Scaffolding protein A-Kinase Anchoring Protein 12 enhances β_2 -adrenoceptor sensitivity in tracheal smooth musclebronchorelaxation

Airway hyper-responsiveness is a key feature of obstructive airway diseases such as asthma and chronic obstructive pulmonary disease which is associated with exaggerated bronchoconstriction. Alleviation of this bronchoconstriction is achieved using bronchodilators such as the cAMP-elevating β_2 -adrenoceptor agonists. In recent years another dimension has been added to cAMP signalling in the form of A-kinase anchoring proteins (AKAPs), which are proteins that scaffold PKA and other proteins, including enzymes and receptors to specific compartments in the cell. Recently, we have shown that airway smooth muscle cells express several AKAPs (1). AKAP5 and AKAP12 are decreased after cell exposure to cigarette smoke extract and in lung tissue of COPD patients (1). Both AKAP5 and AKAP12 are known to interact with the β_2 -adrenoceptor to control itssensitivity by regulating its presence at the cell membrane (2). This study investigates the role of AKAPs in bronchorelaxation of tracheal smooth muscle using β_{2} adrenoceptor agonists, with a special focus on AKAP5 and AKAP12.All experiments were performed in accordance with the national guidelines and approved by the University of Groningen Institutional Animal Care and Use Committee (Groningen, the Netherlands). Outbred, male, specified pathogen-free Dunkin Hartley guinea pigs (Harlan, Heathfield, UK) were terminated with a sharp blow to the head. After dissection of the smooth muscle layer and careful removal of the mucosa and connective tissue, tracheal smooth muscle strips of identical length and width were prepared and mounted in the organ bath for isometric tension measurements. Tracheal strips were pre-contracted with 10 µM methacholine and incubated with or without the PKA-AKAP interaction inhibitor st-Ht31 (50 µM) or control peptide st-Ht31P (50 µM) for 30 min (n=4 per treatment group) followed by a cumulative concentration response curve with β_2 -adrenoceptor agonists fenoterol in 0.5 log increments (10⁻¹⁰- 10^{-5.5} M). Statistical comparison was made using a one-way ANOVA followed by a Dunnet Post-Hoc analysis. Inbred C57BL/6J wild type (n=6), AKAP5-/- (n=5) and AKAP12-/- (n=5) male mice were terminated in a CO₂ chamber and tracheal rings were prepared and mounted on 5-mL DMT Myographs, once mounted, rings were maintained at 37°C. Tracheal rings were pre-contracted with 1 µM methacholine followed by a cumulative concentration response curve with the β_2 -adrenoceptor agonists isoprenaline in 0.5 log increments (10⁻¹⁰- 10⁻⁵ M). Statistical comparisonsweremade using anindependent samples T-test analysis. All data are represented as mean(±S.D.).Pre-incubation with the global PKA-AKAP interaction inhibitor st-Ht31 caused a rightward shift in the relaxation curve (LogEC₅₀ 8.00(\pm 0.35) control vs. 7.47 (\pm 0.16) st-Ht31 p<0.05) with no effect of the control peptide st-Ht31P (LogEC₅₀ 7.93 (±0.14)). No statistically significant was observed on E_{max}. AKAP12^{-/-}mimicked the st-Ht31 effect and induced a significant (p<0.05) rightward shift (LogEC₅₀ 7.55 (±0.08)) compared to wild type mice (LogEC₅₀ 7.73 (±0.14)). AKAP5^{-/-} showed the same response as the wild type (LogEC₅₀ 7.77 (±0.19)). No statistically significant effects were observed on E_{max} in either mouse strain. In summary, AKAPs play a role in β_2 -adrenoceptor sensitivity, in particular AKAP12 seems to maintain an improved response to β_2 -adrenoceptor agonists. Therefore, the loss of AKAP12 seen previously in COPD patients might be of importance to improve the therapeutic efficacy of B2adrenoceptor agonists in these patients.

- 1. Poppinga et al. (2015) Am J Physiol Lung Cell Mol Physiol. 308(8): L766-75.
- 2. Poppinga et al. (2014) Br J Pharmacol. 171(24): 5603-23.