available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/biochempharm

A protective role for proteinase activated receptor 2 in airways of lipopolysaccharide-treated rats

Silvana Morello [a], *Valentina Vellecco* [a], *Fiorentina Roviezzo* [a], *Pasquale Maffia* [a], *Salvatore Cuzzocrea* [b], *Giuseppe Cirino* [a], *Carla Cicala* [a,*]

[a] *Department of Experimental Pharmacology, University of Naples "Federico II", via D. Montesano 49, 80131 Naples, Italy*
[b] *Institute of Pharmacology, School of Medicine, University of Messina, Messina, Italy*

ARTICLE INFO

Article history:
Received 7 July 2005
Accepted 6 October 2005

Keywords:
Airways
Calcitonin gene related peptide
Cyclooxygenase-2
Lipopolysaccharide
Proteinase activated receptor 2
Rat

ABSTRACT

Proteinase activated receptor-2 (PAR2), a seven transmembrane domain G protein coupled receptor, is expressed on airway epithelium and smooth muscle cells and over-expressed in human airways under pathological conditions, such as asthma and chronic obstructive pulmonary disease (COPD). However, the role of PAR2 in airways has not yet been defined. Aim of the present study, was to evaluate the in vitro rat bronchial response to a synthetic peptide activating PAR2 (PAR2-AP; SLIGRL), following an in vivo treatment with bacterial lipopolysaccharide (LPS). Bronchi from LPS-treated animals showed an increased relaxant response to PAR2-AP, compared to naïve animals, the effect was maximum after 20-h pre-treatment and reduced by epithelium removal. Western blot analysis showed an increased PAR2 protein expression on bronchi removed 20 h after LPS treatment. PAR2-AP-induced bronchorelaxation was inhibited by ibuprofen, by the selective cyclooxygenase2 (COX-2) inhibitor, diisopropyl fluorophosphate (DFP) and partially by the calcitonin gene related peptide (CGRP) antagonist, rat-$CGRP_{[8–37]}$. Furthermore, there was a strong immunoreactivity for COX-2 on bronchial epithelium of LPS-treated rats. Prostaglandin E_2 (PGE_2) tissue release and CGRP tissue content were significantly increased following tissue incubation with PAR2-AP. The in vivo LPS treatment in rats strongly increases the bronchorelaxant effect of PAR2-AP, this effect correlates with an increased tissue protein receptor expression and the COX-2 localization on bronchial epithelium. Our work supports a role for PAR2 as a defence mechanism aimed to preserve bronchial functionality under systemic inflammatory conditions; both COX-2-derived PGE_2 and CGRP are involved in this effect.

1. Introduction

Proteinase activated receptor 2 (PAR2), a seven transmembrane domain G protein coupled receptor activated by proteolytic cleavage which, together with other PARs, shows a unique mechanism of autoactivation, is expressed on several animal and human tissues, under physiological conditions [1]. Within the respiratory tract, PAR2 is expressed by airway epithelial and smooth muscle cells, as well as endothelial and vascular smooth muscle cells [1,2]. PAR2 is also expressed by lung macrophages, mast cells, granulocytes, lymphocytes and eosinophils [3,4]. The role of PAR2 in airways is still controversial. Studies performed on animal tissues have demonstrated a protective role for PAR2 in airways; indeed, activation of PAR2 receptors causes an epithelium-dependent relaxation of mouse isolated bronchi, that correlates with

* *Corresponding author*. Tel.: +39 081 678455; fax: +39 081 678403.
E-mail address: cicala@unina.it (C. Cicala).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2005.10.016

PAR2 immunoreactivity in cytoplasmic regions of airway epithelial cells [5], and also an epithelium-dependent relaxation of mouse and guinea pig tracheal rings [6,7]. In vivo, it has been demonstrated that activation of PAR2 produces a protective effect against 5HT-induced bronchoconstriction in rats [5] and histamine-induced bronchoconstriction in guinea pigs [8]. Conversely, it has also been reported that PAR2 activation leads to a sensory neuropeptide-dependent bronchoconstrictor response in vivo [9] and, in vitro, causes constriction of human bronchi [10] and hyperresponsiveness to histamine, in guinea pigs [11].

