BRONCHOCOSTRICTOR EFFECT OF PROTEASE ACTIVATED RECEPTOR-1 ACTIVATING PEPTIDE (PAR-1AP) *IN VITRO* AND *IN VIVO*

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Protease Activated Receptor (PAR-1) is a member of the seven transmembrane domain G protein coupled receptor proteolitically activated by thrombin. Thrombin cleaves the peptide bond Arginina 41-Serina 42 in the N-terminal extracellular domain exposing a new N-terminus which functions as a tethered ligand for the receptor itself (1). The new N-terminal domain interacts with the receptor and initiates intracellular signalling. PAR-1 activation by thrombin may finally result in the activation of transcription factors that regulate the expression of tissue factor, adhesive proteins, growth factors, cytokines and other mediators (2). Syntethic peptides ranging between 5 and 14 aminoacids, called Protease Activated Receptor-1 Activating Peptides (PAR-1APs), corresponding to the N-terminus residue unmasked, are capable of reproducing several cellular effects of thrombin but are devoid of the thrombin catalityc activity (3). Previously, it has been observed that thrombin has a direct action on lung parenchymal smooth muscle, *in vitro* (4). Moreover, it has been demonstrated that thrombin causes bronchoconstriction in guinea pigs through activation of its receptor PAR-1; bronchocostrinction was paralleled by a biphasic change in arterial blood pressure, characterized by a hypotensive phase followed by a hypertensive phase (5).

The aim of this study was to evaluate the mediators and the mechanism involved in the bronchoconstriction induced by a peptide activating PAR-1 (SFLLRN) in guinea pigs, *in vitro* and *in vivo*.

For *in vitro* experiments, guinea pigs (Charles River 300-350g) were sacrificed by decapitation, exsanguinated and lungs were removed. Main bronchi were dissected free of parenchyma and mounted in an isolated organ bath connected to an isometric force transducer. Tissue reactivity was checked by evaluating the response to a single concentration of acetylcholine (30 µM).

For *in vivo* experiments, guinea pigs were anaesthetised with urethane; the jugular vein and the carotid artery were cannulated for drug administration and blood pressure recording respectively. Animals were artificially ventilated and airway resistance was measured through a cannula inserted into the trachea and connected to a bronchospasm transducer interfaced with a computerised system for data analysis, PowerLab. After surgery, a single dose of histamine (10 µg/kg iv.) was administered to evaluate animal airway responsiveness.

Data were expressed as mean ± s.e.m and analysed with a computerized statistical system. Results were analysed by one or two ways ANOVA, followed by Bonferroni's test or Dunnet's test; a value of $P<0.05$ was taken as significant.

In vitro, PAR-1AP (1-100 ìM) caused a concentration dependent bronchocostriction (pEC_{50} 4.50 ± 0.27) , reaching the maximum value of 35.48 ± 5.08 % (n=4) at the concentration of 100 µM. There was no desensitisation after repeated administration of the peptide. The contractile response to PAR-1AP was inhibited following tissue incubation with the COX inhibitor, indomethacin (10 ìM) (Emax 6.67 ± 3.4 % vs. 34.0 ± 8.5 %,

p<0.01; n=3) and tyrosine kinase inhibitor, genistein (2 ìM) (Emax 17.8 ± 8.4. % vs. 46.0 ± 8.0 %, p<0.01; n=3) and by the TXA_2 antagonist, IcI 192.605 (1 ìM) (Emax 25.2 ± 3.1 % vs. 37.2 ± 8.2 %, p<0.05; n=5).

In vivo, intravenous administration of PAR-1AP caused an increase both in airway resistance and in blood pressure. A dose-response curve to the peptide-induced bronchocostriction was performed reaching the value of 7.6 ± 3.0 %; 28.5 ± 7.2 % and 57 ± 4.6 % (n=10), respectively at the doses of 0.1, 0.3 and 1 mg/kg (i.v.). PAR-1AP (0.1 – 1 mg/kg i.v.) also caused a dose dependent hypertension reaching the value of 36 ± 5.3 mmHg at the dose of 1mg/kg. There was a partial desensitisation to peptide bronchocostrinctor effect for repeated administration while the hypertension induced by PAR-1AP was not modified. PAR-1AP (0.3 mg/kg i.v.)-induced bronchocostriction was significantly reduced after animal treatment with IcI 192.605 (0.5 mg/kg i.v.) (17 ± 6.6 % vs. 57.6 ± 5.3 %, n=4; p<0.001) or ibuprofen (10 mg/kg i.v.) (17.4 ± 6.2 % vs. 57.6 ± 5.3 %, n=3; p<0.001). In IcI 192,605 or ibuprofen treated animals, PAR-1AP-induced hypertension was respectively inhibited (10.8 ± 3.7 mmHg vs. 34.6 ± 6.0 mmHg, n=4; p<0.01 and 9.6 ± 1.6 mmHg vs. 34.6 ± 6.0 mmHg, n=3; p<0.01).

Our results suggest that the release of prostanoids, particularly thromboxane A_2, and the activation of the pathway of tyrosine kinase might be the triggers of the bronchoconstrictor effect of PAR-1AP.

References

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