The dopamine D₂ receptor is expressed and sensitizes adenylyl cyclase activity in airway smooth muscle

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Mizuta K, Zhang Y, Xu D, Masaki E, Panettieri RA Jr, Emala CW. The dopamine D₂ receptor is expressed and sensitizes adenylyl cyclase activity in airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 302: L316–L324, 2012. First published September 30, 2011; doi:10.1152/ajplung.00130.2011.—Dopamine receptors are G protein-coupled receptors that are divided into two subgroups, “D₁-like” receptors (D₁ and D₃) that couple to the Gₛ protein and “D₂-like” receptors (D₂, D₃, and D₄) that couple to Gᵢ. Although inhaled dopamine has been reported to induce bronchodilation in patients with asthma, functional expression of dopamine receptor subtypes has never been described on airway smooth muscle (ASM) cells. Acute activation of Gₛ-coupled receptors inhibits adenylyl cyclase activity and cAMP synthesis, which classically impairs ASM relaxation. In contrast, chronic activation of Gᵢ-coupled receptors produces a paradoxical enhancement of adenylyl cyclase activity referred to as heterologous sensitization. We questioned whether the dopamine D₂-like receptor is expressed on ASM, whether it exhibits classical Gᵢ-coupling, and whether it modulates ASM function. We detected the mRNA encoding the dopamine D₂ receptor in total RNA isolated from native human ASM and from cultured human airway smooth muscle (HASM) cells. Immunoblots identified the dopamine D₂ receptor protein in both native human and guinea pig ASM and cultured HASM cells. The dopamine D₂ receptor protein was immunohistochemically localized to both human and guinea pig ASM. Acute activation of the dopamine D₂ receptor by quinpirole inhibited forskolin-stimulated adenylyl cyclase activity in HASM cells, which was blocked by the dopamine D₂ receptor antagonist L-741626. In contrast, the chronic pretreatment (1 h) with quinpirole potentiated forskolin-stimulated adenylyl cyclase activity, which was inhibited by L-741626, the phospholipase C inhibitor U73122, or the protein kinase C inhibitor GF109203X. Quinpirole also stimulated inositol phosphate synthesis, which was inhibited by L-741626 or U73122. Chronic pretreatment (1 h) of the guinea pig tracheal rings with quinpirole significantly potentiated forskolin-induced airway relaxation, which was inhibited by L-741626. These results demonstrate that functional dopamine D₂ receptors are expressed on ASM and could be a novel therapeutic target for the relaxation of ASM.

METHODS

Materials. HASM cells were cultured in M-199 smooth muscle medium containing human fibroblast growth factor, human epidermal growth factor, FBS, and antibiotic-antimycotic which were purchased from Invitrogen (Grand Island, NY). Total RNA from whole human brain was purchased from Clontech (Mountain View, CA). Pertussis toxin (PTX) and protease inhibitor cocktail III was obtained from BD Biosciences (Palo Alto, CA). Total RNA from cortex were used as a positive protein control on immunoblots and from Invitrogen (Grand Island, NY). Lysates of human brain cerebral cortex were used as a positive protein control on immunoblots and were obtained from BD Biosciences (Palo Alto, CA). Total RNA from whole human brain was purchased from Clontech (Mountain View, CA). Pertussis toxin (PTX) and protease inhibitor cocktail III was obtained from EMD Biosciences (San Diego, CA). α-[32P]ATP (800 Ci/mmol), [3H]cAMP (32 Ci/mmol), and [3H]myo-inositol (20 Ci/mmol) were obtained from MP Biomedicals (Irvine, CA). Quinpirole, L-741626, and U73122 were purchased from Tocris Bioscience (Elisville, MO). All other chemicals were obtained from Sigma (St. Louis, MO) unless otherwise stated.

