G_i-Coupled γ-Aminobutyric Acid–B Receptors Cross-Regulate Phospholipase C and Calcium in Airway Smooth Muscle

Kentaro Mizuta[1,2], Fumiko Mizuta[1,2], Dingbang Xu[1], Eiji Masaki[2], Reynold A. Panettieri, Jr.[3], and Charles W. Emala[1]

[1]Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, New York, New York; [2]Department of Dento-Oral Anesthesiology, Tohoku University Graduate School of Dentistry, Sendai, Japan; and [3]Division of Pulmonary, Allergy, and Critical Care, Department of Medicine, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system, and exerts its actions via both ionotropic ($GABA_A$) and metabotropic ($GABA_B$) receptors. Although the functional expression of $GABA_B$ receptors coupled to the G_i protein was reported for airway smooth muscle, the role of $GABA_B$ receptors in airway responsiveness remains unclear. We investigated whether G_i-coupled $GABA_B$ receptors cross-regulate phospholipase C (PLC), an enzyme classically regulated by G_q-coupled receptors in human airway smooth muscle cells. Both the $GABA_B$-selective agonist baclofen and the endogenous ligand GABA significantly increased the synthesis of inositol phosphate, whereas $GABA_A$ receptor agonists, muscimol, and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol exerted no effect. The baclofen-induced synthesis of inositol phosphate and transient increases in $[Ca^{2+}]_i$ were blocked by CGP35348 and CGP55845 (selective $GABA_B$ antagonists), pertussis toxin (PTX, which inactivates the G_i protein), gallein (a $G_{\beta\gamma}$ signaling inhibitor), U73122 (an inhibitor of PLC-β), and xestospongin C, an inositol 1,4,5-triphosphate receptor blocker. Baclofen also potentiated the bradykinin-induced synthesis of inositol phosphate and transient increases in $[Ca^{2+}]_i$, which were blocked by CGP35348 or PTX. Moreover, baclofen potentiated the substance P–induced contraction of airway smooth muscle in isolated guinea pig tracheal rings. In conclusion, the stimulation of $GABA_B$ receptors in human airway smooth muscle cells rapidly mobilizes intracellular Ca^{2+} stores by the synthesis of inositol phosphate via the activation of PLC-β, which is stimulated by $G_{\beta\gamma}$ protein liberated from G_i proteins coupled to $GABA_B$ receptors. Furthermore, crosstalk between $GABA_B$ receptors and G_q-coupled receptors potentiates the synthesis of inositol phosphate, transient increases in $[Ca^{2+}]_i$, and smooth muscle contraction through G_i proteins.

Keywords: G_i protein; $G_{\beta\gamma}$; inositol phosphate; phospholipase C; airway smooth muscle

CLINICAL RELEVANCE

The activation of γ-aminobutyric acid–B receptors in airway smooth muscle by therapeutic agents such as baclofen can potentiate intracellular calcium and the contraction of smooth muscle, and may potentiate bronchoconstriction in patents with asthma.

γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system. GABA acts at two distinct types of receptors, namely, ligand-gated ionotropic $GABA_A$ receptors and G-protein–linked metabotropic ($GABA_B$) receptors. The $GABA_B$ receptor is composed of two subunits ($GABA_BR1$ and $GABA_BR2$), and typically functions as a G_i-protein–coupled receptor. In addition to their well-characterized expression and function in neurons, GABA and functional $GABA_B$ receptors were detected in peripheral tissues such as the adrenal medulla, islets of Langerhans, the placenta, and smooth muscle cells of the urinary bladder and uterus. In airways, a $GABA_B$ receptor agonist, baclofen, can worsen airway responses after the administration of methacholine to patients with asthma (1, 2). Although our recent findings suggest the functional expression of G_i-protein–coupled $GABA_B$ receptors in airway smooth muscle (3) and airway epithelial (4) cells, the functional signaling consequences of $GABA_B$ receptor activation on airway responsiveness remain unclear.

Airway contractile agents, such as acetylcholine, bradykinin, histamine, and tachykinins, initiate the contraction of airway smooth muscle by binding to G_q-coupled receptors. These agonists bind to their cognate heptahelical G-protein–coupled receptors (GPCRs) and activate the G_q protein, promoting its dissociation into $G_q\alpha$ and $G_{\beta\gamma}$ and the exchange of guanosine diphosphate bound to $G\alpha_q$ for GTP. The resulting GTP-$G\alpha_q$ complex activates the β isoforms of phospholipase C (PLC) via the carboxy-terminal region of the enzyme (5). PLC-β catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate inositol 1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 binds to the IP_3 receptor on the sarcoplasmic reticulum and releases Ca^{2+}, leading to a rapid and transient increase in intracellular concentrations of Ca^{2+} ($[Ca^{2+}]_i$). Recently, the activation of the adenosine A_1 receptor was reported to rapidly mobilize intracellular Ca^{2+} ($[Ca^{2+}]_i$) stores by a mechanism dependent on pertussis toxin (PTX)–sensitive G_i proteins and IP_3 signaling in human bronchial smooth muscle cells (6). These findings indicate that not only G_q-coupled receptors but also G_i-coupled receptors can contribute to airway contraction through the synthesis of IP_3 and release of $[Ca^{2+}]_i$ from the sarcoplasmic reticulum. Because the $GABA_B$ receptor is G_i-coupled, these findings led us to hypothesize that the direct activation of the $GABA_B$ receptor may increase the synthesis of inositol phosphate, ($[Ca^{2+}]_i$ and augment contraction in human airway smooth muscle.