There is in vitro and in vivo evidence for an up-regulation of PAR2 by inflammatory stimuli, such as tumour necrosis factor-α (TNF-α) and interleukin-1α (IL-1α) [12], suggesting that the activation of PAR2 may be regulated by inflammatory mediators. Recently, it has been demonstrated an increased PAR2 expression in airways of mice during influenza A virus infection, coupled to a prolonged inhibition of methacoline-induced bronchoconstriction [13]. Similarly, within human airways, PAR2 is over-expressed under pathological conditions, such as asthma and chronic obstructive pulmonary disease (COPD) [2,14,15]. However, it is currently unclear the physiological significance of receptor up-regulation under certain diseases, both in animals and humans.

Injection of bacterial lipopolysaccharide (LPS) to experimental animals causes a systemic inflammatory response characterized by multiple organ dysfunction syndrome (MODS) [16,17]. Within the airways, the effect of LPS appears to be secondary to cytokine release, and it has been postulated that LPS exerts its effects on peripheral terminals of sensory afferents and, through cytokine release, regulates sensory nerves terminal release [18]. A relationship between PAR2 and non adrenergic non cholinergic (NANC) system was first proposed by Steinhoff et al. [19], who postulated a neurogenic mechanism at the basis of the pro-inflammatory effect of PAR2 agonist. Successively, it has been demonstrated that a neurogenic mechanism is also at the basis of colitis [20] and hyperalgesia [21] induced by PAR2 agonists in experimental animals.

Previously, we have shown that an increased protein expression of PAR2 on vascular endothelium and smooth muscle cells following injection of LPS, in rats, correlates with an increased hypothensive response to the injection of the synthetic peptide activating PAR2 (PAR2-AP) [22], suggesting that the increase in cellular PAR2 expression during inflammation might reflect an increased tissue sensitivity to endogenous PAR2 activators.

To further evaluate the role of PAR2 in airways, under inflammatory conditions, in the present study we have investigated the ex vivo effect of LPS on rat bronchial response to a synthetic peptide activating PAR2.

2. Materials and methods

2.1. Animals and LPS treatment

Male Wistar rats (200–250 g; Charles River, Milan, Italy) were slightly anaesthetized with enflurane and intravenously injected, through the caudal vein, with LPS from *Escherichia coli* (serotype 0127:B8; 14.0×10^6 U/kg) or with an equal volume of sterile saline (NaCl 0.9%; 1 mL/kg). The dose of LPS used was a threshold dose, chosen on the basis of a previous study performed on rats [22]. LPS or saline injected animals were randomized and sacrificed at different times after treatments.

2.2. Functional study

After 4, 12 and 20 h from treatment with LPS, animals were sacrificed by cervical dislocation, exsanguinated and lungs were removed and placed into a Petri dish containing Krebs solution of the following composition (nM): NaCl, 115.3; KCl, 4.9; $CaCl_2$, 1.46, $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0 and glucose 11.1; termosted at 37 °C and oxygenated (CO_2, 5%; O_2, 95%). Main bronchi were dissected free of parenchyma and mounted in 2.5 mL isolated organ bath containing Krebs solution, at 37 °C, oxygenated (95% O_2 and 5% CO_2), and connected to an isometric force transducer (Ugo Basile) under a resting tension of 0.5 g. After about 60 min equilibration period, tissue reactivity was checked by evaluating the response to a single concentration of acetylcholine (Ach, 30 μM). After washing, a cumulative concentration response curve to PAR2-AP (1–100 μM), or to the control peptide (LSIGRL, 1–100 μM), was performed on tissue pre-contracted with carbachol (1 μM). The effect of PAR2-AP was also evaluated on bronchi after epithelium destruction, obtained by leaving tissue in contact with distilled water for 22 s. Epithelium destruction was then confirmed by histological examination. Furthermore, as positive control, to ascertain that distilled water challenge did not alter the ability of airway smooth muscle to relax, we tested relaxation in response to exogenous Prostaglandin E_2 (1 μM) before and after epithelium destruction.

