RELAXANT EFFECT OF PAR2-ACTIVATING PEPTIDE ON ISOLATED BRONCHI FROM LPS-TREATED RATS

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Protease activated receptor 2 (PAR2) is a member of seven transmembrane domain G protein coupled receptor activated by proteases. Following cleavage of the amino terminal sequence of the extracellular exodomain, the new unmasked amino terminal sequence acts as a tethered ligand, activating the receptor itself. In absence of proteolysis, PAR2 can be activated by synthetic peptides mimicking the sequence of the receptor tethered ligand, termed PAR2-activating peptides (PAR2-APs) (Dery et al., 1998). There is in vitro and in vivo evidence that PAR2 is protective in airways of experimental animals *in vitro* and *in vivo* (Coocks et al., 1999, Cicala et al., 2001), nonetheless, the role of PAR2 in physiology and/or pathology has not been clarified. We have previously shown that following injection of E.coli lipopolysaccharide (LPS) to rats, there is an increased vascular response to PAR2-activating peptide (PAR2-AP), paralleled by an increased PAR2 expression on endothelium and vascular smooth muscle, suggesting that this receptor can participate to modulate systemic inflammation triggered by endotoxin (Cicala et al., 1999). In this study, we have evaluated the effect of PAR2-AP (SLIGRL) on contractility of isolated bronchus from LPS treated rats. Male Wistar rats (Charles River, 250-300 g) were slightly anaesthetized with enflurane and intravenously injected with LPS (14 x 10^6 U / kg) or with saline. After 4, 12, 20 and 48 hours from LPS or vehicle treatment, rats were sacrificed by cervical dislocation after exposure to CO_2 and their lungs were removed and placed in a Petri dish containing Krebs solution. Main bronchi were dissected free of parenchyma, mounted in 2.5 ml isolated organ bath containing Krebs solution, at 37°C and oxygenated (95 % O_2 and 5 % CO_2), and connected to an isometric force transducer. Tissues were preloaded with 0.5 g and allowed to equilibrate for at least 90 min, during this period the tissue was washed about 6 –7 times, with fresh Krebs solution. After equilibration, tissue reactivity was checked by evaluating the response to a single concentration of Ach (30 μM). After washing, the effect of PAR2-AP (SLIGRL, 1 – 100 μM) was evaluated on tissue pre-contracted with carbachol (1 μM). Relaxation are evaluated as percentage reduction of maximal tension, expressed as mean ± standard error and analysed by two way ANOVA. PAR2-AP significantly relaxed (15.40 ± 2.35 %, n = 8) control tissue only at highest concentration tested (100 μM). Bronchi from LPS treated animals showed an increased response to PAR2-AP that reached the maximum 20 h after LPS treatment (E max 46.97 ± 9.13 %; pEC50 4.9 ± 0.19; n = 16). This increased response was inhibited following tissue incubation with ibuprofen (10 μM) (E max 13.01 ± 2.1 % vs. 32.86 ± 3.03 %, $p < 0.01$; n = 6), and partially reduced by the selective COX1 inhibitor, FR 122047 (0.1 μM), the selective COX2 inhibitor, DFP (10 μM) and the calcitonin gene related peptide antagonist (ratCGRP [8-37], 1 μM), while it was unaffected by propranolol (10 μM). Our results demonstrate that PAR2-AP modulate bronchial responsiveness in LPS treated rats through a mechanism involving prostaglandins and calcitonin gene related peptide.

References

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