In addition, many examples indicate that the synthesis of IP_3 and $[Ca^{2+}]_i$ signaling by one type of G-protein–coupled receptor

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Correspondence and requests for reprints should be addressed to Charles W. Emala, M.D., Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, 630 W. 168th St., P&S Box 46, New York, NY 10032. E-mail: cwe5@columbia.edu

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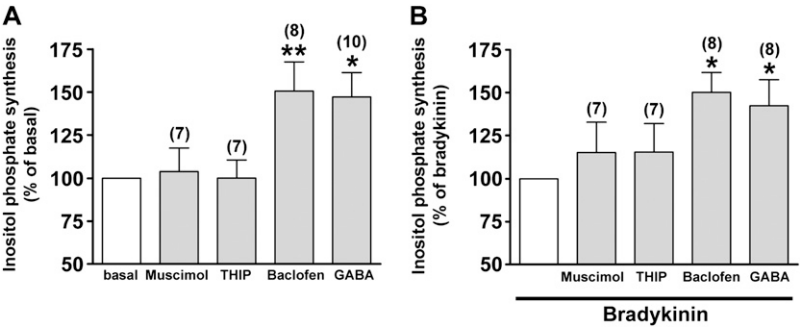

Figure 1. (*A*) Effects of γ-aminobutyric acid (GABA) receptor agonists (i.e., $GABA_A$ receptor agonist; 100 μM muscimol hydrobromide or 100 μM 4,5,6,7-tetrahydroisoxazolo [5,4-c]pyridin-3-ol [THIP], $GABA_B$ receptor agonists; 100 μM baclofen, a nonselective GABA receptor agonist; and 100 μM GABA) on the synthesis of inositol phosphate in native cultured human airway smooth muscle cells. Data are shown as percentages of basal concentrations. $*P < 0.05$ and $**P < 0.01$, compared with basal concentrations. (*B*) Effects of GABA receptor agonists ($GABA_A$ receptor agonist; 100 μM muscimol hydrobromide or 100 μM THIP, $GABA_B$ receptor agonists; 100 μM baclofen, a nonselective GABA receptor agonist; and 100 μM GABA) on the synthesis of inositol phosphate stimulated by 1 μM bradykinin in native cultured human airway smooth muscle cells. Data are shown as percentage of bradykinin alone. $*P < 0.05$, compared with bradykinin alone (control). Data represent means ± SEM. Numbers of experiments are shown in parentheses.

can be influenced by the stimulation of a different type of GPCR (7–9). For example, in addition to directly activating PLC, the adenosine A_1 receptor also "potentiates" the PLC responses mediated by a range of G_q-coupled receptors in several cell types (10, 11). Likewise, in smooth muscle cells, the dual activation of G_i-coupled adenosine A_1 receptors and G_q-coupled bradykinin receptors synergistically stimulates the synthesis of IP_3 and an increase in $[Ca^{2+}]_i$ in the ductus deferens tumor cell line MF-2 smooth muscle cells (12). The crosstalk between G_i and G_q pathways in airway smooth muscle regulates bronchial contractility and relaxation (13). These findings led us to hypothesize that the activation of $GABA_B$ receptors could potentiate the G_q-coupled receptor–mediated synthesis of IP_3, $[Ca^{2+}]_i$, and contraction signaling in airway smooth muscle cells.

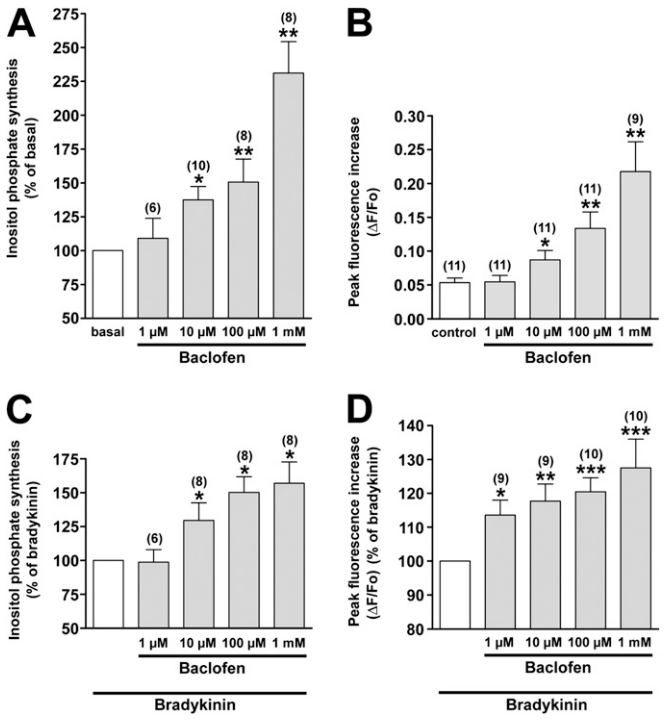

Figure 2. Dose-dependent effects of baclofen on (*A*) the synthesis of inositol phosphate (shown as percentages of basal concentrations) and (*B*) the peak (transient) increase in intracellular Ca^{2+} (shown as change in fluorescence [ΔF] from baseline fluorescence [Fo]) in human airway smooth muscle cells. $*P < 0.05$ and $**P < 0.01$, compared with basal concentrations. The dose-dependent effects of baclofen on (*C*) the bradykinin-stimulated synthesis of inositol phosphate and (*D*) the peak intracellular increase of Ca^{2+} in human airway smooth muscle cells are shown as a percentage of the response to 1 μM bradykinin. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$, compared with bradykinin. Data represent means ± SEM. Numbers of experiments are shown in parentheses.

