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ScienceDirect
Life Sciences 80 (2007) 2290–2293
Life Sciences
www.elsevier.com/locate/lifescie

Administration of keratinocyte growth factor (KGF) modulates the pulmonary expression of nicotinic acetylcholine receptor subunits α7, α9 and α10

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Received 23 October 2006; accepted 10 January 2007

Abstract

Administration of recombinant human keratinocyte growth factor (rHuKGF, Δ23N-KGF, palifermin) protects the lung against a variety of injurious stimuli. The exact mechanisms leading to lung protection are unknown. Alterations in the non-neuronal cholinergic system of the lung might be involved, as vital pulmonary functions are regulated by acetylcholine. Here, we investigated the effect of KGF on the expression of nicotinic acetylcholine receptor subunits α7, α9 and α10 in rat lungs. Adult rats were treated via intratracheal instillation with rHuKGF or with an equivalent volume of PBS. The expression of nicotinic acetylcholine receptor subunits was analyzed by real-time RT–PCR, immunoblotting and immunohistochemistry. Treatment with rHuKGF led to a decreased expression of nicotinic receptor subunit α7 in the total lung. In contrast, the expression of the receptor subunits α9 and α10 was up-regulated. In conclusion, nicotinic acetylcholine receptors are differentially regulated by KGF treatment in vivo, which might result in changes in the biological effects of acetylcholine.

Keywords: Keratinocyte growth factor; Nicotinic acetylcholine receptor; Lung

Introduction

Keratinocyte growth factor (KGF) plays important roles in lung development, inflammation and repair (for review: Ware and Matthay, 2002). After intratracheal application of rHuKGF (Δ23N-KGF, palifermin) the lungs are protected against various injurious stimuli including hyperoxia, bleomycin, acid aspiration, mechanical damage, radiation, infections, and graft-versus-host disease (Ware and Matthay, 2002). Various mechanisms seem to be involved in the protective effect of KGF. It induces proliferation of alveolar epithelial type II cells (AEIIs), stabilization of surfactant homeostasis, improvement of barrier function of both vascular endothelia and alveolar epithelia, increased fluid clearance from the alveoli, as well as anti-inflammatory functions in macrophages (Ware and Matthay, 2002).

The effects of KGF are in several aspects quite similar to those of stimulators of α7 nicotinic acetylcholine receptors (nAChR) which are homopentameric ligand-gated ion channels with preferred permeability for calcium (Lukas et al., 2000). Activation of this pathway has a pronounced anti-inflammatory effect upon macrophages (Pavlov et al., 2003), induces proliferation of various pulmonary cells (Dasgupta et al., 2006), and prenatal nicotine exposure significantly increases the number of AEIIs in fetal monkey lung (Sekhon et al., 1999). In the nervous system, expression of the α7 nAChR subunit is controlled by several growth factors (Kawai et al., 2002; Zhou et al., 2004). On this background we asked whether pulmonary α7 nAChR expression is also controlled by KGF, which would

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0024-3205/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.lfs.2007.01.024

imply interaction between two pro-proliferative and anti-inflammatory pathways in the lung. In addition to α7 nAChR, we included nAChR subunits α9 and α10 in our study, because they share several important characteristics such as preferred ligands, ion permeability, and the ability to assemble to functional pentamers (α9 with or without additional α10 subunits) without participation of additional β-subunits.

Methods

Bioactive rHuKGF (Δ23N-KGF, palifermin) was provided by Amgen Inc. (Thousand Oaks, CA, USA). Male LEW rats weighing 200–270 g were supplied by Harlan Winkelmann (Borchen, Germany). Animal care and animal experiments were performed following the current version of the German Law on the Protection of Animals as well as the NIH principles of laboratory animal care.

