Functional expression of the GABA_B receptor in human airway smooth muscle

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Am J Physiol Lung Cell Mol Physiol 291: L923–L931, 2006. First published July 7, 2006; doi:10.1152/ajplung.00185.2006.—\gamma-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system and exerts its actions via both ionotropic (GABA_A/GABA_C) and metabotropic (GABA_B) receptors (R). The GABA_B receptors have been detected in nonneuronal cells in peripheral tissue. Although the GABA_B receptor has been shown to function as a prejunctional inhibitory receptor on parasympathetic nerves in the lung, the expression and functional coupling of GABA_B receptors to Gi, in airway smooth muscle itself have never been described. We detected the mRNA encoding multiple splice variants of the GABA_B1R and GABA_B2R in total RNA isolated from native human and guinea pig airway smooth muscle and from RNA isolated from cultured human airway smooth muscle (HASM) cells. Immunoblots identified the GABA_B1R and GABA_B2R proteins in human native and cultured airway smooth muscle. The GABA_B1R protein was immunohistochemically localized to airway smooth muscle in guinea pig tracheal rings. Baclofen, a GABA_B receptor agonist, elicited a concentration-dependent stimulation of [35S]GTP_S binding in HASM homogenates that was abrogated by the GABA_B receptor antagonist CGP-35348. Baclofen also inhibited adenylyl cyclase activity and induced ERK phosphorylation in HASM. Another GABA_B receptor agonist, SKF-97541, mimicked while pertussis toxin blocked baclofen’s effect on ERK phosphorylation, implicating Gi protein coupling. Functional GABA_B receptors are expressed in HASM. GABA may modulate an uncharacterized signaling cascade via GABA_B receptors coupled to the G_i protein in airway smooth muscle.

G protein; adenylyl cyclase; mitogen-activated protein kinase; [35S]GTP_S binding; guinea pig; trachea

GAMMA-AMINOBUTYRIC ACID (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). GABA acts at two distinct types of receptors, ligand-gated ionotropic GABA_A and GABA_C receptors, and G protein-linked metabotropic (GABA_B) receptors (R). The GABA_B receptor is composed of two subunits (GABA_B1R and GABA_B2R), and to date four splice variants of the human GABA_B1R have been described (2, 15, 17, 26). The GABA_B receptor typically functions as a G_i protein-coupled receptor and is fully functional only when both subunits are expressed and are linked in a heterodimeric assembly (2). In the CNS, presynaptically located GABA_B receptors suppress neurotransmitter release by inhibiting voltage-sensitive Ca^{2+} channels (14).

Postsynaptically, GABA_B receptors stimulation causes inhibition of adenylyl cyclase via the G_i protein as well as activation of the Kir3 type potassium channels by liberated G_{i3};3;3-subunits (15). GABA_B receptors are also capable of directly interacting with transcription factors and can regulate gene transcription regulation upon stimulation (29). In addition to their presence on neurons, GABA and functional GABA_B receptors have been detected in peripheral tissues such as adrenal medulla, islets of Langerhans, placenta and smooth muscle cells of the urinary bladder and uterus. However, the expression and functional coupling of GABA_B receptors to G_i in airway smooth muscle has never been described.

It is known that GABA_B-specific agonists decrease airway responsiveness to various bronchoconstricting agents by modulating presynaptic acetylcholine release from parasympathetic nerves (6, 28). On the other hand, a GABA_B receptor agonist, baclofen, can worsen airway responses following the administration of methacholine to asthmatic patients (7). This paradoxical enhancement by baclofen of airway responsiveness led us to hypothesize that there may be postganglionic (i.e., smooth muscle) GABA_B functional receptors that couple to the G_i protein, known to impair relaxation of airway smooth muscle (3, 23).

In the present study, we investigated the expression of GABA_B receptors in native guinea pig, human airway smooth muscle (HASM), and cultured HASM cells, and assessed the functional coupling of the GABA_B receptor to the G_i protein by demonstrating adenylyl cyclase inhibition and ERK activation.

