

MUSCARINIC STIMULATION OF HUMAN LUNG FIBROBLAST PROLIFERATION

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Airway remodelling is associated with chronic inflammatory and obstructive airway diseases, and fibroblasts appear to be crucially involved. Clinical observations showing that the long-acting muscarinic antagonist tiotropium delays the decline in lung function in COPD (Anzueto *et al.*, 2005) suggest, that cholinergic mechanisms may contribute to structural changes. Moreover, tiotropium was found to attenuate the increase in airway smooth muscle mass and myosin expression induced by repeated allergen challenges (Gosens *et al.*, 2005).

The aim of the present study was to investigate whether lung fibroblast proliferation is modulated by muscarinic mechanisms.

The human lung fibroblast cell line MRC-5 and primary human lung fibroblasts (obtained by outgrowth technique from isolated human lung tissue) were cultured in the absence and presence of FCS as indicated below. The expression profile of muscarinic receptors was characterised by semi-quantitative RT-PCR. Proliferative activity was measured by (³H)-thymidine incorporation.

MRC-5 fibroblasts express mRNA encoding different subtypes of muscarinic receptors ($M_2 > M_3 > M_4$, traces of M_5 and no M_1). The muscarinic agonists carbachol (up to 10 μ M) or oxotremorine (10 μ M) stimulated [³H]-thymidine incorporation with maximum increases between about 40% and 100% under various experimental conditions. When cells were cultured 24 h in presence of 10% FCS followed by 48 h in the absence of FCS, [³H]-thymidine incorporation (within the last 24 h of the culture period) amounted to 6,411 \pm 610 d.p.m. (mean \pm s.e.mean, n=21) under control conditions. Carbachol (10 μ M) or oxotremorine (10 μ M) (present during the FCS-free period) stimulated [³H]-thymidine incorporation by 53 \pm 9% (n=14) and 61 \pm 9% (n=9), respectively. When cells were cultured 48 h in the absence of FCS, carbachol (10 μ M) or oxotremorine (10 μ M) stimulated [³H]-thymidine incorporation (within the last 24 h of the culture period) by 81 \pm 10% (n=6) and 100 \pm 26% (n=6), respectively. The non-selective muscarinic antagonist atropine (1 μ M) and the long-acting muscarinic antagonist tiotropium (0.1 μ M) had no effect on their own, but prevented the stimulatory effect of 10 μ M carbachol or 10 μ M oxotremorine indicating the involvement of specific muscarinic receptors. Likewise, primary human lung fibroblasts express also mRNA for muscarinic receptors ($M_2 > M_3$) and 10 μ M carbachol stimulated (³H)-thymidine incorporation under various culture conditions between about 30% and 100% in an atropine- and tiotropium-sensitive manner.

In conclusion, human lung fibroblast proliferation can be stimulated by muscarinic receptor activation. This effect might be of relevance in remodelling processes during chronic airway diseases.

Gosens R *et al.* (2005) *Am. J. Respir. Crit. Care Med.* **171**:1096-1102.

Anzueto A *et al.* (2005) *Pulm. Pharmacol. Ther.* **18**:75-81.

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