2.3. Western blot analysis

Tissue samples removed as described above, were homogenised on ice in the following lysis buffer: Tris–HCl pH 7.5, 50 mM; NaCl, 150 mM; sodium ortovanadate, 1 mM; β-glycerophosphate, 20 mM; EDTA, 2 mM; DTT, 1 mM; PMSF 1 mM; leupeptin, 5 μg/mL; aprotinin, 5 μg/mL, pepstatin, 5 μg/mL. Protein concentration was measured by Bradford reagent using BSA as a standard. Protein samples (30 μg) were briefly boiled and subjects to electrophoresis on an SDS 10% polyacrylamide gel and transferred onto a nitrocellulose transfer membrane using standard procedure (Protran, Schleicher & Schuell, Germany). The membranes were placed in 5% non-fat milk for 1 h at room temperature and then incubated with rabbit anti PAR2 polyclonal antibody, 1:500, overnight at 4 °C. After three washes of 5 min each in PBS–Tween 20 (0.1%, v/v), membranes were incubated with the secondary antibody (1:5000) conjugated with horseradish peroxidase, antirabbit IgG for 2 h at 4 °C and then three 5-min washes were performed. The signal was detected with ECL (enhanced chemiluminescence) System according to the manufacturer's instructions (Amersham Pharmacia Biotech; Milan, Italy).

2.4. Effect of inhibitors

To investigate on mediators involved in the bronchorelaxant effect of PAR2-AP, functional experiments were also per-

formed on bronchial rings incubated with a non selective cyclooxygenase1/cyclooxigenase2 (COX-1/COX-2) inhibitor, ibuprofen (10 μM, −20 min); the selective COX-1 inhibitor, FR 122047 (0.1 μM, −20 min); the selective COX-2 inhibitor, diisopropyl fluorophosphate (DFP, 10 μM, −20 min); the non selective β adrenergic antagonist, propranolol (10 μM, −10 min); the calcitonin gene related peptide (CGRP) antagonist, rat-$CGRP_{[8–37]}$ (1 μM, −10 min) or the neurokinin 1 receptor (NK1R) antagonist, L703606 (1 μM, −10 min).

2.5. Prostaglandin E_2 (PGE_2) assay

In some cases, after performing functional experiments as described above, organ bath fluid was collected and 50 μL aliquots were assayed for PGE_2 content by an enzyme immune assay (EIA). Samples collected from preparations stimulated with PAR2-AP (1–100 μM) were compared to an appropriate time-control. PGE_2 content was expressed as pg PGE_2/mL.

2.6. CGRP assay

In another set of experiments, main bronchi from naive and 20 h LPS pre-treated rats were removed as described above, cut in rings and placed in a 24-well plate containing Krebs solution, 1 mL/well. Tissues were pre-contracted with carbachol (1 μM) and then stimulated with a single concentration of PAR2-AP, ranging from 1 to 100 μM, or with the only vehicle, following the same conditions of the functional study. After 5 min, each ring was snap frozen with liquid nitrogen and stored at −80 °C. Successively, from each ring CGRP was extracted following manufacturer extraction protocol and assayed by an enzyme immune assay (EIA). CGRP content in each sample was expressed as pg per mg protein determined by Bradford assay.

2.7. Histological analysis

Main bronchi removed as described above were fixed in 10% (v/v) buffered formalin for 48 h. Sections were cut (6 μm thick) and stained with haematoxylin and eosin to demarcate cell type. The sections were analyzed by using a standard light microscope (×20 objective) and photographed under low power. Histological examination was performed on tissue samples from naive and LPS-treated animals, to evaluate the effect of LPS treatment on epithelial integrity, and from tissue after treatment with distilled water, to verify epithelial destruction.

2.8. Immunohistochemical localization of COX-2

Main bronchi removed as described above were fixed in 10% (v/v) buffered formalin for 48 h and 6 μm sections were prepared from paraffin embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% (v/v) H_2O_2 in 60% (v/v) methanol for 30 min. The sections were permeablized with 0.1% (w/v) Triton X-100 in PBS for 20 min. Non-specific adsorption was minimised by incubating the section in 2% (v/v) normal goat serum in PBS for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin. The sections were then incubated overnight with primary anti-COX-2 antibody, 1:500 dilution or with control solutions including buffer alone or non specific purified rabbit IgG. Specific labelling was detected with a biotin-conjugated anti-rabbit IgG and avidin–biotin peroxidase complex. The counterstain was developed with DAB (brown colour) and nuclear fast red (red background). To verify the binding specificity of the relevant antibody for COX-2, some sections were also incubated with the primary antibody only (no secondary antibody) or with secondary antibody only (no primary antibody). In these situations, no positive staining was found in the sections indicating that the immunoreactions were positive in all the experiments carried out.