MATERIALS AND METHODS

Materials

SmGM-2 smooth muscle medium was obtained from Lonza (Walkersville, MD). Fluo-4AM and Pluronic F-127 were obtained from Molecular Probes (Eugene, OR). [^{3}H]*myo*-inositol (20 Ci/mmol) was obtained from MP Biomedicals (Irvine, CA). CGP35348, CGP55845, gallein, and U73122 were obtained from Tocris Bioscience (Ellisville, MO). Pertussis toxin was obtained from Calbiochem (San Diego, CA). Xestospongin C was obtained from Cayman Chemical (Ann Arbor, MI). All other chemicals were obtained from Sigma (St. Louis, MO) unless otherwise stated.

Cell Culture

Primary cultures of human airway smooth muscle cells were obtained from lung transplant donors in accordance with procedures approved by the University of Pennsylvania Committee on Studies Involving Human Beings, as previously described (14). Cells were grown to confluence on 96-well (calcium study) or 24-well (inositol phosphate study) plates in culture medium (SmGM-2, supplemented with 5% FBS, 5 μg/ml insulin, 1 ng/ml human fibroblast growth factor, 500 pg/ml human epidermal growth factor, 30 μg/ml gentamicin, and 15 ng/ml amphotericin B; Lonza) at 37°C in an atmosphere of 5% CO_2–95% air. Human airway smooth muscle cells cultured at these conditions retain the expression of both $GABA_A$ and $GABA_B$ receptors between passages 3 and 8 (3, 15).

Inositol Phosphate Assays

The synthesis of total ^{3}H-inositol phosphates was measured in confluent human airway smooth muscle cells in 24-well tissue culture plates, as described elsewhere (16, 17). In brief, after overnight loading with ^{3}H-*myo*-inositol (10 μCi/ml or 20 Ci/mmol) in inositol-free and serum-free Dulbecco's modified Eagle's medium (Chemicon, Temecula, CA), each well was washed three times (37°C, with 500 μl Hanks' balanced salt solution and 10 mM LiCl). The incubation of cells with GABA receptor agonists (GABA, muscimol hydrobromide, 4,5,6,7-tetrahydroisoxazolo [5,4-c]pyridin-3-ol [THIP], or baclofen) in a final volume of 300 μl at 37°C for 30 minutes was performed in the absence or presence of 1 μM bradykinin. In some experiments, cells were pretreated with inhibitors, namely, 100 μM CGP35348 (a $GABA_B$ receptor antagonist, 30 minutes before the addition of baclofen), 10 nM CGP55845 (a potent $GABA_B$ receptor antagonist, 30 minutes before the addition of baclofen), 100 ng/ml pertussis toxin (PTX; 4 hours before the addition of baclofen), 100 μM gallein (a $G_{\beta\gamma}$ signaling inhibitor (18, 19), 30 minutes before the addition of baclofen), or 5 μM U73122 (a PLC inhibitor, 30 minutes before the addition of baclofen). The reactions were terminated, and total [^{3}H]inositol phosphates were recovered by chromatography (16).

