Glucocorticoids Rescue TGF-β1-Mediated β2-Adrenergic Receptor Dysfunction by Attenuating Gene Expression

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Glucocorticoids (GCs) and β-adrenergic receptor (β2AR) agonists decrease asthma symptoms in most patients. GC treatment results in gene expression changes in human airway smooth muscle (HASM), thereby modulating the inflammation and airway reactivity that are hallmarks of asthma. Our previous studies showed that the pro-fibrotic cytokine, transforming growth factor β-1 (TGF-β1), may be blunting the effects of intracellular cAMP to induce airway relaxation by increasing its breakdown through upregulation of enzymes called phosphodiesterases (PDEs). We hypothesize that dexamethasone (DEX), a GC, rescues TGF-β1-induced β2-agonist hyporesponsiveness by attenuating PDE4D gene expression in HASM cells. HASM cells were stimulated in serum-free F12 media with dexamethasone (DEX, 1000 nM; 30 min) prior to TGF-β1 (10 ng/mL; 18 hr). Subsequently, HASM cells were treated with β_2-agonist, isoproterenol (ISO, 1 μM; 5 min) or G_s activator, cholera toxin (CTX, 0.25 μg/mL). HASM cells were lysed and intracellular cAMP levels were determined by chemiluminescent immunoassay. RNA was isolated and purified from HASM cells using the RNeasy Mini Kit. cDNA was generated using SuperScript IV First-Strand Synthesis System. Relative cDNA quantification was performed using qRT-PCR. Overnight TGF-β1 treatment significantly decreased ISO-induced cAMP levels by 44.82% $\pm$ 8.56 (P=0.0064) and DEX rescued the reduction in cAMP levels induced by TGF-β1 treatment (25.83% $\pm$ 12.66; P=0.057 compared to TGF-β1 treatment). Additionally, TGF-β1 significantly attenuated CTX-induced cAMP levels (59.97% $\pm$ 10.01; P=0.004), which was then reversed by DEX pretreatment (71.47% $\pm$ 7.11; P=0.0017 compared to TGFβ1 treatment). Our RNAseq data shows little difference between *pde4d* expression in the presence or absence of DEX alone. We found that DEX (100 nM pretreatment) significantly decreases TGF-β1-induced *pde4d* expression (77.09% $\pm$8.17; P=0.0002). Our data show that TGF-β1 induces β2AR hyporesponsiveness through attenuation of β_2AR agonist- and G_s-induced cAMP production via *pde4d* upregulation in HASM. Reversal of the effects of TGF-β1 by DEX suggests a novel mechanism underlying GC-dependent effects on β2AR hyporesponsiveness in asthma.