Cell culture. Primary cultures of HASM cells came from two sources. Cells were obtained from Lonza (Walkersville, MD) or at the University of Pennsylvania, which were obtained from lung transplant donors in accordance with procedures approved by the University of Pennsylvania Committee on Studies Involving Human Beings as...
Table 1. Primer sequences for dopamine D2-like receptor subtypes

<table>
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<tr>
<th>Target</th>
<th>Sequence of Primer</th>
<th>Amplicon Size, bp</th>
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<tbody>
<tr>
<td>Human dopamine D2</td>
<td>FP: 5’-GGA CCT CCG TCA AGA CCA TGA GCC GTA-3’</td>
<td>287</td>
</tr>
<tr>
<td></td>
<td>RP: 5’-GGA GCA GAG TCA GAG GTG GAT CTT CAG-3’</td>
<td></td>
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<tr>
<td>Human dopamine D3</td>
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<td>241</td>
</tr>
<tr>
<td></td>
<td>RP: 5’-ACT GTA AAG CTC TGG GAA CAC TGG GCA-3’</td>
<td></td>
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<tr>
<td>Human dopamine D4</td>
<td>FP: 5’-GCT CTC CTT GTC CTG CGG CTC TGC TGC TAC T-3’</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>RP: 5’-CAC GGC CAC GGC CAC GAC CAC CTT GT-3’</td>
<td></td>
</tr>
</tbody>
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FR, forward primer; RP, reverse primer.
biotin. Slides were rinsed with PBST and incubated overnight at 4°C in primary antibody against dopamine D2 receptor protein (for human tracheal ring sections: rabbit polyclonal 1:2,000; HP013, DAKO) or mouse inositol phosphates was measured in confluent HASM cells in 24-well tissue culture plates as described previously (19, 23). Briefly, after overnight loading with [3H]-inositol (10 μM; 30 min before the addition of quinpirole). Cells were then washed twice with PBS before stimulation with 100 nM forskolin (M) in cell culture medium before being washed with PBS. In acute experiments, 100 μl of warm PBS were added to each well. Subsequently, 50 μl of 3x adenylyl cyclase buffer (19) were added directly to the wells (to achieve final concentrations of 50 μM HEPES pH 8.0, 50 mM NaCl, 0.4 mM EGTA, 1 mM CaCl2, 7 μM MgCl2, 0.1 mM ATP (20 μCi/ml 32P-ATP), 0.1 mg/ml BSA, 50 U/ml creatine phosphokinase, and 7 mM phosphocreatine) in the absence (basal activity) or presence of 10 μM forskolin ± 1 μM quinpirole (dopamine D2-like receptor agonist) ± 1 μM L-741626 (selective dopamine D2 receptor antagonist) and the plates were incubated at 37°C for 15 min. In chronic experiments examining the sensitization of adenylyl cyclase activity, HASM cells were initially pretreated with either vehicle, 1 μM L-741626 (30 min), 5 μM U73122 [phospholipase C (PLC) inhibitor; 30 min], 100 nM GF109203X [proteinkinase C (PKC) inhibitor; 1 h], or 100 ng/ml PTX (G i protein blocker; 4 h) and then treated for 1 h with quinpirole (1 μM) in the M-199 culture medium at 37°C in a humidified incubator with 5% CO2. Cells were then washed twice with PBS before stimulation with 10 μM forskolin for 15 min at 37°C in the presence of L-741626 (1 μM) in those wells pretreated with L-741626 to prevent activation of dopamine D2 receptor by residual quinpirole (48).

For all experiments, the reactions were terminated by the addition of 100 μl stop buffer [50 mM HEPES pH 7.5, 2 mM ATP, 0.5 mM cAMP (0.5 μCi/ml 3H-cAMP), and 2% SDS] and synthesized 32P-cAMP was separated and quantitated by sequentially column chromatography over Dowex and alumina as described previously (37).

**Inositol phosphate assays.** Synthesis of total [3H]inositol phosphates was measured in confluent HASM cells in 24-well tissue culture plates as described previously (19, 23). Briefly, after overnight loading with [3H]-myo-inositol (10 μCi/ml, 20 Cl/mmol) in inositol-free and serum-free DMEM (Chemoicon, Temecula, CA), plates were washed three times (37°C, 500 μl HBSS with 10 mM LiCl). Incubation of cells with quinpirole (1 μM) in a final volume of 300 μl (HBSS with 10mM LiCl) at 37°C for 30 min was performed in the absence or presence of PTX (100 ng/ml; 4 h before the addition of quinpirole), U73122 (5 μM; 30 min before the addition of quinpirole), or L-741626 (1 μM; 30 min before the addition of quinpirole). Incubation of the cells with L-741626 (1 μM) alone in a final volume of 300 μl at 37°C for 30 min was also performed to determine whether L-741626 by itself exerts any effect on inositol phosphate synthesis. Reactions were terminated, and total [3H]inositol phosphates were recovered by column chromatography (19).