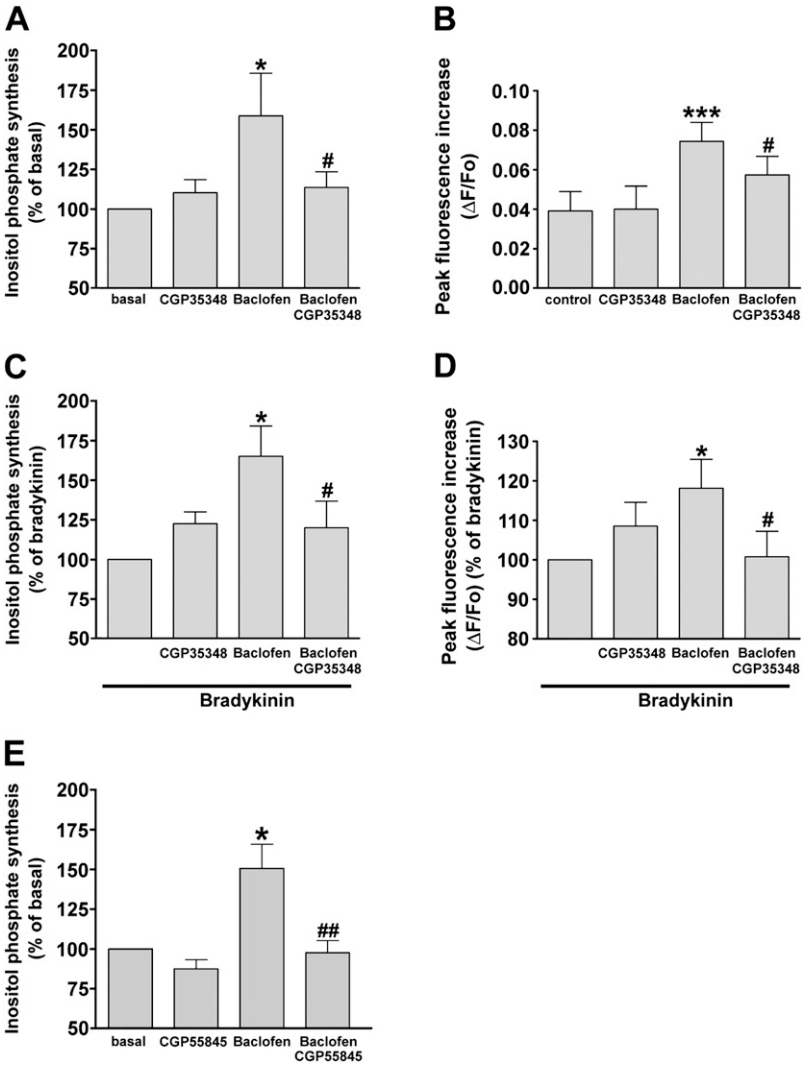

Figure 3. Effects of selective $GABA_B$ receptor antagonist (100 μM CGP35348) on (*A*) the synthesis of inositol phosphate induced by 100 μM baclofen (shown as percentage of basal concentration) ($n = 8$) and (*B*) the peak increase in intracellular Ca^{2+} (shown as change in fluorescence [ΔF] from baseline fluorescence [Fo]) ($n = 8$) in human airway smooth muscle cells. $^*P < 0.05$ and $^{***}P < 0.001$, compared with basal concentrations. $^{\#}P < 0.05$, compared with baclofen. Effects of a selective $GABA_B$ receptor antagonist (100 μM CGP35348) on the potentiation induced by 100 μM baclofen on the (*C*) synthesis of inositol phosphate ($n = 8$) and (*D*) peak increase in intracellular Ca^{2+} ($n = 7$) stimulated by 1 μM bradykinin in human airway smooth muscle cells, shown as a percentage of the response to 1 μM bradykinin. $^*P < 0.05$, compared with bradykinin. $^{\#}P < 0.05$, compared with bradykinin/baclofen. (*E*) Effect of another potent selective $GABA_B$ receptor antagonist (10 nM CGP55845) on the synthesis of inositol phosphate induced by 100 μM baclofen (shown as percentage of basal concentration) ($n = 7$) in human airway smooth muscle cells. $^*P < 0.05$, compared with basal concentration. $^{\#\#}P < 0.05$, compared with baclofen. Data represent means ± SEM.

Experiments were repeated at least six times on human airway smooth muscle cells obtained from the same source.

Measurement of $[Ca^{2+}]_i$

Details regarding the measurement of $[Ca^{2+}]_i$ are provided in the online supplement.

In Vitro Effects of $GABA_B$ Receptor Agonist on Guinea Pig Airway Smooth Muscle Contraction

Determination of the *in vitro* effects of the $GABA_B$ receptor agonist on guinea pig airway smooth muscle contraction was performed as previously described (17). Please see the online supplement for details.

Statistical Analysis

Statistical analysis was performed using repeated-measures of ANOVA, followed by a Bonferroni posttest comparison using GraphPad Instat version 3.0.6 software (GraphPad Software, Inc., San Diego, CA). Data are presented as means ± SEM. $P < 0.05$ was considered significant.

RESULTS

We first examined the effects of GABA receptor agonists (GABA, i.e., a nonselective GABA receptor agonist; muscimol hydrobromide and THIP, $GABA_A$ receptor agonists; and baclofen, a $GABA_B$ receptor agonist) on the synthesis of inositol phosphate in human airway smooth muscle cells. Both GABA (100 μM) and baclofen (100 μM) significantly increased the synthesis of inositol phosphate ($P < 0.05$, $n = 10$, and $P < 0.01$, $n = 8$, respectively), whereas $GABA_A$ receptor agonists (100 μM muscimol hydrobromide and 100 μM THIP) exerted no effect (Figure 1A). In addition, both GABA and baclofen-potentiated bradykinin (1 μM) induced the synthesis of inositol phosphate ($P < 0.05$, $n = 8$, and $P < 0.05$, $n = 8$, respectively), whereas $GABA_A$ receptor agonists did not affect bradykinin-induced synthesis of inositol phosphate (Figure 1B). Baclofen alone significantly stimulated both the synthesis of inositol phosphate (an increase of 231% ± 23.2%, compared with basal concentrations [$P < 0.01$, $n = 8$] at 1 mM baclofen) (Figure 2A) and transient increases in $[Ca^{2+}]_i$ ($\Delta F/Fo = 0.218 \pm 0.044$ at 1 mM baclofen, $P < 0.01$, $n = 9$) (Figure 2B) at concentrations ranging from 10 μM to 1 mM in a concentration-dependent manner. Baclofen also elicited a concentration-dependent potentiation of the bradykinin (1 μM)–induced synthesis of inositol phosphate (an increase of 157% ± 15.8%, compared with bradykinin alone [$P < 0.05$, $n = 8$] at 1 mM baclofen) (Figure 2C) and a transient increase in $[Ca^{2+}]_i$ (an increase of 128% ± 8.50%, compared with bradykinin alone [$P < 0.001$, $n = 10$] at 1 mM baclofen) (Figure 2D).

A $GABA_B$ receptor–selective antagonist, CGP35348 (100 μM), blocked the baclofen (100 μM)–stimulated synthesis of inositol phosphate (Figure 3A) and the transient increase in $[Ca^{2+}]_i$ ($P < 0.05$, $n = 8$, and $P < 0.05$, $n = 8$, respectively) (Figure 3B).

