. Additional doses of doxacurium chloride were administered as necessary, and additional doses of anesthetic were administered at regular time intervals after paralysis. Mice were ventilated with 100% oxygen at a constant volume of 8-10 ml/kg and a frequency of 125 breaths/min. Measurement of airway pressure was made at a side port of the tracheal cannula connected to a Validyne differential pressure transducer. Electrocardiogram electrodes were placed subcutaneously for the measurement of heart rate. For studies involving vagal stimulation, the right and left branches of the vagus nerve were isolated from the carotid artery, placed on respective stimulating electrodes, and immersed in mineral oil. Electrocardiogram signals and tracheal pressure were acquired by computer and analyzed using peak detection software. Airway responses were denoted as airway pressure time index (APTI), which was calculated as the sum of the MCh-induced change in peak airway pressure integrated with respect to time. APTI was calculated every 10 s from the time of injection until the peak pressure plateaued (2 min). Others have validated this measure as a reasonable index of airway responsiveness (6, 10, 27). Average heart rate was calculated using the instantaneous heart rate derived from the electrocardiogram R-R interval.

Experimental Protocols

MCh injection. Increasing doses of MCh (25, 50, 100, and 150 µg/kg) were injected intravenously into anesthetized, paralyzed, and ventilated mice. Respiratory efforts were closely monitored to prevent their interference with accurate measurement of tracheal pressure. Mice were hyperinflated 1 min before each MCh injection to establish a constant volume history and respiratory mechanics. Each MCh injection was separated by a 5-min recovery period.

Vagal stimulation. A separate group of mice was surgically prepared for vagal stimulation by placing a length of nerve on platinum electrodes. A section of nerve proximal to the electrodes was crushed to prevent afferent nerve stimulation. Nerves were activated with a supramaximal stimulus (16 V, 2-ms duration) to ensure recruitment of all vagal efferents for a period of 10 s and at various stimulus frequencies (5, 10, 15, and 20 Hz). Bilateral vagal stimulation was randomized in the following order: 10, 15, 5, and 20 Hz, and a 5-min recovery period (between stimulations) was allowed. Mice were injected with propranolol (1 mg/kg) and doxacurium chloride (0.25 mg/kg) before the initiation of the vagal stimulation frequency response protocol.

In vitro tissue preparation and protocols. The trachea was immediately removed from mice (that had been killed) and placed in ice-cold oxygenated Krebs solution containing (in mM): 118 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgCl2, 25.0 NaHCO3, 1.2 NaH2PO4, and 11 glucose, pH 7.35-7.40. Five to seven cartilage rings were cut, and smooth muscle was exposed. The tracheal preparation was mounted with small pins and surgical suture (4.0 silk) between platinum ring electrodes under an initial load of 0.4-0.6 g in an organ tissue bath (Radnoti, Monrovia, CA) containing Krebs solution. The Krebs solution was bubbled continuously with a mixture of 95% O2 and 5% CO2 and maintained at 37°C ± 1°C. Tracheal ring preparations were allowed to equilibrate for at least 60 min before being adjusted to optimal length. The isometric force of tracheal smooth muscle was measured using a calibrated force displacement transducer (Grass) and acquired using a computer data acquisition program (CODAS, DATAQ). A Grass S88 stimulator was used to produce electric field stimulation of tissues by passing current pulses between ring electrodes. In all protocols, pulses of 2-ms duration, 50 V, and 20 Hz, were applied for 10 s. These stimulus parameters were chosen based on preliminary studies that demonstrated maximal contractile tension with little or no direct effect on smooth muscle, as assessed by the ability of 1 µm of tetrodotoxin to abolish the response. For the KCl depolarization protocol, equimolar KCl was substituted for NaCl in the Krebs solution (32).