In vitro effects of chronic treatment with dopamine D2 receptor agonist on guinea pig airway smooth muscle relaxation. All studies were approved by the Columbia University’s Institutional Animal Care and Use Committee. Force measurements were performed on closed guinea pig tracheal rings suspended in organ baths as previously described (23). Briefly, Hartley male guinea pigs (~400 g body wt) were anesthetized with 50 mg/kg ip pentobarbital, the tracheas were removed promptly and dissected into closed rings comprised of two cartilaginous rings from which mucosa, connective tissue, and epithelium were removed. Silk threads were tied to the rings such that the threads were at each end of the posterior aspect of the ring (lacking in cartilage), ~180° from one another. One thread was attached to a fixed point at the bottom of 4-ml organ baths (Radnoti Glass Technology, Monrovia, CA), and the opposing thread was attached to a Grass FT03 force transducer (Grass-Telefactor, West Warwick, RI) coupled to a computer via Biopac hardware and Acknowledge 7.3.3 software (Biopac Systems, Goleta, CA) for continuous digital recording of muscle tension. The rings were suspended in 4 ml of KH buffer solution (composition in mM: 118 NaCl, 5.6 KCl, 0.5 CaCl2, 0.2 MgSO4, 25 NaHCO3, 1.3 NaH2PO4, and 5.6 glucose) with 10 μM indomethacin (DMSO vehicle final concentration of 0.01%), which was continuously bubbled with 95% O2-5% CO2 at pH 7.4, 37°C. The rings were equilibrated at 1 g of isotonic tension for 1 h with new KH buffer added every 15 min. All rings were precontracted with two cycles of cumulatively increasing concentration of acetylcholine (0.1 μM-1 mM) with extensive buffer washes between and after these two cycles with resetting of the resting tension to 1.0 g. Rings were chronically pretreated with or without the dopamine D2-like receptor agonist quinpirole (10 μM) for 1 h to chronically activate the dopamine D2-like receptor to test for adenylyl cyclase sensitization. Rings were then contracted with acetylcholine (an individual EC50 calculated for each ring). Following the achievement of a stable contraction (typically 15 min), forskolin (5 μM) was added to the buffer in the baths. In separate experiments, rings were pretreated with the dopamine D2 receptor selective antagonist L-741626 (10 μM) 30 min before the quinpirole (10 μM) treatment. Control-contracted rings received vehicle (KH buffer) to serve as time controls.

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

**RESULTS**

**RT-PCR analysis of dopamine D2-like receptors in airway smooth muscle.** Initially, we assessed the expression of mRNA encoding dopamine D2-like receptors (D2, D3, and D4) in freshly isolated HASM and in primary cultures of HASM cells. Total RNA from whole brain was used as a positive control. Freshly dissected native HASM expressed mRNA encoding the dopamine D2 receptor (Fig. 1). Messenger RNA encoding the dopamine D3 and D4 receptors was not detected in freshly dissected airway smooth muscle from human upper airways despite their detection in control human brain RNA. In primary cultures of HASM cells, mRNA encoding the dopamine D2 and D4 receptors was detected whereas D3 was not detected (Fig. 1).

**Immunoblot analysis of dopamine D2-like receptors in airway smooth muscle.** Lysates prepared from freshly dissected native HASM, freshly dissected native guinea pig airway smooth muscle and cultured HASM cells were subjected to immunoblot analysis using a specific antidopamine D2 receptor antibody (Fig. 2). Immunoreactive bands of appropriate molecular mass of ~50 kDa for dopamine D2 receptor were identified airway smooth muscle from all three sources. The
antibody used to detect the dopamine D₂ receptor protein is capable of identifying two isoforms of the dopamine D₂ receptor (D₂L and D₂S). The higher band of the appropriate molecular mass for dopamine D₂L (50.6 kDa) was identified in primary cultures of HASM cells and in freshly isolated airway smooth muscle from guinea pig, while the D₂S (47.4 kDa) was identified in freshly isolated airway smooth muscle from human and guinea pig (Fig. 2).