MATERIALS AND METHODS

Cells were cultured in SmGM-2 smooth muscle medium (Cambrex, Walkersville, MD). [35S]GTP_S (1,250 Ci/mmol) was obtained from Perkin Elmer (Boston, MA). [α-32P]ATP (800 Ci/mmol) and [3H]cAMP (32 Ci/mmol) were obtained from MP Biomedicals (Irvine, CA). Human brain protein was obtained from BD Biosciences (Palo Alto, CA). All other chemicals were obtained from Sigma (St. Louis, MO) unless otherwise stated.

Cell culture. Primary cultures of HASM 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 (20). The cells were grown to confluence on 24- (adyenyl cyclase) or 6-well (immunoblotting) 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).
tericin B, Cambrex) at 37°C in 5% CO₂-95% air. In all studies, culture media was not changed for 72 h (conditioned media) before the beginning of treatment with GABA₂ agonists.

For analysis of ERK phosphorylation, cells were treated with GABA₂ agonists (baclofen or SKF-97541) for 5 min in 72 h-conditioned culture medium. Pertussis toxin (100 ng/ml) was preincubated for 4 h prior to the addition of GABA₂ agonists. After treatment, cells were rinsed with cold phosphate-buffered saline (PBS), and ice-cold lysis buffer [50 mM Tris·HCl, pH 8.0, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, 1:200 dilution of protease inhibitor cocktail III (Calbiochem, San Diego, CA), 1 mM Na₃VO₄, 1 mM NaF] was added. Thereafter, the whole cell lysates were sonicated four times on ice for 15 s, and the protein concentration was determined. Cell lysates were solubilized by heating at 95°C for 5 min in sample buffer (final concentrations: 50 mM Tris·HCl, pH 6.8, 2.5% SDS, 6% glycerol, 2.5% 2-mercaptoethanol, bromophenol blue) and were stored at -20°C.

Isolation of smooth muscle from human trachea and guinea pig trachea. Studies were approved by Columbia University’s Institutional Review Board and deemed not human subjects research under 45 CFR 46. Human trachea came from two sources. Snap-frozen tracheas obtained at autopsy from nonasthmatic adults within 8 h of death were obtained from the National Disease Research Exchange (Philadelphia, PA). Additional tracheas were obtained from discarded regions of healthy donor lungs harvested for lung transplantation at Columbia University. Lung transplant excess tissue was transported to Columbia University. Pertussis toxin (100 ng/ml) was preincubated for 4 h prior to the addition of GABAB agonists. After treatment, cells were rinsed with cold phosphate-buffered saline (PBS), and ice-cold lysis buffer [50 mM Tris·HCl, pH 7.4, 1 mM EDTA, 1:200 dilution of protease inhibitor cocktail III (Calbiochem, San Diego, CA), 1 mM Na₃VO₄, 1 mM NaF] was added. Thereafter, the whole cell lysates were sonicated four times on ice for 15 s, and the protein concentration was determined. Cell lysates were solubilized by heating at 95°C for 5 min in sample buffer (final concentrations: 50 mM Tris·HCl, pH 6.8, 2.5% SDS, 6% glycerol, 2.5% 2-mercaptoethanol, bromophenol blue) and were stored at -20°C.

Immunoblotting. Whole cell or tissue lysates were electrophoresed (8–10% SDS-PAGE) and immunoblotted using antibodies directed against GABA₂R1 (rabbit 1:1,000, sc-14006; Santa Cruz Biotechnology, Santa Cruz, CA), GABA₂R2 (rabbit 1:1,000, AB5848; Chemicon, Temecula, CA), and total or phospho-ERK (both rabbit 1:1,000, nos. 9102 and 9101, respectively; Cell Signaling Technology, Danvers, MA). Epitopes were visualized with horseradish peroxidase-conjugated secondary antibodies (goat anti-rabbit, 1:5,000, Santa Cruz Biotechnology) using ECL Plus (Amersham Biosciences) and developed on film (Kodak BioMax light film; Kodak, Rochester, NY). Film development was developed such that band intensities were within the linear range of film responses, and band intensities were quantified using Quantity One software (BioRad, Hercules, CA).

