Endothelin-1 induced activiation of ERK – MAP kinase pathway mediates stimulation of collagen synthesis in human lung fibroblasts

Ahmedat AS1, Warnken-Uhlich W1, Juergens UR2, Racké K2. 1Univ. Bonn, Inst. Pharmacology, Germany, 2Univ. Hospital, Med. Clinic II, Germany.

There is increasing evidence that endothelin (ET) is involved in the pathogenesis of pulmonary hypertension and fibrotic remodelling processes associated with chronic inflammatory, obstructive airway diseases. Recently we reported that human lung fibroblasts express a functional endothelinergic system. They are able to synthesize and release ET-1, are endowed with ET-A and ET-B receptors and ET-1 can mediate stimulatory effects on proliferation and collagen synthesis (e.g. Ahmedat et al. (2010) Naunyn-Schmiedeberg´s Arch Pharmacol 381 (Suppl. 1):56). The present study aimed to explore intracellular pathways involved in ET-1-induced stimulation of lung fibroblast collagen synthesis.

MRC-5 human lung fibroblasts were cultured and [^{3}H]-proline incorporation into cellular proteins served as a measure of collagen synthesis. In addition activation of ERK - MAP kinase was studied by detection of phospho-ERK1/2 by Wester blot analysis.

[^{3}H]-proline incorporation during 24 h incubation period under control conditions amounted to 30,557±2163 d.p.m. (n=45). ET-1 (1 and 10 nM) caused an increase in [^{3}H]-proline incorporation by 55±8% and 79±6%, resp. The muscarinic agonist oxotremorine (10 µM) caused an increase by 46±8%. Pertussis toxin (50 ng/ml, 6 h prior to [^{3}H]-proline exposure), which caused a reduction in basal [^{3}H]-proline incorporation by 36±1%, prevented oxotremorine-induced [^{3}H]-proline incorporation, but did not affect the stimulatory effect of ET-1 (10 nM). On other hand, the stimulation of [^{3}H]-proline incorporation by ET-1 (10 nM), like that by oxotremorine (10 µM) was largely attenuated by 10 µM PD 098059 and prevented by 30 µM PD 098059 (a specific inhibitor of the MAP kinase-activating enzyme).

ET-1 (10 and 100 nM) caused, like oxotremorine (10 µM) a rapid (within 5 min) and transient increase in phospho-ERK 1 / 2. Pertussis toxin pretreatment resulted in large attenuation of the effect of oxotremorine, but did not affect that of ET-1. ET-1-induced activation of ERK 1 / 2 was antagonized by the ET-A receptor selective antagonist BQ123 (1 µM), but not affected by the ET-B receptor selective antagonist BQ788 (100 nM).

ET-1-induced stimulation of collagen synthesis in human lung fibroblasts is mediated via G_q coupled ET-A receptors and involves activation of the ERK - MAP kinase pathway.

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