Immunohistochemical detection of dopamine D₂ receptor expression in guinea pig airway smooth muscle. To confirm the localization of dopamine D₂ receptor protein to airway smooth muscle, immunohistochemistry was performed using a rabbit polyclonal antibody that recognizes the dopamine D₂ receptor proteins in paraffin sections of both human and guinea pig tracheal rings. Specific immunoreactivity was detected in the airway smooth muscle layer of both human and guinea pig trachea (indicated by brown color) with no staining in the negative control sections using an isotype-specific rabbit IgG or mouse IgG2a control primary antibodies. In addition, no specific immunoreactivity was detected in the airway epithelial layer of both human and guinea pig trachea (Fig. 3).

Agonist-induced adenylyl cyclase activity in HASM cells. Dopamine D₂ receptor-mediated acute inhibition and chronic activation of adenylyl cyclase activity via the Gᵢ protein are well known in neurons (48). To examine whether a dopamine D₂ receptor agonist has similar effects on adenylyl cyclase activities in cultured HASM cells, we measured adenylyl cyclase activity in the presence or absence of the dopamine D₂ receptor agonist quinpirole. Acute activation of the dopamine D₂ receptor with quinpirole (1 μM) significantly inhibited forskolin-stimulated adenylyl cyclase activity (P < 0.01; n = 13), which was significantly blocked by pretreatment with the Gᵢ inhibitor PTX (100 ng/ml; 4 h; P < 0.05; n = 13) or the dopamine D₂ receptor antagonist L-741626 (1 μM; P < 0.05; n = 13; Fig. 4A). In contrast, chronic pretreatment with the same drugs resulted in a heterologous sensitization of adenylyl cyclase activity. The chronic (1 h) pretreatment with quinpirole (1 μM) significantly enhanced the forskolin-stimulated adenylyl cyclase activity (P < 0.001; n = 14), which was significantly blocked by the selective dopamine D₂ receptor antagonist L-741626 (1 μM; P < 0.05; n = 14) in a PTX (100 ng/ml; 4 h)-insensitive manner (NS; n = 14; Fig. 4B).

Agonist-induced stimulation of inositol phosphate synthesis in HASM cells. Classically receptors that couple to the Gᵢ protein activate PLC increasing inositol phosphates and intracellular calcium. Similarly, in Gᵢ-coupled receptors, cross-talk to PLCβ₁ from liberated Goα or Gβγ subunits has been described in other cell types (1, 14, 16, 42). To examine whether the dopamine D₂ receptor agonist stimulate inositol phosphate synthesis in cultured HASM cells, we measured the quinpirole-induced inositol phosphate synthesis in the presence or absence of L-741626, U73122, or PTX. Quinpirole (1 μM) significantly increased inositol phos-

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**Fig. 1.** Representative gel images of RT-PCR analysis of total RNA using primers specific for each of the known human dopamine D₂-like receptor subtypes (D₂L, D₂S, and D₃). Total RNA extracted from primary cultures of human airway smooth muscle (HASM) cells or freshly dissected human tracheal airway smooth muscle was analyzed. Lane 1: base pair standards; lane 2: negative control water blanks; lane 3: total RNA from primary cultured HASM cells; lane 4: total RNA from freshly dissected native human airway smooth muscle; lane 5: total RNA from whole human brain.

**Fig. 2.** Representative immunoblot analyses using antibodies against the dopamine D₂ receptor using protein prepared from cultured HASM cells (100 μg), freshly dissected native human tracheal airway smooth muscle (SM; 100 μg), and freshly dissected native guinea pig (GP) tracheal SM (100 μg) identifying two known isoforms of the dopamine D₂ receptor (D₂L and D₂S). White spaces between the 3 lanes indicate that these lanes were located on the same immunoblot but were not located in neighbouring lanes on the original gel and immunoblot image.