Treatment of rats with rHuKGF, Western blot analysis, and immunohistochemistry have been described before (Grau et al., 2006). Briefly, adult rats were treated twice via intratracheal instillation (day 1 and day 2) with rHuKGF (5 mg KGF per kg body weight) or with an equivalent volume of PBS (200–270 μl). At day 4, the right lungs were either fixed and embedded in paraffin for immunohistochemistry ($n=4$ each) or snap-frozen for immunoblotting (at least $n=5$ each) and real-time RT–PCR ($n=5$ each). The expression of nAChR subunits α9 and α10 was investigated by immunohistochemistry using mono-specific polyclonal antibodies raised against synthetic peptides (Lips et al., 2002; Nguyen et al., 2000). Bound primary antibodies were detected by the mouse EnVision® peroxidase system and DAB. The specificity of the antibody binding was tested by adding excess amounts of peptide to the primary antibody dilution, which drastically reduced the staining intensity. Immunoblotting was performed with the anti-α10 antiserum and a monoclonal antibody to the nAChR subunit α7 (clone No. 306, Sigma, Deisenhofen, Germany). Additionally, the mRNA expression of the α7, α9 and α10 subunits was analyzed by real-time RT–PCR using the i-cycler (Bio-Rad, Munich, Germany) in combination with the IQ SYBR Green Real-Time PCR Supermix (Bio-Rad). The expression of α7 (forward primer: ACATTGACGTTCGCTGGTTC, reverse primer: CTACGGCGCATGGTTACTGT, L31619), α9 (forward primer: CGTGGGATCGAGACCAGTAT, reverse primer: TCATATCGCAGCACCACATT, AY574257) and α10 (forward primer: TCTGACCTCACAACCCACAA, reverse primer: TCCTGTCTCAGCCTCCATGT, AF196344) was normalized with β2-microglobulin (forward primer: TGTCTCAGTTCCACCCACCT, reverse primer: GGGCTCCTTCAGAGTGACG, NM_012512) as a housekeeping gene.

The means of the protein and mRNA expression values obtained from control-treated animals were normalized to 1 arbitrary unit and the expression data for the rHuKGF-treated

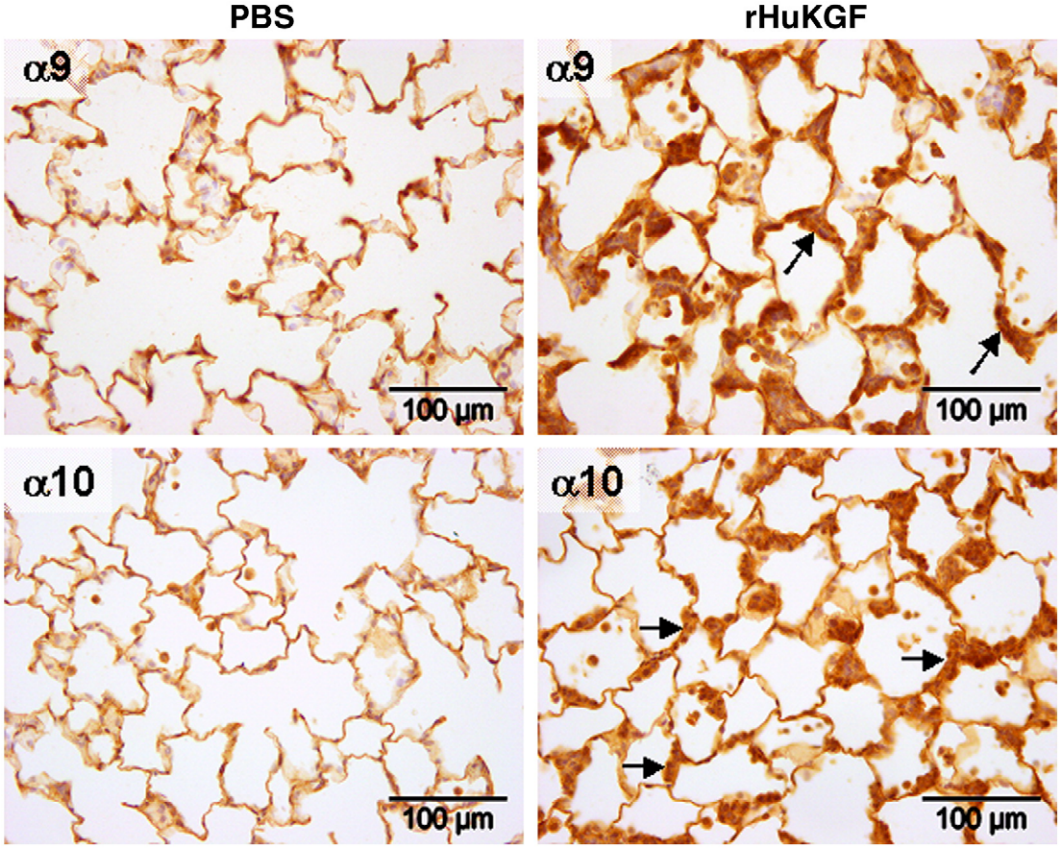

Fig. 1. Paraffin sections of the parenchyma of rat lungs treated with PBS (control) and recombinant human keratinocyte growth factor (rHuKGF), respectively. Treatment with rHuKGF results in a pronounced hyperplasia of alveolar epithelial cells type II (arrows). Detection of α9 and α10 nAChR subunits was performed by immunohistochemistry (brown staining). The slides were slightly counterstained with hemalum.