Protocols. Four protocols were conducted on each tracheal ring preparation with washout between protocols. The protocols, in order of occurrence, were as follows: 1) complete depolarization with 90 mM KCl; 2) cumulative concentration response to carbachol; 3) relaxation over 60 min after precontracting with carbachol to 70% of maximum; and 4) relaxation with increasing concentrations of isoproterenol after precontracting with carbachol to 70% of maximum. In a separate experiment, tissues were precontracted with 90 mM KCl and relaxed with increasing doses of isoproterenol. In a second group of mice, the following protocol was conducted: 1) complete depolarization with 90 mM KCl; 2) cumulative concentration response to
carbachol; and 2) relaxation with increasing concentrations of isoprote-nerol after blocking M2 receptors with 0.3 μM methoctramine and precontracting with carbachol to 78% of maximum.

Western blots. Airway tissues including lung and trachea were removed and stored at −80°C until assayed for protein. Stored tissues analyzed for GRK5 protein were homogenized using liquid nitrogen and mortar and pestle. The homogenized tissue was solubilized in 800 μl of RIPA buffer (50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 5 mM EDTA, 10 mM NaF, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, protease inhibitors) and centrifuged (100,000 g) for 30 min at 4°C. Solubilized proteins were separated using 10% SDS-PAGE, transferred to nitrocellulose membranes, immunoblotted [monoclonal anti-GRK5 (A16/17)] (33), and visualized using autoradiography.

Statistical analyses. Statistical analyses were carried out using the computer software GraphPad Prism or SPSS. Differences between genotype, drug dose, and time after challenge were analyzed for MCh using multiway ANOVA in a general linear model with genotype, time, and dose as factors. Values are reported as means ± SE. To determine differences within a protocol for a mouse genotype, a paired two-tailed t-test was used unless more than two cases existed, in which case a one-way ANOVA for repeated measures was used, and differences were identified with Tukey’s honest significant difference post hoc test. Results were considered significantly different if P ≤ 0.05.

RESULTS

GRK5 expression. Figure 2 shows that GRK5 is indeed expressed in mouse lung and trachea and is, therefore, readily available to exert a physiological effect on GPCRs in those tissues. Notice that GRK5 remains measurable in tracheal tissue even after epithelial tissue has been denuded, suggesting that GRK5 is expressed in airway smooth muscle cells. Others have previously shown that GRK5 is expressed in the mouse heart (35), thus cardiac samples are not presented here.

In vivo airway response to bilateral vagal stimulation and MCh. Control tracheal pressure was not different between WT and MCh. heart (35), thus cardiac samples are not presented here. Notice that GRK5 remains measurable in tracheal tissue even after epithelial tissue has been denuded, suggesting that GRK5 is expressed in airway smooth muscle cells. Others have previously shown that GRK5 is expressed in mouse lung and trachea and is, therefore, readily available to exert a physiological effect on GPCRs in those tissues.

Fig. 2. GRK5 protein expression by Western blotting. Whole lung, trachea, and epithelium-denuded trachea from wild-type (WT) animals were subjected to immunoblotting using an anti-GRK3 antisera (A16/17) (33). All WT tissues exhibited a 67-kDa band having migration consistent with that of control GRK5 protein. GRK5 control protein is loaded in the fourth lane from the left.

stimulation of these receptors in GRK5−/− mice should result in excessive inhibition of junctional acetylcholine release. Thus one might observe decreased airway responsiveness to vagal stimulation in GRK5−/− mice. Both WT and GRK5−/− mice demonstrated a frequency-dependent increase in APTI during bilateral vagal stimulation. However, at stimulation frequencies of 5 and 15 Hz, average APTI values were significantly greater in GRK5−/− mice relative to WT mice (Fig. 3), a trend that was also apparent for the other stimulus frequencies. These results suggest that M2 muscarinic autoinhibitory receptors are not modulated by GRK5 but that airways of GRK5−/− mice are hyperresponsive to vagal stimulation. To assess whether the enhanced airway response to vagal stimulation in GRK5−/− mice was mediated by M2 muscarinic receptors on airway smooth muscle, we measured APTI during intravenous administration of MCh. Figure 4 shows that the airway responses to 25 and 50 μg/kg of MCh were not enhanced in GRK5−/− mice and that the effect of β2-AR antagonist (propranolol) on the airway response to 50 μg/kg of MCh was not different between WT and GRK5−/− mice. These data suggest that GRK5 is not an important regulator of