**Fig. 3.** Agonist-induced adenylyl cyclase activity in HASM cells. Dopamine D₂ receptor-mediated acute inhibition and chronic activation of adenylyl cyclase activity via the Gᵢ protein are well known in neurons (48). To examine whether a dopamine D₂ receptor agonist has similar effects on adenylyl cyclase activities in cultured HASM cells, we measured adenylyl cyclase activity in the presence or absence of the dopamine D₂ receptor agonist quinpirole. Acute activation of the dopamine D₂ receptor with quinpirole (1 μM) significantly inhibited forskolin-stimulated adenylyl cyclase activity (P < 0.01; n = 13), which was significantly blocked by pretreatment with the Gᵢ inhibitor PTX (100 ng/ml; 4 h; P < 0.05; n = 13) or the dopamine D₂ receptor antagonist L-741626 (1 μM; P < 0.05; n = 13; Fig. 4A). In contrast, chronic pretreatment with the same drugs resulted in a heterologous sensitization of adenylyl cyclase activity. The chronic (1 h) pretreatment with quinpirole (1 μM) significantly enhanced the forskolin-stimulated adenylyl cyclase activity (P < 0.001; n = 14), which was significantly blocked by the selective dopamine D₂ receptor antagonist L-741626 (1 μM; P < 0.05; n = 14) in a PTX (100 ng/ml; 4 h)-insensitive manner (NS; n = 14; Fig. 4B). In addition, as shown in Fig. 5, this sensitization of adenylyl cyclase activity by chronic activation of the dopamine D₂ receptor with quinpirole was significantly blocked by either the PLC inhibitor U73122 (5 μM; P < 0.05; n = 8; Fig. 5A) or the PKC inhibitor GF109203X (100 nM; P < 0.05; n = 9; Fig. 5B).

Agonist-induced stimulation of inositol phosphate synthesis in HASM cells. Classically receptors that couple to the Gᵢ protein activate PLC increasing inositol phosphates and intracellular calcium. Similarly, in Gᵢ-coupled receptors, cross-talk to PLCβ₁ from liberated Goα or Gβγ subunits has been described in other cell types (1, 14, 16, 42). To examine whether the dopamine D₂ receptor agonist stimulate inositol phosphate synthesis in cultured HASM cells, we measured the quinpirole-induced inositol phosphate synthesis in the presence or absence of L-741626, U73122, or PTX. Quinpirole (1 μM) significantly increased inositol phos-
phate synthesis ($P < 0.05; n = 8$), which was significantly blocked by L-741626 ($P < 0.05; 1 \mu M$; Fig. 6A). Pretreatment the cells with U73122 (5 $\mu M$; 30 min) also significantly blocked the quinpirole-induced inositol phosphate synthesis, whereas the pretreatment with PTX (100 ng/ml; 4 h) did not significantly block quinpirole-induced synthesis of inositol phosphates (NS; $n = 5$; Fig. 6B).

Functional studies of dopamine $D_2$ receptor function in intact guinea pig airway rings. Molecular identification of multiple subunits of dopamine $D_2$ receptors led us to question
whether chronic activation of the functional dopamine D2 receptor could modulate airway smooth muscle tone. Guinea pig tracheal rings suspended in organ baths subjected to chronic activation of the dopamine D2 receptor with quinpirole (10 μM) demonstrated significantly increased forskolin (5 μM)-induced relaxation of an acetylcholine (EC50)-induced contraction. Under identical conditions the pretreatment of tracheal rings with the selective dopamine D2 receptor antagonist L-741626 (10 μM; Fig. 7) significantly attenuated the potentiation induced by quinpirole (10 μM; Fig. 7).

DISCUSSION

The primary findings of the present study include the first demonstration of dopamine D2 receptor mRNA and protein expression in human and guinea pig airway smooth muscle cells and the ability of a dopamine D2 receptor-selective agonist to facilitate the relaxation against acetylcholine-induced contraction in airway smooth muscle through adenylyl cyclase sensitization.

The dopamine D2-like receptor is ubiquitously expressed in the central nervous system, and the modulation of neuronal activity by dopamine has been extensively studied. Numerous studies (17, 18, 20, 25, 28, 40) have also indicated the expression of functional dopamine D2 receptors in peripheral and nonneuronal cells such as pulmonary artery (36), renal tubules, glomeruli (postganglionic sympathetic nerve terminals), and zona glomerulosa cells of the adrenal cortex.

Identification of mRNA in freshly isolated HASM ensures that native expression of the dopamine D2-like receptor (D2, D3, and D4) is being identified. However, even with careful dissection freshly isolated tissue is contaminated by other cells types that could give rise to cDNA products during sensitive RT-PCR amplification. Therefore, we also evaluated the expression of the dopamine D2-like receptor in established primary cultures of HASM cells, which are a homogenous population of airway smooth muscle cells. Despite their cellular purity, cultured cell systems can result in an altered phenotype of protein expression during culture. Despite these limitations, there was remarkable agreement but some differences between the expression of dopamine D2-like receptor in freshly isolated vs. cultured HASM. We detected mRNA encoding the dopamine D2 receptor in dual sources of HASM studied including human freshly isolated airway smooth muscle and cultured HASM cells. In contrast, the dopamine D4 receptor was not identified in freshly isolated HASM but was readily detected in cultured HASM cells. This