2.9. Drugs

Ach, aprotinin, carbachol, ibuprofen, LPS, PGE_2, propranolol, haematoxylin, eosin, nuclear fast red were purchased from Sigma (Italy). DTT, PMSF, leupeptin, pepstatin were purchased from ICN Pharmaceutical, S.r.l. (Milan, Italy). Bradford reagent was purchased from Bio-rad Laboratories (Segrate, MI). Rabbit anti PAR2 polyclonal antibody and goat anti COX-2 polyclonal antibody were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Antirabbit IgG conjugated to horseradish peroxidase was purchased from Dako (Denmark). ECL system was from Amersham Pharmacia Biotech (Milan, Italy). Endogenous biotin blocking, biotin-conjugated anti-rabbit IgG and avidin biotin peroxidase complex and DAB were purchased from DBA (Milan, Italy). FR122047 was a generous gift of Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan) and DFP was a generous gift of Prof. T. Warner, William Harvey Institute (London, UK). rat-$CGRP_{[8–37]}$ and NK1R antagonist, L703606, were purchased from Tocris (UK). EIA kits were from Cayman, SpiBio (France). All salts were purchased from Carlo Erba (Italy). The peptide SLIGRL and its scramble control, LSIGRL, were synthesised at the Department of Medicinal Chemistry, University of Naples ''Federico II'', Italy, as previously described [8].

2.10. Statistical analysis

All data are expressed as mean ± S.E.M. of at least five independent experiments. PAR2-AP effect on pre-contracted bronchial rings was expressed as mean ± S.E.M. of the percentage reduction of maximal tension. Concentration response curves were represented by a non linear regression and compared by two ways ANOVA followed by Bonferroni's test for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Functional studies

On tissue from naïve animals, PAR2-AP caused a relaxation reaching the maximum value (E_{max}) of 15.40 ± 2.35% ($N = 8$) at the highest concentration tested (100 μM), that was unaffected by epithelium removal. Bronchi from LPS-treated animals showed an increased response to PAR2-AP. The time-course