Fig. 3. Airway response to increasing frequency of vagal stimulation is enhanced in GRK5−/− mice relative to WT mice. Airway pressure time index (APTI) was calculated over a 30-s period beginning at the initiation of a 10-s vagus nerve stimulation at 5, 10, 15, or 20 Hz. Mice were anesthetized and paralyzed with doxacurium chloride. Data points are means ± SE for n = 10 WT and n = 12 GRK5−/−. *GRK5−/− significantly different from WT mice (P < 0.05) at 5 and 15 Hz.

Fig. 4. Airway response to intravenous administration of methacholine (MCh). Response to 25 and 50 μg/kg of MCh is not different between WT (filled bars) and GRK5−/− (open bars) mice. Propranolol had no effect on the airway response to 50 μg/kg of MCh (50 pst-prop). Data points are means ± SE for n = 7 WT and n = 8 GRK5−/−.
the M_3 muscarinic receptors that mediate contraction of airway smooth muscle.

In vitro tracheal smooth muscle contractile response to carbachol and isoproterenol. To further explore GRK5 regulation of airway muscarinic receptors, we assessed the in vitro response of tracheal smooth muscle to the muscarinic receptor agonist carbachol. The maximum absolute tracheal smooth muscle tension developed in response to 10 μM carbachol (maximal contraction) or 90 mM KCl was not different between WT and GRK5^{-/-} mice (Table 1). The sensitivity of the contractile response to carbachol also was the same in WT and GRK5^{-/-} mice (Fig. 5). These data provide additional support for the idea that GRK5 is not an important regulator of M_3 muscarinic receptor-mediated contraction of airway smooth muscle.

Having ruled out a regulatory effect of GRK5 on prejunctional M_2 muscarinic receptors and postjunctional M_3 muscarinic receptors, we focused on the possibility that GRK5 might desensitize M_2 muscarinic receptors located on airway smooth muscle. If this were the case, then the physiological response mediated by these receptors (opposition of G_{αs}-mediated relaxation) would be more effective in mice lacking the GRK5 protein (GRK5^{-/-}). To test M_2 muscarinic receptor-mediated inhibition of G_{αs}-mediated relaxation, we precontracted tracheal smooth muscle with carbachol and then exposed it to increasing concentrations of isoproterenol, a β_2-AR agonist that causes G_{αs}-mediated airway smooth muscle relaxation. Both WT (70 ± 4% of maximum) and GRK5^{-/-} (68 ± 1% of maximum) mice were precontracted to similar tensions (Fig. 6A). However, tracheal smooth muscle from GRK5^{-/-} mice was more resistant to the relaxation effects of isoproterenol (Fig. 6B). This observation was not a result of decreased responsiveness to isoproterenol alone (Fig. 7A) or inherent time-related differences in relaxation (Fig. 7B). When tissue was precontracted with KCl, rather than a muscarinic agonist, β_2-AR-mediated relaxation was not different in GRK5^{-/-} mice relative to WT mice (Fig. 7A). Furthermore, time-series control data show that WT and GRK5^{-/-} mice sustain their carbachol-induced contractions similarly over an interval equal to that of the isoproterenol relaxation challenge (Fig. 7B). Thus the relaxatory effect of isoproterenol was diminished in GRK5^{-/-} mice precontracted with carbachol but not KCl. One mechanism to explain this observation is that β_2-AR-mediated relaxation is excessively opposed by enhanced activation of an M_2 muscarinic receptor-mediated pathway in GRK5^{-/-} mice. To test this hypothesis, we precontracted tracheal tissue with carbachol to 78% of maximum and relaxed it with isoproterenol in the presence of the selective M_2 muscarinic receptor antagonist methacholine. As shown in Fig. 7C, when M_2 muscarinic receptors are pharmacologically inhibited, tracheal tissue relaxes similarly in GRK5^{-/-} and WT mice. Together, the in vivo and in vitro airway data suggest that M_2 receptors on airway smooth muscle cells are desensitized, at least in part, by GRK5, whereas M_3 receptors on these same cells and M_2 autoreceptors on the vagus nerves are not.