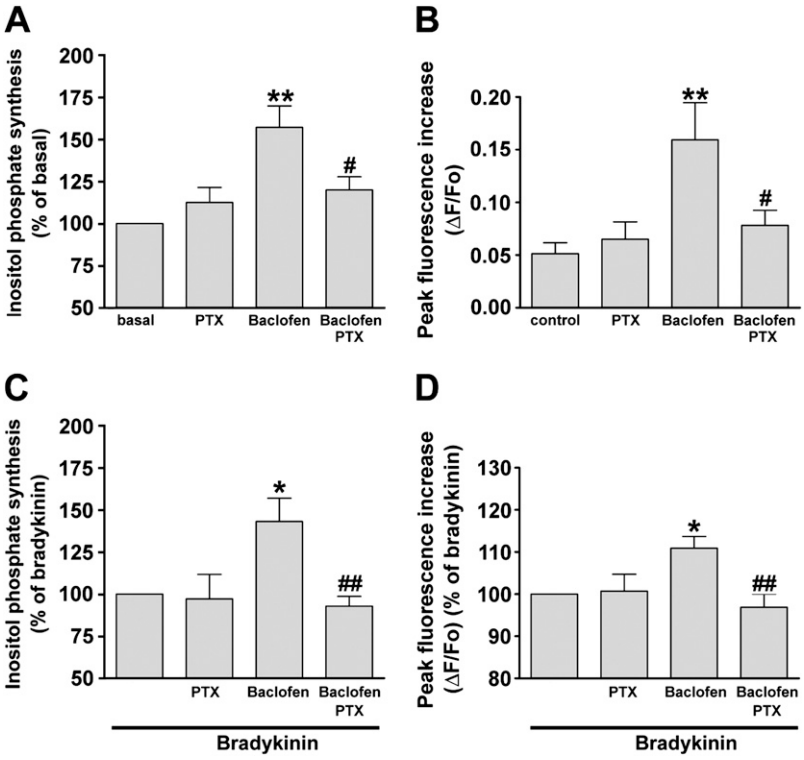

Figure 4. Effects of 4-hour pretreatment with pertussis toxin (PTX; 100 ng/ml) on (*A*) the synthesis of inositol phosphate (shown as percentage of basal concentration) ($n = 12$) and (*B*) peak increase in intracellular Ca^{2+} (shown as change in fluorescence [ΔF] from baseline fluorescence [Fo]) ($n = 8$) induced by 100 μM baclofen in human airway smooth muscle cells. $^{**}P < 0.01$, compared with basal concentration. $^{\#}P < 0.05$, compared with baclofen. (*C* and *D*) Effects of 4-hour pretreatment with 100 ng/ml PTX on the potentiation stimulated by 1 μM bradykinin in (*C*) the synthesis of inositol phosphate ($n = 7$) and (*D*) the peak increase in intracellular Ca^{2+} ($n = 6$) induced by 100 μM baclofen in human airway smooth muscle cells. $^{*}P < 0.05$, compared with bradykinin. $^{\#\#}P < 0.01$, compared with bradykinin/baclofen. The data are presented as a percentage of the response to 1 μM bradykinin. Data represent means ± SEM.

CGP35348 (100 μM) also blocked the baclofen (100 μM)–induced potentiation of the bradykinin-induced synthesis of inositol phosphate (Figure 3C) and the transient increase in $[Ca^{2+}]_i$ ($P < 0.05, n = 8$ and $P < 0.05, n = 7$, respectively) (Figure 3D). In addition, another potent $GABA_B$ receptor–selective antagonist, CGP55845 (10 nM), blocked the baclofen (100 μM)–stimulated synthesis of inositol phosphate ($P < 0.01$, $n = 7$) (Figure 3E).

We examined whether the G_i protein takes part in the $GABA_B$ receptor–mediated activation of IP_3 synthesis and the transient increase in $[Ca^{2+}]_i$. Pretreatment of human airway smooth muscle cells with pertussis toxin (PTX; 100 ng/ml; 4 hours), an inhibitor of G_i proteins, significantly blocked the synthesis of inositol phosphate (Figure 4A) and the transient increase in $[Ca^{2+}]_i$ (Figure 4B) in response to 100 μM baclofen ($P < 0.05$, $n = 12$, and $P < 0.05$, $n = 8$, respectively). PTX also blocked the baclofen-induced potentiation of the bradykinin (1 μM)–induced synthesis of inositol phosphate (Figure 4C) and the transient in $[Ca^{2+}]_i$ ($P < 0.01, n = 7$, and $P < 0.01, n = 7$, respectively) (Figure 4D). Pretreatment with PTX alone exerted no significant effect on the synthesis of inositol phosphate or the transient increase in $[Ca^{2+}]_i$ in the presence or absence of bradykinin (Figure 4).

G_i-coupled receptors, including the $GABA_B$ receptor, have the capacity to initiate or modulate signaling through the actions of the liberated $G_i\alpha$ or $G_{i\beta\gamma}$ subunits (20). Although

