Bronchial response to protease-activated receptor stimulation of airway lumenal and adventitial surfaces

H.W. Mitchell, H.L. Goh, N. Asokananthan, R.S. Lan, G.A. Stewart and A. Sharma, School of Biomedical and Chemical Sciences, University of Western Australia, Nedlands, WA, Australia.

A recently characterised family of G protein coupled receptors, protease-activated receptors (PARs), modulate inflammatory and regulatory signals in the airway. There are currently four PARs (PAR1, PAR2, PAR3 and PAR4) that have been cloned and characterised. Trypsin is an endogenous activator of PAR2 and PAR4. Activating these PARs has been shown to release PGE_2 from airway epithelial cells and modulate smooth muscle tone in isolated airway preparations (Cocks *et al*., 1999; Lan et *al*., 2001). It is uncertain how these different actions of PARs expressed on the various cell types in the airway may modulate airway function where lumenal and adventitial surfaces can be separately accessed by PAR activators. The present study investigates the actions of trypsin (300 μg/ml) and PAR agonist peptides (100-400 mM) on isolated whole airways in which the epithelial and adventitial surfaces can be separately exposed to PAR agonists. Bronchial airways were dissected from the lungs of pigs. Side branches were ligated and segments were placed in a bath at 37°C so that lumenal and adventitial surfaces were bathed in Krebs solution. A pressure transducer measured airway lumenal pressure, from which airway responses were assessed. Lumenal Krebs solution was assayed for PGE_2 by ELISA. Trypsin added to the adventitia produced a short latency (<5 min) inhibition of carbachol-induced tone. However, trypsin added to the airway lumen produced a delayed (>45 min) suppression of acetylcholine dose-contraction curve. Moreover, both trypsin and the PAR2 agonist increased PGE_2 production. Indomethacin pre-treatment blocked production of PGE_2, but had no effect on trypsin-induced relaxation by either route. The PAR1, 2 and 3 agonists had not effect on airway tone, but the PAR4 agonist produced short latency relaxation that was blocked by indomethacin. The study confirms that trypsin relaxes airways and releases PGE_2. Moreover, the effects of trypsin are highly dependent on its route of delivery, suggesting the contribution of different cell types by each route of exposure. However, the results observed with trypsin and the PAR2 agonist appear to dissociate the possible link between PGE_2 release by PAR activation and subsequent airway relaxation in this whole airway preparation. These findings suggest that the functional responses to trypsin are likely to be mediated by a receptor other than the established PAR1, PAR2, PAR3 or PAR4.

Cocks, T.M., Fong, B., Chow, J.M., Anderson, G.P., Frauman, A.G., Goldie, R.G., Henry, P.J., Carr, M.J., Hamilton, J.R. & Moffatt, J.D. (1999) *Nature*, 398, 156-160.

Lan, R.S., Knight, D.A., Stewart, G.A. & Henry, P.J. (2001) *British Journal of Pharmacology,* 132, 93-100.