Immunohistochemistry. Guinea pig tracheal rings were fixed using 10% formalin for 24 h at room temperature for GABA₂R1 immunostaining and using 4% paraformaldehyde/1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 4 h at 4°C for GABA₂ immunostaining. Tracheal rings were paraffin embedded, sectioned (5 μm), dewaxed in xylene, and rehydrated in a graded alcohol series to water. Endogenous peroxidase was blocked in 0.3% hydrogen peroxide. Heat-

### Table 1. GABA₄R1 and GABA₄R2 primer sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (from 5' to 3')</th>
<th>Product, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human R1a</td>
<td>5' CTG GCC AAC GGC TCC TGG ACA GAT ATG GAC</td>
<td>R1a: 1529</td>
</tr>
<tr>
<td></td>
<td>3' AGT TTC TGT GAG ACG AAG CCG AAT GTG TGG ATG A</td>
<td>R1c: 1343</td>
</tr>
<tr>
<td>Human R1b</td>
<td>5' GAT GGA GCC GCC TCC CCG GCA CCC TCT TA</td>
<td>272</td>
</tr>
<tr>
<td></td>
<td>3' GGC CCG ATG TAC ACT GGC CCG CGT TCT GA</td>
<td></td>
</tr>
<tr>
<td>Human R1e</td>
<td>5' GAA GAA GAT CTA CTA TGA CAG GAC GAA GGA TGA</td>
<td>R1a/b/c: 302</td>
</tr>
<tr>
<td></td>
<td>3' CCA GGC AGA AGC CAG CAG CTA AAG CCA GTG AG</td>
<td>R1e: 150</td>
</tr>
<tr>
<td>Human R2</td>
<td>5' GGA CCT GGA TGC CCG CTC GCT GCT GTA</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>3' GCG TTC GGC TCG CTC CAT GCT GTA</td>
<td></td>
</tr>
<tr>
<td>Guinea pig R1</td>
<td>5' CCA GTG GCT CCC GGA TGG CTT GGA GA</td>
<td>446</td>
</tr>
<tr>
<td></td>
<td>3' GGC CAG ACT AAA GGC CAG ACC CAG GAG CCA GA</td>
<td></td>
</tr>
<tr>
<td>Guinea pig R2</td>
<td>5' AGC TGG TGG TGG TGG GGG GCA GTC TAC</td>
<td>348</td>
</tr>
<tr>
<td></td>
<td>3' GGA AGG AGA CAG CGG CCC AAA TGA TAG AGA TGA</td>
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</tr>
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</table>
mediated antigen retrieval was performed with 10 mM sodium citrate buffer, pH 6.0 for 30 min. An avidin biotin blocking kit (Vector Laboratories, Peterborough, UK) was used (in 10% serum in PBS) to block endogenous biotin. Slides were rinsed with PBS and incubated overnight at 4°C in primary antibody against GABABR1 (rabbit, sc-14006, Santa Cruz Biotechnology) or GABA (mouse, MAB316, Chemicon) at a concentration of 1:250 or 1:50 in 2% serum in PBS, respectively. A tracheal ring section was incubated with the appropriate isotype IgG antibody as a negative control. A tracheal ring section was also incubated with a primary antibody directed against human γ- and α-smooth muscle actin (1:10,000, mouse, MAB1522, Chemicon) to identify smooth muscle in trachea. Following overnight incubation at 4°C, slides were washed with PBS, and primary antibodies were detected using biotinylated anti-mouse or anti-rabbit antibodies (Vector Laboratories) at a concentration of 1:100. The antigen antibody complex was then visualized by the enzymatic reduction of 3,3-diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin and dried, and cover slides were mounted using Poly-mount (Polysciences, Warrington, PA).