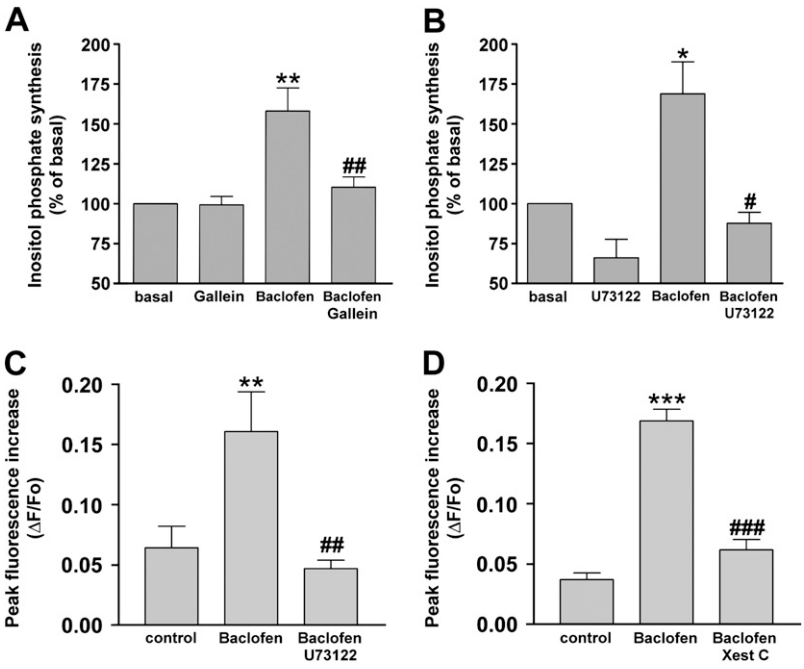

Figure 5. Effects of (*A*) $G_{\beta\gamma}$ signaling inhibitor gallein (100 μM; 30-minute pretreatment; $n = 5$) and (*B*) the phospholipase C-β (PLC-β) inhibitor U73122 (5 μM; 30-minute pretreatment; $n = 5$) on the synthesis of inositol phosphate induced by 100 μM baclofen in human airway smooth muscle cells. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with basal concentration. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with baclofen. Effects of (*C*) U73122 (5 μM; 30-minute pretreatment; $n = 7$) and (*D*) the inositol 1,4,5-triphosphate (IP_3) receptor inhibitor xestospongin C (Xest C; 20 μM; 30-minute pretreatment; $n = 6$) on the peak intracellular Ca^{2+} increase induced by 100 μM baclofen (shown as change in fluorescence [ΔF] from baseline fluorescence [Fo]) in human airway smooth muscle cells. $^{**}P < 0.01$ and $^{***}P < 0.001$, compared with basal concentration. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$, compared with baclofen. Data represent means ± SEM.

PTX inhibits the activation of the G_i protein, it does not distinguish between signaling events mediated through $G_i\alpha$ versus $G_{i\beta\gamma}$ subunits. Therefore, we examined whether $G_{i\beta\gamma}$ subunits participate in the $GABA_B$ receptor–mediated activation of inositol phosphate synthesis and the transient increase in $[Ca^{2+}]_i$. Pretreatment of human airway smooth muscle cells with gallein (100 μM; 30 minutes), a $G_{\beta\gamma}$ inhibitor (18, 19), significantly inhibited the synthesis of inositol phosphate elicited by baclofen (100 μM). Pretreatment with gallein by itself did not exert any significant effect on the synthesis of inositol phosphate (Figure 5A).

We next questioned whether $G_{\beta\gamma}$ subunits liberated from G_i proteins after activation of the $GABA_B$ receptor cross-regulated the activity of PLC-β. PLC-β promotes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) into IP_3 and DAG, and IP_3 in turn liberates calcium from sarcoplasmic reticulum stores. Although $G_q\alpha$ and $G_{\beta\gamma}$ dissociated from G_q-coupled receptors are the classic signaling molecules that activate PLC-β (5, 20), PLC-β was also shown to be activated by $G_{\beta\gamma}$ proteins liberated from G_i proteins (21, 22). We therefore examined whether PLC-β modulates the baclofen-induced activation of inositol phosphate synthesis and the transient increase in $[Ca^{2+}]_i$. The presence of U73122 (5 μM), an inhibitor of PLC, significantly inhibited both the synthesis of inositol phosphate and the transient increase in $[Ca^{2+}]_i$ in response to baclofen (100 μM) ($P < 0.05$, $n = 5$, and $P < 0.01$, $n = 7$, respectively) (Figures 5B and 5C). In addition, in the presence of xestospongin C (20 μM), a cell-permeable inhibitor of the IP_3 receptor, the baclofen (100 μM)–induced transient increase in $[Ca^{2+}]_I$ was significantly inhibited ($P < 0.001$, $n = 6$) (Figure 5D).

Finally, we examined whether baclofen potentiates the contraction of airway smooth muscle induced by substance P (1 μM). Baclofen (100 μM) significantly potentiated the substance P–induced contraction of guinea pig tracheal airway smooth muscle (Figure 6).

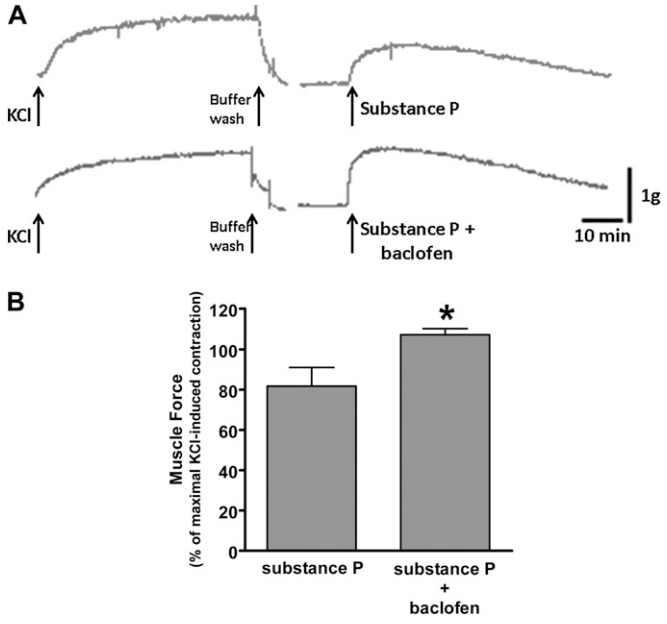

Figure 6. The $GABA_B$ receptor agonist, baclofen, potentiates substance P–induced contractions in guinea pig tracheal rings. (*A*) Representative tension tracing in guinea pig tracheal ring illustrates the potentiation of substance P (1 μM)–induced contraction by the $GABA_B$ receptor agonist baclofen (100 μM). *Upper tracing*: control (substance P alone). *Lower tracing*: baclofen + substance P. (*B*) Baclofen (100 μM) significantly potentiated the contraction induced by substance P (1 μM). Data represent means ± SEM. *$P < 0.05$, compared with substance P ($n = 7$).