Fig. 5. A: effect of phospholipase C (PLC) inhibitor (U73122; 5 μM) on forskolin (10 μM)-stimulated adenylyl cyclase activity in cultured HASM cells induced by chronic activation of dopamine D2 receptor by quinpirole (1 μM; n = 8). *P < 0.05, compared with forskolin alone. **P < 0.05, compared with forskolin + quinpirole. B: effect of protein kinase C (PKC) inhibitor (GF109203X; 100 nM) on forskolin (10 μM)-stimulated adenylyl cyclase activity in cultured HASM cells induced by chronic activation of dopamine D2 receptor by quinpirole (1 μM; n = 9). Data represent means ± SE. *P < 0.05, compared with forskolin alone. **P < 0.05, compared with forskolin + quinpirole. In each experiment, values were determined in triplicate.
Fig. 6. A: effect of the selective dopamine D2 receptor antagonist L-741626 (1 μM) on quinpirole (1 μM)-stimulated inositol phosphate synthesis in HASM cells (n = 8). In separate experiments, the effect of L-741626 by itself on inositol phosphate synthesis was measured (n = 6). Data represent means ± SE, presented as percentages of basal values. *P < 0.05, compared with quinpirole. #P < 0.05, compared with quinpirole. In each experiment, values were determined in triplicate. B: effects of PTX (100 ng/ml) or U73122 (5 μM) on quinpirole (1 μM)-stimulated inositol phosphate synthesis in HASM cells (n = 5). Data represent means ± SE, presented as percentages of basal values. *P < 0.01, compared with basal. **P < 0.05, compared with quinpirole. In each experiment, values were determined in triplicate.

raises the possibility that there is a phenotypic switch from the dopamine D2 receptor to the D4 receptor in cultured HASM cells, the functional consequence of which is unknown.

Identification of mRNA encoding dopamine D2 receptor in airway smooth muscle led us to investigate its protein expression by immunoblotting and immunohistochemistry. In agreement with our mRNA analysis, the dopamine D2 receptor protein was identified in airway smooth muscle from human and guinea pig airway smooth muscle and cultured HASM cells. The antibody used to detect dopamine D2 receptor identified the two isoforms of the dopamine D2 receptor, named long (D2L) and short (D2S) isoforms (13), which differ by an insertion of 29 amino acids in the third intracellular loop of the D2L receptor. We resolved two immunoreactive bands of 47.4 kDa (D2S) and 50.6 kDa (D2L) in freshly dissected guinea pig airway smooth muscle. In contrast, only a single band corresponding to 47.4 kDa was identified in freshly dissected HASM, whereas only the larger 50.6-kDa band was identified in cultured HASM cells. The smooth muscle-specific expression of dopamine D2 receptor was further confirmed immunohistochemically in both human and guinea pig airway smooth muscle. Although RNA of all dopamine D2-like receptors have been reported to be expressed in HASM cells by using expression microarrays (15), the present study is the first to demonstrate the protein expression of dopamine D2 receptor localized to airway smooth muscle.

After demonstrating the mRNA and protein expression of dopamine D2 receptor in airway smooth muscle, we sought to assess its bronchodilatory effect. Numerous studies have demonstrated that the acute activation of Gi-coupled receptors inhibits adenylyl cyclase activity, whereas chronic activation of these inhibitory receptors results in a compensatory increase in cAMP accumulation (47). Consistent with these previous findings (48), our findings also suggest that acute activation of dopamine D2 receptor on HASM cells inhibited the adenylyl cyclase activity by a PTX-sensitive pathway, while chronic activation of dopamine D2 receptor stimulated adenylyl cyclase activity but not via a Gi pathway.

This heterologous sensitization of adenylyl cyclase represents an adaptive response to persistent activation of several Gi-coupled receptors including dopamine D2 and D4, μ-opioid, δ-opioid, α2-adrenergic, M3 and M4 muscarinic, CB1 cannabinoid, and 5HT1A serotonin receptors (2-5, 7, 22, 30, 31, 39, 41, 43, 44, 48). Although long-term (18 h) agonist pretreatment has been reported to sensitize the adenylyl cyclase activity, short-term agonist pre-treatment (2 h) still caused sensitization (48) and was apparent after 20–30 min of agonist treatments (24). The sensitization of adenylyl cyclase has also been described in HASM cells (7, 8).