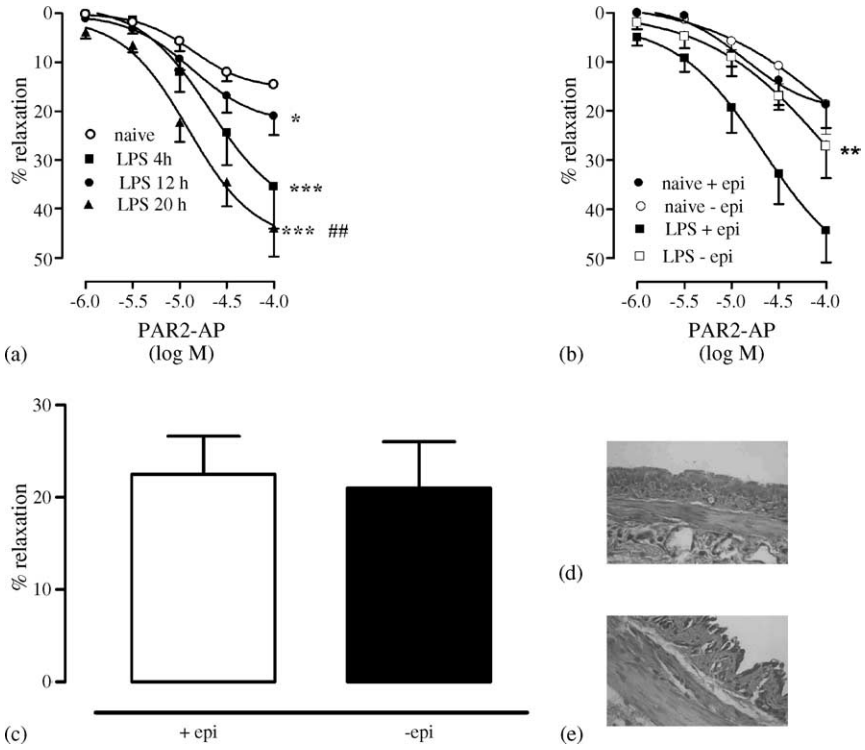

Fig. 1 – (a) Effect of PAR2-AP (1–100 μM) on rat bronchial rings of naïve rats (open circle) and of LPS-treated rats (closed symbols). LPS (14.0×10^6 U/kg i.v.) treatment was performed 4, 12 or 20 h before the in vitro functional study. $^{*}P < 0.05$ and $^{***}P < 0.001$ vs. naive; $^{\#\#}P < 0.01$ vs. 4 and 12-h pre-treatment (two-way ANOVA; N = 8–16). (b) Effect of epithelial destruction (open symbols) on relaxation induced by PAR2-AP (1–100 μM) on bronchial rings of naive and of LPS-treated (−20 h) rats. (c) Effect of epithelial destruction on relaxation induced by exogenous PGE_2 (1 μM; N = 4). Insert Photomicrographs of histological examination of rat bronchial tissue with an intact epithelium (d) and after epithelium destruction by contact with distilled water (e).

study showed that response was maximum on tissue removed 20 h after LPS treatment (E_{max} 46.57 ± 4.45%; $-\log EC_{50}$ 4.91 ± 0.19; N = 16) and was significantly reduced by epithelium removal (Fig. 1). Thus, all the following experiments were performed on bronchi removed from rats after 20 h in vivo pre-treatment with intravenous LPS. The control peptide, LSIGRL, was inactive (data not shown).

3.2. *Western blot analysis*

Western blot analysis showed an increased PAR2 protein expression in bronchi from LPS-treated rats (20-h pre-treatment) compared to bronchi from naïve animals, that was confirmed by densitometry (Fig. 2).

3.3. *Effect of inhibitors*

The epithelial-dependent effect of the peptide was abolished by ibuprofen (10 μM) and significantly inhibited by the selective COX-2 inhibitor, DFP (10 μM) ($-\log EC_{50}$ 4.4 ± 0.15 versus 4.8 ± 0.08, N = 4; $P < 0.05$). On the contrary, response to PAR2-AP was unaffected by the selective COX-1 inhibitor, FR 122047 (0.1 μM). Furthermore, relaxation induced by PAR2-AP was also partially reduce by the CGRP antagonist, rat $CGRP_{[8–37]}$ (1 μM) (E_{max}, 32.13 ± 2.87% versus 43.27 ± 2.52%, N = 6; $P < 0.01$), while it was unaffected by propranolol (10 μM) and by the NK1R antagonist, L-703606 (1 μM; Fig. 3) at a concentration able to inhibit exogenous substance P-induced relaxation by 80% (7.00 ± 1% versus 34.67 ± 2.33%, N = 3; $P < 0.01$).

3.4. *PGE_2 assay*

PGE_2 levels measured in the organ bath fluid collected after performing a cumulative concentration response curve to PAR2-AP (1–100 μM) were significantly increased compared to PGE_2 control levels (Fig. 4).

3.5. *CGRP assay*

In the tissues from LPS-treated animals, the content of CGRP was significantly increased compared to tissue from naïve rats. CGRP levels were further increased by tissue incubation with PAR2-AP (Fig. 5). Conversely, PAR2-AP did not modify basal CGRP content obtained from naïve animal tissues (data not shown).

3.6. *Immunoistochemistry*

Tissue from LPS-treated animals showed a strong positive immunoreactivity for COX-2 compared to tissue obtained from naive animals; the enzyme was preferentially localized in the bronchial epithelium (Fig. 6).

