β2-Adrenoceptors And Muscarinic Receptors Mediate Opposing Effects On Endothelin-1 (ET-1) Expression In Human Lung Fibroblasts

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ET-1 appears to be involved in the pathogenesis not only of pulmonary hypertension, but also of fibrotic remodeling processes associated with chronic obstructive airway diseases. Since human lung fibroblasts, a potential source of ET-1 (Ahmedat et al., 2010), have been shown to be controlled by muscarinic and β-adrenoceptor mechanisms (e.g. Racké et al., 2008), the present study explored a possible muscarinic and β-adrenergic modulation of ET-1 expression in human lung fibroblasts.

MRC-5 human lung fibroblasts were cultured for up to 24 h in absence or presence of test substances, followed by prepro-ET-1 (ppET-1) mRNA determination by qPCR.

The muscarinic agonist oxotremorine (10 µM) induced an increase in ppET-1 mRNA by 182±31% (mean±s.e.mean, n=18), an effect prevented by 10 nM tiotropium, which alone had no effect. The long acting β2-adrenoceptor agonist olodaterol (10 and 100 nM) caused a reduction of ppET-1 mRNA expression by 43±2% and 45±11%, resp.. The effect of 10 nM olodaterol was pre-vented by the β2-adrenoceptor selective antagonist ICI 118,551 (1 µM), but not affect by the β1-a-drenoceptor selective antagonist CGP 20712 (3 µM). Olodaterol (10 nM) strongly opposed the stimulatory effect of 10 µM oxotremorine, in presence of both drugs, an insignificant increase by 14±7% was observed. The inhibitory effect of olodaterol was mimicked by the selective protein kinase A (PKA) agonist 6-Bnz-cAMP (500 µM), which caused a reduction in ppET-1 mRNA expres-sion by 63±7%, whereas the selective Epac (exchange protein activated by cAMP) agonist 8-CPT-2'-O-Me-cAMP (100 µM) caused only a marginal inhibition by 22±6%. An increase in ppET-1 mRNA expression by 184±31% caused by TGF-β in a sub-maximally effective concentration of 0.3 ng/ml was effectively opposed by 10 and 100 nM olodaterol, resulting in an inhibition comparable to that in absence of TGF-β (by 38±9% and 39±9%, resp.). However, the increase in ppET-1 mRNA expres-sion by 620±16% caused by 1 ng/ml TGF-β, a maximally effective concentration, was not signifi-cantly affected by 10 or 100 nM olodaterol. Likewise, the PKA-agonist 6-Bnz-cAMP (500 µM) opposed the increase in ppET-1 mRNA expression caused by 0.3 ng/ml TGF-β (an inhibition by 49±6%), but not that caused by 1 ng/ml TGF-β. TGF-β caused, with an IC50 of 0.3 ng/ml, a marked down-regulation of β2-adrenoceptor mRNA expression, maximally by 87±2% within 6 h.

ET-1 expression in human lung fibroblasts is regulated by stimulatory muscarinic receptors and inhibitory β2-adrenoceptors. The effect of β2-adrenoceptors may be mediated via PKA, as it is mimicked by direct activation of PKA. ET-1 expression in human lung fibroblasts is markedly up-regu-lated by TGF-β, but only effects of sub-maximally effective concentrations of TGF-β are opposed by the β2-adrenoceptor - PKA pathway, in part because of TGF-β-induced down-regulation of β2-adrenoceptors. Since ET-1 can promote pro-fibrotic features in human lung fibroblasts, inhibition of ET-1 expression could contribute to long-term beneficial effects of long-acting β2-adrenoceptor ago-nists such as olodaterol and long-acting muscarinic antagonists such as tiotropium.