**RESULTS**

**RT-PCR analysis of GABA_B isoforms in airway smooth muscle.** RT-PCR analysis demonstrated mRNA encoding four splice variants of the human GABA_B1 protein (Fig. 2, A–C) and the GABA_B2 protein (Fig. 2D) in both native human tracheal smooth muscle and cultured HASM cells. Several splice variants of the GABA_B1 protein are expressed in human tissues, and we used primer sets designed to distinguish between each of these GABA_B1 variants. The expression of mRNA encoding the GABA_B1a and 1c variants was examined using a common primer set and was distinguishable by virtue of the omitted exons in GABA_B1c, resulting in a smaller PCR product for GABA_B1c (1,343 bp) compared to GABA_B1a (1,529 bp; Fig. 2A). We identified PCR products corresponding to the expected size of GABA_B1a and GABA_B1c in both native human tracheal airway smooth muscle and cultured HASM cells (Fig. 2A). Additionally, mRNA encoding the splice variant GABA_B1b was detected in both native HASM and cultured HASM cells (Fig. 2B). We then confirmed the existence of GABA_B1e using primers designed to flank the omitted exons of GABA_B1e and therefore could distinguish 1e from other isoforms (i.e., 1a, b, and c). We observed two PCR products of predicted sizes corresponding to splice variants GABA_B1a, b, and c (302 bp) and GABA_B1e (150 bp) in human trachea and cultured HASM cells (Fig. 2C). In human brain, used as a positive control, GABA_B1e was detected as a faint band, mRNA encoding GABA_B2 was also detected in native and cultured HASM (Fig. 2D). Guinea pig airway smooth muscle also expressed mRNA encoding both the GABA_B1a and GABA_B2 protein (Fig. 2E).
whole brain from both species (Fig. 3A). Whereas the larger 130-kDa band corresponds to the expected molecular mass of GABA\(_B\)R1\(_a\), the 100-kDa band corresponds to the molecular mass of either GABA\(_B\)R1\(_b\) or GABA\(_B\)R1\(_c\). In contrast, only the larger immunoreactive band corresponding to the GABA\(_B\)R1\(_a\) protein was identified in cultured HASM cells (Fig. 3A). An antibody raised against the GABA\(_B\)R2 protein identified a single immunoreactive band of \(~110\) kDa in protein samples from whole human brain and both native and cultured HASM (Fig. 3B), consistent with the predicted size for GABA\(_B\)R2. This antibody was weakly reactive using a protein sample from whole guinea pig brain (data not shown), and thus this antibody was considered not sufficiently reactive with guinea pig protein samples to analyze guinea pig airway smooth muscle expression.

**Immunohistochemical analysis of GABA\(_B\)R1 and GABA expression in guinea pig trachea.** To confirm the protein localization of GABA\(_B\)R1 to airway smooth muscle in guinea pig airways, immunohistochemistry was performed. GABA\(_B\)R1 immunoreactivity in guinea pig trachea was detected in airway smooth muscle, airway epithelium, and tracheal cartilage chondrocytes (Fig. 4A) with no staining in the negative control studies with rabbit IgG (Fig. 4B). The identity of the airway smooth muscle layer (and vascular smooth muscle) was con-
firmed using an anti-α-smooth muscle actin antibody (Fig. 4C), and no staining was seen with control mouse IgG2a (Fig. 4D).

Next, we examined whether the endogenous ligand for the GABA_B receptor, GABA, was expressed in guinea pig airways. GABA was localized to epithelium, chondrocytes, and an area immediately adjacent to airway smooth muscle with limited staining in small punctate areas within the airway smooth muscle itself (Fig. 5, A and C). No specific staining was apparent within these structures using a negative control, mouse IgG1 (Fig. 5, B and D).