Heart rate response to bilateral vagal stimulation and MCh. We next tested the contribution that GRK5 may make to the regulation of cardiac chronotropy. Resting heart rate was not significantly different between anesthetized, intact WT (476 ± 11 beats/min, n = 6) and GRK5^{-/-} (486 ± 17 beats/min, n = 6) mice. Propranolol caused a similar and significant decline in heart rate in WT (−18 ± 4 beats/min) and GRK5^{-/-} (−26 ± 4 beats/min) mice.

To determine whether GRK5 regulates cardiac muscarinic receptor sensitivity, we measured heart rate during bilateral vagal nerve stimulation and before and after administration of the muscarinic receptor agonist MCh. At a stimulation fre-

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**Table 1. Maximum contractile tension developed**

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<tr>
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<th>Wild Type</th>
<th>GRK5^{-/-}</th>
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<tr>
<td>Carbachol max</td>
<td>1,565 ± 297 mg, n = 6</td>
<td>1,324 ± 263 mg, n = 4</td>
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<tr>
<td>90 mM KCl</td>
<td>887 ± 169 mg, n = 6</td>
<td>751 ± 161 mg, n = 4</td>
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**Fig. 5.** Effect of carbachol on tracheal smooth muscle isometric force generation is not different between WT (■) and GRK5^{-/-} (○) mice. Force generated at 10 μM carbachol is defined as "maximum" force of contraction. All other values are expressed as a percentage of maximum force. Data points are means ± SE for n = 6 WT and n = 4 GRK5^{-/-}. Nonlinear regression for sigmoidal dose response was calculated.

**Fig. 6.** Effect of isoproterenol on tracheal smooth muscle precontracted with carbachol. A: tracheal tissue from WT (solid bar) and GRK5^{-/-} (open bar) mice was precontracted to similar tensions. B: relaxation in response to log increases in isoproterenol concentration is inhibited in GRK5^{-/-} (○) mice relative to WT (■) mice. Data points are means ± SE for n = 6 WT and n = 4 GRK5^{-/-}. Nonlinear regression for sigmoidal dose response was calculated. *Significant difference between WT and GRK5^{-/-} (P < 0.05).
and 4). This bradycardia was not different in GRK5 M2 muscarinic receptor desensitization (16). Consistent with pronounced central cholinergic supersensitivity and impaired frequency of 10 Hz, both WT and GRK5−/− mice demonstrated a significant bradycardia followed by heart rate recovery (Fig. 8A). Neither the heart rate decrease nor the heart rate recovery was different between genotypes. Intravenous injection of MCh produced a time- and dose-dependent reduction in heart rate in both WT and GRK5−/− mice (P < 0.001 all cases; Fig. 8B). This bradycardia was not different in GRK5−/− mice relative to WT mice. These data suggest that M2 muscarinic receptor-mediated bradycardia is not significantly modulated by GRK5.

DISCUSSION

Our previous work showed that GRK5−/− mice demonstrate pronounced central cholinergic supersensitivity and impaired M2 muscarinic receptor desensitization (16). Consistent with those findings, this study indicates that airway smooth muscle M2 muscarinic receptor function is exaggerated in GRK5−/− mice. However, the current study also shows that GRK5 is unimportant for regulating cardiac chronotropy through direct regulation of cardiac M2 muscarinic receptors.