Previous studies (7) have shown that the chronic activation of the Gi-coupled receptors with their agonist induced adenylyl cyclase sensitization in HASM cells in PKC-insensitive, PTX-sensitive manner, suggesting that this sensitization is Gi-protein mediated. However, in the present study, adenylyl cyclase sensitization induced by chronic activation of the dopamine D2 receptor on HASM cells was not attenuated by PTX, while both a PLC inhibitor and a PKC inhibitor significantly blocked this sensitization. In addition, activation of the dopamine D2 receptor stimulated inositol phosphate synthesis in a PLC-sensitive manner. Classically Gi-coupled receptors are known to stimulate inositol phosphate synthesis through PLC. However, evidence exists in multiple cell types that activated Gα or Gβγ subunits released from Gi-coupled receptor can also cross-activate PLC, which is located downstream of the Gαγ-coupled receptor pathway (1, 14, 16, 42). PLC promotes the hydrolysis of phosphoinositol 4,5-bisphosphate into inositol phosphates and 1,2-diacylglycerol, which promotes the translocation of PKC from cytoplasm to the membrane and its subsequent activation. Rashid et al. (35) stated that dopamine D2 receptor stimulates PLC via a cross-talk mechanism through Gα12 or Gβγ subunits released from the dopamine D2 receptor. However, this cross-talk mechanism is inconsistent with our findings in that both the adenylyl cyclase sensitization induced by chronic activation of the dopamine D2 receptor and inositol phosphate synthesis induced by activation of this receptor were not attenuated by PTX, indicating that dopamine D2 receptor agonist has promiscuous effects on G protein coupling. There is increasing evidence that the dopamine D2 receptor is capable of forming a heterodimer with the dopamine D1 receptor and acts as a Gi-coupled receptor (35). Yan et al. (49) also suggested that the dopamine D2 receptor that classically couples to Gt can couple to the Gt/PLCβ pathway, causing an elevation of intracellular Ca2+ and activation of PKC. In our studies, the dopamine D1 receptor as well as the D2 receptor protein is expressed in airway smooth muscle (data not shown), suggesting that sensitization of the adenylyl cyclase through Gtβγ pathway may be triggered through dopamine D1-D2 receptor heterodimerization. However, detailed mechanisms of this in airway smooth muscle cells have not been investigated.

PKC is involved in the mechanism of heterologous sensitization of adenylyl cyclase isofrom VI after activation of Gi-coupled receptors (21, 45, 47). For example, in Chinese hamster ovary...
cells, prolonged \( \delta \)-opioid receptor activation sensitizes subsequent stimulation of adenylyl cyclase isoform VI phosphorylation, and PKC attenuated the sensitization of cAMP response (43, 44). In contrast, in HEK293 cells expressing both recombinant adenylyl cyclase VI and the dopamine D2L receptor, a PKC inhibitor did not attenuate adenylyl cyclase VI sensitization after persistent D2L receptor activation (6). These findings indicate that the mechanism of adenylyl cyclase sensitization differ between cell types. The present study indicated that, in HASM cells, adenylyl cyclase sensitization are mediated via PKC. The purpose of such adenylyl cyclase sensitization in HASM cells is unclear but may involve the need to maintain cAMP synthesis in the face of persistent Gi-coupled receptor activation.

In cases of bronchoconstriction, the administration of dopamine, systemically or topically, has a bronchodilatory effect (11, 12, 26). In the present study, chronic pretreatment of the guinea pig tracheal rings with a dopamine D2 receptor agonist potentiated forskolin-induced airway relaxation. These findings suggest that the continuous use of a dopamine D2 receptor agonist with a stimulator of adenylyl cyclase such as a \( \beta_2 \)-adrenergic receptor agonist (i.e., \( \beta_2 \)-adrenergic receptor agonist) would cooperatively relax the airway smooth muscle through sensitization of adenylyl cyclase activity.

In summary, we demonstrate for the first time the molecular expression of the dopamine D2 receptor in human and guinea pig airway smooth muscle. We demonstrate that a selective dopamine D2 receptor agonist can potentiate the relaxation of intact airway smooth muscle contracted with acetylcholine. These studies suggest that dopamine D2 receptor on airway smooth muscle would be a novel pharmacologic target for the relaxation of airway smooth muscle.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

REFERENCES