**Functional coupling of the GABA_B receptor to G_i signaling pathways in HASM.** Demonstration of GABA_B receptor mRNA and protein in airway smooth muscle led us to determine whether the receptor demonstrated classical coupling to the G_i protein by evaluating 1) agonist-induced enhanced [35S]GTPγS binding, 2) agonist-induced inhibition of adenylyl cyclase activity, and 3) pertussis toxin-sensitive, agonist-induced activation of mitogen-activated protein kinase (MAPK ERK).

**Agonist-enhanced [35S]GTPγS binding in native HASM.** Baclofen, a specific GABA_B agonist, elicited a concentration-dependent stimulation of [35S]GTPγS binding with a 152 ± 6% increase above basal values (P < 0.001, n = 3) at 1 mM baclofen (Fig. 6A). A GABA_B antagonist, CGP-34358 (1 mM), blocked baclofen (300 μM)-induced increases (P < 0.001, n = 3) in [35S]GTPγS binding (Fig. 6B). These results suggest that GABA_B receptors are functionally coupled to a G protein in HASM.

**Agonist-induced inhibition of adenylyl cyclase activity in human cultured airway smooth muscle cells.** GABA_B receptor-mediated inhibition of adenylyl cyclase activity via coupling to
the G\textsubscript{i} protein is well known in neurons (12). To examine whether baclofen inhibits the adenylyl cyclase activity in cultured HASM cells, we measured 10 \(\mu\)M forskolin-stimulated adenylyl cyclase activity in the presence or absence of 100 \(\mu\)M baclofen. Baclofen significantly inhibited forskolin-stimulated adenylyl cyclase activity (\(P < 0.01, n = 8\); Fig. 7).

**Agonist-induced activation of ERK in cultured HASM cells.** Many Gi-coupled receptors are known to activate the ERK pathway via G protein \(\beta\gamma\)-subunits (11). ERK activation was identified by increased phospho-ERK on immunoblots and expressed as a ratio of phospho/total ERK for each sample. Baclofen (100 \(\mu\)M, 5 min) significantly increased phosphorylation of ERK (Fig. 8, left; \(n = 3, P < 0.05\)). In separate experiments, a second selective G\textsubscript{AB}B agonist, SKF-97541, also increased ERK phosphorylation (Fig. 8, right; \(P < 0.01, n = 3\)). Four-hour pretreatment of cells with pertussis toxin (100 ng/ml) abrogated increases in ERK phosphorylation by either G\textsubscript{AB}B agonist (\(P < 0.05\) and \(P < 0.001\) for pertussis toxin + agonist vs. baclofen or SKF-97541 alone, respectively, \(n = 3\)) confirming the role of G\textsubscript{i} in G\textsubscript{AB}B receptor activation of ERK.

**DISCUSSION**

The present study is the first to demonstrate that functional G\textsubscript{AB}B receptors are expressed in both native human and guinea pig airway smooth muscle and cultured HASM cells. Both G\textsubscript{AB}B\textsubscript{R1} and G\textsubscript{AB}B\textsubscript{R2} subunits were detected at the level of mRNA (by RT-PCR) and protein (by immunoblot) at appropriate molecular weights. Immunocytochemistry localized the G\textsubscript{AB}B\textsubscript{R1} subunit to airway smooth muscle in guinea pig tracheal rings. The G\textsubscript{AB}B\textsubscript{R} receptor agonist baclofen significantly increased GTP\textsubscript{y}S binding (implying G protein coupling) in a manner sensitive to the G\textsubscript{AB}B receptor antagonist CGP-35348. Baclofen also inhibited adenylyl cyclase...
activity and induced ERK phosphorylation, which was abrogated by pretreatment of cultured HASM cells with pertussis toxin, confirming classical coupling of the GABAB receptor to G\textsubscript{i} protein signaling pathways in these cells.