DISCUSSION

To summarize the main findings of this study, $GABA_B$ receptor agonists stimulate the synthesis of inositol phosphate and the mobilization of $[Ca^{2+}]_i$ in human airway smooth muscle cells. These responses are mediated by $GABA_B$ receptors that are coupled to PTX-sensitive G_i proteins, which crosstalk via $G_i\beta\gamma$ subunits to activate PLC-β. The activation of PLC-β, in turn, synthesizes inositol phosphates, which mobilize calcium from intracellular stores. In addition, the $GABA_B$ receptor agonist potentiates the bradykinin-stimulated synthesis of inositol phosphate and a transient increase in $[Ca^{2+}]_i$. Moreover, we demonstrated that the $GABA_B$ agonist baclofen potentiates a G_q-coupled contraction of guinea pig airway smooth muscle. Because $[Ca^{2+}]_i$ is an important regulator of airway smooth muscle cell contraction, these findings suggest that the $GABA_B$ receptor may contribute to the regulation of bronchomotor tone in the airways.

In the present study, GABA, the natural endogenous ligand for $GABA_A$ and $GABA_B$ receptors, and baclofen, a selective $GABA_B$ receptor agonist, stimulated both the synthesis of inositol phosphate and the transient increase in $[Ca^{2+}]_i$, which were significantly blocked by two selective $GABA_B$ receptor antagonists (CGP35348 and CGP55845). In contrast, selective agonists for the $GABA_A$ receptor (muscimol and gaboxadol [THIP]) did not stimulate the synthesis of inositol phosphate and the transient increase in $[Ca^{2+}]_i$ in human airway smooth muscle cells. These findings suggests that the $GABA_B$ receptor, but not the $GABA_A$ receptor, is the GABA receptor subtype that is coupled with the synthesis of inositol phosphate and the transient increase in $[Ca^{2+}]_i$ in human airway smooth muscle cells. These findings are novel, because the synthesis of inositol phosphate and transient increases in calcium in direct response to the $GABA_B$ receptor agonist have not been previously demonstrated in airway smooth muscle. Because calcium is an important regulator of airway smooth muscle cell contraction, these findings suggest that the $GABA_B$ receptor may also exert direct effects that regulate bronchomotor tone in the airways.

Classically, the activation of PLC-β, resulting in the synthesis of inositol phosphate and the release of sarcoplasmic reticulum calcium, was linked to G_q-coupled receptors. In the present study, the baclofen-induced synthesis of inositol phosphate and the transient increase in $[Ca^{2+}]_i$ were attenuated by PTX (an inhibitor of G_i protein), gallein (a $G_{\beta\gamma}$ signaling inhibitor), U73122 (a PLC inhibitor), and xestospongin C (an IP_3 receptor inhibitor). Crosstalk among $GABA_B$ receptors to activate PLC-β through G_i-protein βγ subunits is possible, because several studies in a variety of cells and tissues demonstrated that the mobilization of $[Ca^{2+}]_i$ is stimulated by G_i-coupled receptors such as the adenosine A_1 receptor, involving the activation of PLC-β by $G_{\beta\gamma}$ subunits (6, 21–26). In the airways, the activation of adenosine A_1 receptors in allergic rabbit airway smooth muscle was shown to cause the production of IP_3 via a PTX-sensitive G_i-protein–coupled PLC, and this signaling mechanism may be involved in airway contractile responses (27). Ethier and Madison also showed that adenosine A_1 receptors induce the release of $[Ca^{2+}]_i$ from the sarcoplasmic reticulum through the activation of PLC in human bronchial smooth muscle cells (6). Taken together, our findings suggest that baclofen stimulated the responses of $[Ca^{2+}]_i$ via $GABA_B$ receptors, and dissociated $G_{\beta\gamma}$ subunits from $GABA_B$ receptors activated PLC-β, which hydrolyzes PIP_2 into DAG and IP_3, followed by the mobilization of calcium from the sarcoplasmic reticulum (Figure 7A).

In patients with asthma, the $GABA_B$ receptor agonist baclofen can worsen airway responses after the administration of methacholine, a G_q-coupled M_3-muscarinic receptor agonist