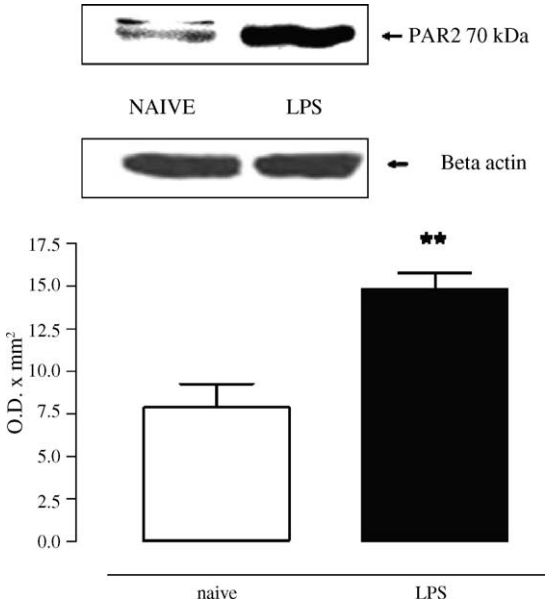

Fig. 2 – Upper panel: detection of PAR2 by Western blot analysis in bronchial tissue of naive and LPS-treated (−20 h) rats. Lower panel: densitometric analysis showing that there is a remarkable increase of PAR2 expression in tissue of LPS-treated rats compared to tissue from naïve rats. The blot is representative of three separate experiments. $^{**}P < 0.01$ vs. naive.

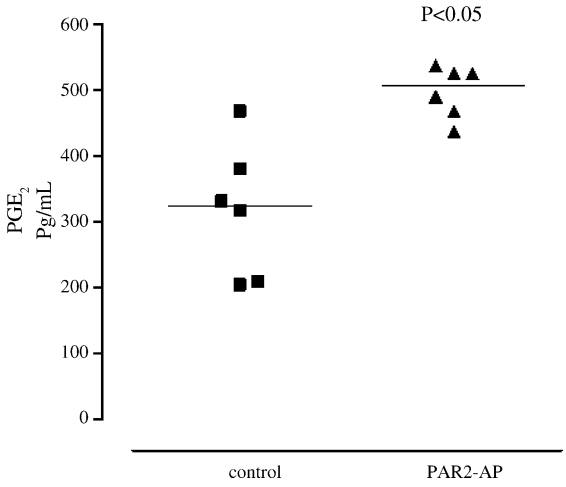

Fig. 4 – PGE_2 levels detected in the organ bath fluid after performing a cumulative concentration response curve to PAR2-AP (1–100 μM) on bronchial rings from rats pre-treated with LPS (−20 h). Samples collected from preparations stimulated with PAR2-AP were compared to an appropriate time-control.

4. Discussion

The main finding of our study is that main bronchi isolated from LPS-treated rats show an increased relaxant response to PAR2-AP. Time course experiments show that this effect is maximum at 20 h. Conversely, tissues from naïve animals