Several studies indicate the possible expression of functional GABAB receptors in peripheral and nonneuronal cells such as pancreatic beta cells (4), adrenocortical cells (18), cardiomyocytes (16), chondrocytes (27), and osteoblasts (9). Furthermore, GABABR1 mRNA expression was detectable using RT-PCR analysis in many peripheral organs including heart, spleen, lung, liver, intestine, kidney, stomach, adrenal gland, testis, ovary, and urinary bladder (5). Although mRNAs encoding GABABR1a and 1b have been reported in RNA isolated from whole lung (5), the present study is the first to demonstrate the expression of both the GABABR1 and GABABR2 localized to airway smooth muscle.

Recent evidence has shown that GABAB receptors must exist as a heterodimer to form a functional Gi protein-coupled receptor in the plasma membrane (2). The presence of both subunits in airway smooth muscle cells suggests that they can assemble functional GABAB receptor heterodimers. We demonstrated that baclofen was effective in activation of G protein as well as inhibition of adenylyl cyclase, thus showing that functional GABAB receptors are expressed in HASM. To date, four different splice variants (GABABR1a, b, c, and e) of GABABR1 have been identified in the human. Indeed, we identified not only the expression of major splice variants (GABABR1a and 1b) but also minor variants 1c and 1e in HASM. The GABABR1e splice variant encodes a truncated protein lacking the transmembrane and intracellular domains and is known to be present in a variety of peripheral human tissues. At the present time, there are no confirmed GABABR2 splice variants (2). The GABABR1e is suggested to compete for heterodimerization with the GABABR2 subunit and affect the formation of functional GABAB receptors in a dominant negative manner (26). In the present study, a high concentration of baclofen was needed to achieve significant stimulation of [35S]GTP\textsubscript{S} binding. This may be due to the low expression of GABAB receptors in airway smooth muscle relative to brain. However, the expression of the GABABR1e subunit in airway smooth muscle may decrease the number of functional GABAB receptors.

Although we identified mRNA encoding all four known splice variants of the GABABR1 subunit and the GABABR2 subunit in RNA isolated from freshly dissected human and guinea pig airway smooth muscle, RNA isolated from freshly dissected tissues invariably will contain some RNA from nonmuscle cells despite careful dissection. Therefore, to confirm the smooth muscle cell-specific expression of the GABABR1 and GABABR2 subunits, we analyzed RNA isolated from homogenous cultures of HASM cells and confirmed the expression of the same splice variants of GABABR1 and GABABR2 specifically in airway smooth muscle cells. Furthermore, immunohistochemistry in guinea pig tracheal rings localized the GABABR1 protein to the airway smooth muscle layer. In HASM, the GABABR1 antibody we used for immunoblot analysis reacted strongly and apparently nonspecifically with a 50-kDa protein making its use for immunocytochemis-
try in HASM unreliable. Interestingly, the GABA\textsubscript{B}R1 protein was also abundantly expressed in tracheal epithelial cells and chondrocytes. Whereas the identification of the GABA\textsubscript{A} receptor on chondrocytes has been previously described (9, 27), the identification of the GABA\textsubscript{B} receptor on airway epithelium is novel and suggests that multiple cell types in the airway may be responsive to endogenous GABA.

The endogenous ligand for GABA\textsubscript{B} receptors, GABA, has also been identified in many peripheral tissues, especially in endocrine organs such as the pituitary, pancreas, testis, gastrointestinal tract, ovary, placenta, uterus, and adrenal medulla (10). Peripheral GABA has been suggested to act not only as a neurotransmitter or neuromodulator in the autonomic nervous system but also as a hormone or trophic factor in nonneuronal tissue (19). To identify the existence and localization of GABA in airway, we performed immunohistochemical analysis using a specific antibody against GABA in guinea pig trachea. In the present study, we detected GABA immunoreactivity in the connective tissue near the smooth muscle as well as tracheal epithelium and cartilage chondrocytes. This result suggests that GABA may bind to GABA\textsubscript{B} receptors in airway smooth muscle, although the origin of GABA in airway is still unclear.