Although M3 muscarinic receptors are the primary mediators of airway smooth muscle constriction, M2 receptors, expressed by airway smooth muscle of many species, including humans and mice, frequently outnumber M3 muscarinic receptors (13, 17, 29, 37). This relatively large concentration of M2 muscarinic receptors contributes to airway smooth muscle contraction predominantly through inhibition of relaxation (8, 30). However, in the absence of M3 muscarinic receptors, M2 muscarinic receptors can directly promote airway smooth muscle contraction (39). Our in vitro results show that when tracheal smooth muscle is precontracted with a muscarinic agonist, β2-AR-mediated relaxation is significantly attenuated in GRK5−/− mice relative to WT mice and that this difference disappears when M3 muscarinic receptors are pharmacologically antagonized. Thus our data indicate that GRK5 is likely an important contributor to the desensitization of M2 muscarinic receptors on airway smooth muscle, thereby regulating the ability of these receptors to oppose airway smooth muscle relaxation. Our results are in contrast to a study by Matsui et al. (30), which showed that M2 muscarinic receptor activation does not oppose isoproterenol-induced relaxation of murine tracheal smooth muscle. However, this same study demonstrated that M2 muscarinic receptor activation inhibits forsko-

Fig. 7. Effect of time and isoproterenol on the magnitude of tracheal tissue contraction. Tracheal tissue from WT (●) and GRK5−/− (○) mice showed no difference in relaxation response to isoproterenol when precontracted with KCl (A) or when precontracted with carbachol and antagonized with methoctramine (C). Data points are means ± SE for n = 5 (A) and 4 (C) WT and n = 5 (A) and 4 (C) GRK5−/− mice, respectively. Nonlinear regression for sigmoidal dose response was calculated. B: time series control data for the isoproterenol relaxation protocols shows that the magnitude of the 70% maximum contraction is maintained similarly in WT and GRK5−/− mice. Data points are means ± SE for n = 4 WT and n = 4 GRK5−/−.

Fig. 8. Effect of GRK5 on activation of cardiac muscarinic receptors. Effect of 10 Hz bilateral vagal nerve stimulation (A) and 25 and 50 µg/kg doses of MCh (B) on heart rate in anesthetized, paralyzed WT (closed symbols) and GRK5−/− (open symbols) mice. Resting heart rate was not different between genotypes during control conditions. Genotype had no effect on bradycardic response to vagal stimulation or MCh injection. Heart rate responses to other stimulation frequencies or MCh doses were not different between WT and GRK5−/− mice. Means ± SE are shown. Two separate groups of mice were used. A: WT (●), n = 10; GRK5−/− (○), n = 10. B: WT (25 µg/kg MCh ●; 50 µg/kg MCh ○), n = 6; GRK5−/− (25 µg/kg MCh ●; 50 µg/kg MCh ○), n = 6.
lin-induced cAMP-mediated relaxation, thus supporting the existence of a pathway for M2 muscarinic receptor-mediated inhibition of tracheal smooth muscle relaxation. Methodological differences may underlie the inconsistency between our study and that of Matsui et al. We administered increasing doses of isoproterenol to submaximally contracted tissue to characterize relaxation, whereas Matsui et al. used a maximal dose of isoproterenol before contracting airway smooth muscle with increasing doses of oxotremorine-M. Because tissues were maximally relaxed in the Matsui study, the efficacy of M2 muscarinic receptors may have been masked. There are a number of reports from other species that support the notion that M2 muscarinic receptors oppose β2-AR-mediated relaxation. Through a pertussis toxin-sensitive mechanism, stimulation of airway smooth muscle M2 muscarinic receptors inhibits adenyl cyclase activity (8, 30, 37) and significantly reduces the open-state probability of KCa channels (25, 26), two events promoted by β2-AR stimulation (24).

Potassium ion channels exist in high density in airway smooth muscle membrane of many species, including mice (7, 25, 31). Activation (opening) of murine tracheal smooth muscle KCa channels results in relaxation (28). Thus stimulation of M2 muscarinic receptors opposes β2-AR-mediated relaxation through regulation of second messenger systems or membrane ion channels.