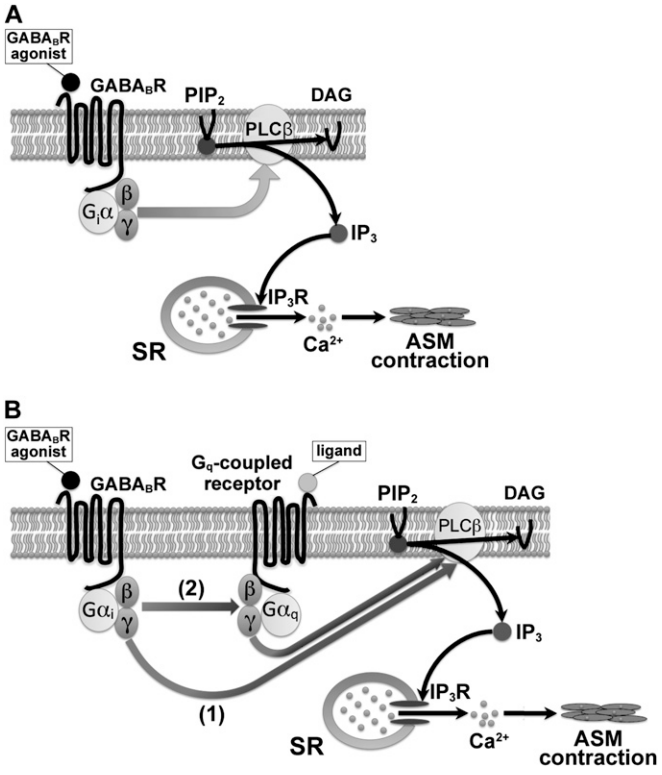

Figure 7. Schematic drawings of $GABA_B$ receptor ($GABA_BR$)–mediated synthesis of IP_3 and intracellular Ca^{2+} signaling under (*A*) basal or (*B*) Gq-coupled receptor activation in human airway smooth muscle. (*A*) The $G_{\beta\gamma}$ subunit, dissociated from the $GABA_B$ receptor, activates PLC-β, which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) into 1,2-diacylglycerol (DAG) and IP_3. IP_3 binds to IP_3 receptors (IP_3R) located on the sarcoplasmic reticulum (SR). The activation of IP_3 receptors results in an efflux of Ca^{2+} into the cytosol, which induces airway smooth muscle (ASM) contraction. (*B*) Activation of the $GABA_B$ receptor potentiates the G_q-coupled receptor–mediated synthesis of IP_3 and intracellular Ca^{2+} signaling. Hypothetically, two mechanisms are involved: (*1*) the Gβγ subunit, dissociated from the $GABA_B$ receptor, directly activates PLC-β; and (*2*) the $GABA_B$ receptor provides the $G_{\beta\gamma}$ subunit directly to the G_q-coupled receptor, which facilitates activation of the G_q signaling pathway.

(2). This result indicates that activation of the G_i-coupled $GABA_B$ receptor could potentiate the airway contraction mediated via the activation of G_q-coupled receptors. Likewise, in the present study, the $GABA_B$ receptor agonist potentiated the guinea pig airway contraction induced by substance P, a G_q-coupled neurokinin receptor agonist. Moreover, the $GABA_B$ receptor agonist potentiated the synthesis of inositol phosphate and the $[Ca^{2+}]_i$ transients induced by activation of the G_q-coupled bradykinin receptor in a PTX-sensitive manner. Previous findings suggest that the stimulation of G_i-coupled receptors can enhance the inositol phosphate signals triggered by an activation of G_q-coupled receptors, even if stimulation of the G_i-coupled receptors alone exerts no effect on inositol phosphates (28–30). In cerebellar Purkinje cells, activation of the $GABA_B$ receptor increases the calcium responses generated by the G_q-coupled metabotropic glutamate receptors, and $G_{\beta\gamma}$ is responsible for this effect (31, 32). Quitterer and colleagues also proposed that crosstalk is mediated by $G_{\beta\gamma}$ exchange between G_i-coupled and G_q-coupled receptors (33). In the present study, the $G_{\beta\gamma}$ blocker significantly inhibited the baclofen-induced synthesis of inositol phosphate and the calcium transients, indicating that activation of the $GABA_B$ receptor dissociates the $G_{\beta\gamma}$ subunit, which in turn enhances bradykinin-induced activities. However, the mechanism of such enhancement by $G_{\beta\gamma}$ subunits continues to be debated (9). The direct stimulation of PLC-β by free $G_{\beta\gamma}$ subunits was reported (34), and this stimulation can occur additively after that by $G_q\alpha$ (35). However, the question arises of whether the direct stimulation of PLC-β by liberated $G_{\beta\gamma}$ constitutes the only mechanism for the enhancing effect caused by the activation of G_i-coupled receptors. $G_{\beta\gamma}$ subunits derived from G_i-coupled adenosine A_1 receptors were suggested to form a heterotrimeric complex with G_q-guanosine diphosphate that is activated by thyrotropin receptors and leads to potentiated PLC-β activity (22). Similarly, the adenosine A_1 receptor and α_{2c}-adrenoceptors provide $G_{\beta\gamma}$ subunits directly to G_q-coupled bradykinin B_2 or P2Y nucleotide receptors, resulting in an enhanced binding of GTP to G_q and enhanced signaling (33). These findings suggest that $G_{\beta\gamma}$ subunits dissociated from G_i-coupled receptors could enhance the synthesis of inositol phosphate and $[Ca^{2+}]_i$ signaling by accelerating G-protein reassociation and by facilitating activation of the G_q signaling pathway (Figure 7B).

Evidence is increasing that such crosstalk between the $GABA_B$ receptor and the metabotropic glutamate 1A receptor does not require their physical interaction, but relates instead to functional crosstalk between their signaling pathways (36, 37). These findings suggest that crosstalk between G_i-coupled and G_q-coupled receptors could exist, even though these receptors are not physically associated.

In conclusion, $GABA_B$ receptors are coupled to calcium signaling, a second messenger pathway important to the regulation of bronchomotor tone. Calcium responses are mediated by $GABA_B$ receptors coupled to PTX-sensitive G proteins and $G_{\beta\gamma}$ subunits, and depend on the activation of PLC-β and the mobilization of calcium from the sarcoplasmic reticulum. Furthermore, activation of the $GABA_B$ receptor potentiates the synthesis of inositol phosphate, the mobilization of calcium, and the contraction in airway smooth muscle induced by activation of the G_q-coupled receptor. These findings indicate that activation of the $GABA_B$ receptor can directly regulate the mobilization of calcium in airway smooth muscle cells, and may induce bronchoconstriction.

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