After demonstrating the mRNA and protein expression of GABA\textsubscript{B} receptors in airway smooth muscle, we sought to confirm its coupling to G proteins in general and its specific classical coupling to the G\textsubscript{i} protein. Activation of GTP binding is a standard measure of receptor coupling to heterotrimeric G proteins, and indeed the GABA\textsubscript{B} receptor agonist baclofen enhanced GTP binding in HASM. Two G\textsubscript{i}-specific coupling pathways were investigated using the GABA\textsubscript{B} agonist baclofen. Inhibition of adenyl cyclase is a well-known effect of G\textsubscript{i} protein activation and is known to occur in airway smooth muscle in response to activation of several G\textsubscript{i}-coupled receptors (e.g., M\textsubscript{2} muscarinic receptor). Activation by phosphorylation of ERK is a ubiquitous signaling pathway following G\textsubscript{i} activation, and pertussis toxin is a widely used tool to inactivate and implicate G\textsubscript{i} proteins in signaling events. Indeed, baclofen inhibited adenyl cyclase and activated ERK phosphorylation in a pertussis toxin-sensitive manner in HASM cells, confirming the coupling of GABA\textsubscript{B} receptors to G\textsubscript{i} proteins in these cells. The specificity of baclofen's effect at the GABA\textsubscript{B} receptor in the present study is supported by the finding that the antagonist CGP-35348 blocked the effect of baclofen in GTP\gammaS binding and by the finding that another GABA\textsubscript{B} agonist, SKF-97541, could mimic the baclofen's effect on ERK activation.

The physiological role of GABA\textsubscript{B} receptors in airway smooth muscle and of GABA-ergic modulation of intercellular cAMP or ERK activation is at present unclear. Because cAMP is known to induce relaxation of airway smooth muscle, an inhibitory effect of baclofen on adenyl cyclase suggests that GABA\textsubscript{B} receptor activation in airway smooth muscle could inhibit cAMP-mediated relaxation. A well known example of a G\textsubscript{i}-coupled receptor that modulates airway smooth muscle relaxation is the M\textsubscript{2} muscarinic receptor. The M\textsubscript{2} muscarinic receptor couples to G\textsubscript{i}, inhibits adenyl cyclase, and is known to inhibit \(\beta_2\)-adrenoceptor-induced smooth muscle relaxation (25), whereas M\textsubscript{2} muscarinic receptor antagonists are known to facilitate isoprenaline- and forskolin-mediated relaxation of acetylcholine-induced contraction of airway smooth muscle (8). These findings support the idea that the GABA\textsubscript{B} receptor could modulate airway smooth muscle tone via activation of the G\textsubscript{i} protein. Consistent with this, GABA\textsubscript{B} receptors have been implicated in modulation of contractility in the rabbit uterus (22), where the receptors appear to be nonneuronal, and are most likely expressed in smooth muscle cells.

Baclofen can increase cell proliferation in a GABA\textsubscript{B} receptor antagonist-sensitive manner in rat growth plate chondrocytes (27). In the present study, we found that baclofen induced ERK phosphorylation. This result was consistent with observations in HEK-293 cells transfected with GABA\textsubscript{B}R1 and R2 subunits (1). The requirement for ERK activation in HASM mitogenic signaling pathways has been well established (21). Since hyperplasia and hypertrophy of smooth muscle is considered to contribute to airway hyperresponsiveness in asthma (30), stimulation of GABA\textsubscript{B} receptors coupling to ERK activation would theoretically favor HASM cell proliferation and be associated with airway hyperresponsiveness. Further investigations are required to identify the physiological and possibly pathophysiological role of GABA\textsubscript{B} receptors in airway smooth muscle cells.

Although the source of the endogenous ligand GABA for GABA\textsubscript{B} receptors in airway is unclear at present, GABA may modulate an uncharacterized signaling cascade via GABA\textsubscript{B} receptors expressed in airway smooth muscle. This signaling cascade could be a target for new therapeutic interventions in controlling airway tone.

GRANTS

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*GABA_B RECEPTOR IN AIRWAY SMOOTH MUSCLE*