M2 muscarinic receptor-mediated inhibition of β2-AR-mediated relaxation may be of functional significance in humans. Severe asthmatics demonstrate reduced responsiveness to doses of muscarinic receptor agonists. We propose that the bronchodilators that act through M2 muscarinic receptors on airway smooth muscle of mice and that this mechanism is responsible for the elevation of APTI during vagal stimulation. This trend, elevated APTI in GRK5−/− mice, should not be observed during intravenous MCh injection for a variety of reasons. First, nonadrenergic, noncholinergic relaxation does not occur during MCh infusion. Second, the inhibitory role of M2 muscarinic receptors is more pronounced at a low level of contraction (37). Our APTI data show much smaller airway responses to vagal stimulation than to MCh injections. Finally, intravenous administration of MCh induces transient hypotension and baroreflex release of norepinephrine (NE). Although one might expect NE-induced relaxation of airway smooth muscle to be opposed by activation of M2 muscarinic receptors, this NE has no effect on the resultant airway response, as evidenced by the lack of effect of propranolol on the APTI response to 50 μg/kg of MCh (Fig. 4).

Heart rate is primarily controlled by the opposing influences of the sympathetic and parasympathetic branches of the autonomic nervous system. Postganglionic cardiac parasympathetic fibers synapse onto cardiac pacemaker cells at the sinoatrial node where they exert an inhibitory effect on heart rate. Vagal release of acetylcholine induces bradycardia through activation of M2 muscarinic receptors expressed on these pacemaker cells. The cardiac chronotropic responses to vagal stimulation or MCh injection were not different between GRK5−/− and WT mice, indicating that M2 muscarinic receptors at the sinoatrial node are not appreciably desensitized by GRK5.

We showed that GRK5 is not important for desensitizing autoinhibitory M2 muscarinic receptors located prejunctionally on postganglionic airway nerves. Prejunctional muscarinic receptors exert an inhibitory influence on acetylcholine release, and if GRK5 were important for desensitizing these receptors, one would expect unchecked autoinhibition of acetylcholine release in GRK5−/− mice to result in a reduced airway response to vagal stimulation. Similarly, GRK5 is not important for regulating M3 muscarinic receptors in mouse airway smooth muscle. This result contrasts with our previous findings in GRK3-deficient mice that showed that the loss of GRK3 enhances airway responsiveness to MCh injection mediated by M3 muscarinic receptors (42). Together, our studies provide in vivo proof that GRKs selectively regulate individual muscarinic receptor subtypes.

This study provides insight into the functional role of GRK5 and contributes to the growing body of literature describing in vivo regulation of GPCR function by GRKs. Our results suggest that GRK5 regulates M2, but not M3, muscarinic receptors on airway smooth muscle. In addition, we show that M2 muscarinic receptors located prejunctionally on the vagus nerve or in cardiac pacemaker cells are not regulated by GRK5.
These results, combined with our previous study (16), conclusively demonstrate not only that GRK5 selectively regulates individual GPCR subtypes (i.e., M2, but not M3, muscarinic receptors in airway smooth muscle), but that GRK5 regulation of a particular receptor subtype is tissue specific (i.e., GRK5 regulates central and airway smooth muscle M2 muscarinic receptors but not cardiac or vagal M2 muscarinic receptors). This paradigm of tissue and receptor specificity may be applicable to all GRKs.

Interestingly, severe asthmatics can become refractory to bronchodilators that act at β2-ARs. Studies in animal airways show that excessive activation of postjunctional M2 muscarinic receptors in airway smooth muscle), but that GRK5 regulation of M2 muscarinic receptors but not cardiac or vagal M2 muscarinic receptors. These results, combined with our previous study (16), conclude that excessive activation of M2 receptors may result in excessive M2 muscarinic receptor-mediated inhibition of relaxation, thus making β2-ARs appear refractory.

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