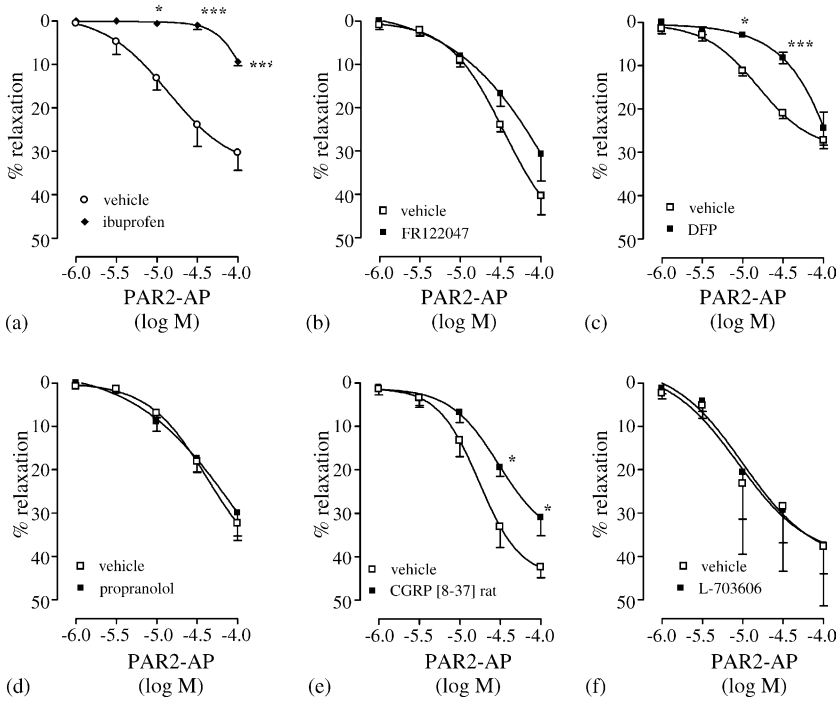

Fig. 3 – Concentration response curves to PAR2-AP on bronchial rings of LPS-treated (−20 h) rats: (a) effect of the COX-1/COX-2 dual inhibitor, ibuprofen (10 μM); (b) effect of the selective COX-1 inhibitor, FR122047 (0.1 μM); (c) effect of the selective COX-2 inhibitor, DFP (10 μM); (d) effect of the non selective β adrenergic antagonist, propranolol (10 μM); (e) effect of the CGRP antagonist, rat $CGRP_{[8–37]}$ (1 μM); (f) effect of NK1R antagonist, L-703606 (1 μM). $^{*}P < 0.05$ and $^{***}P < 0.001$ vs. vehicle.

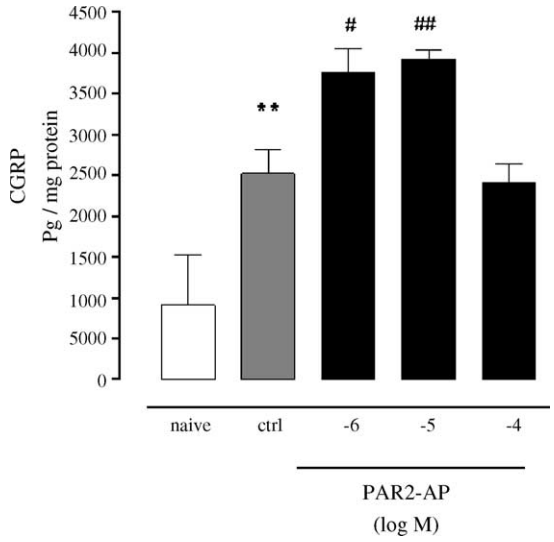

Fig. 5 – CGRP tissue content after rat bronchial ring incubation with PAR2-AP (1–100 μM). $^{**}P < 0.01$ vs. naïve; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. control ($N = 6$).

respond to PAR2-AP with a very small relaxation, only evident at the highest peptide concentration tested (100 μM).

The increased response to PAR2-AP was paralleled by an increased PAR2 protein expression in tissue derived from LPS-treated animals. This finding is in agreement with previous data showing an increased PAR2 expression under inflammatory conditions, either in vitro or in vivo [2,12,13,15,22,23], and further suggests that receptor up-regulation might reflect an increased tissue sensitivity to PAR2 endogenous activators, under certain diseases.

It is known that epithelium plays an important role in the maintenance of airway functionality and that during inflammation it represents the source of a wide array of mediators, but also a surface were receptors are newly expressed [24,25]. Previous studies have demonstrated that the bronchorelaxant effect due to PAR2 agonists is dependent upon epithelial PGE_2 [5,6]. Here, we show that the increased sensitivity to PAR2-AP effect of main bronchi from LPS-treated animals was partially dependent upon epithelium integrity, while control responses were not. However, even after epithelium destruction, responses obtained on tissue removed 20 h after LPS treatment did not return to control value, indicating that, together with epithelium, also smooth muscle cells contribute to the increased tissue sensitivity to PAR2-AP. This increased sensitivity to PAR2-AP of tissues from LPS-treated animals, evident at a low peptide concentration (10 μM) that was inactive on control tissues, was inhibited by the COX-1/COX-2 inhibitor, ibuprofen, and by the selective COX-2 inhibitor, DFP, but not by the selective COX-1 inhibitor, FR122047, suggesting that the prostanoid responsible for PAR2-AP-mediated bronchorelaxation is likely derived from COX-2 rather than COX-1 activity. This finding is in agreement with results obtained by Lan et al. [6,13] who performed experiments on mouse airways. Since PGE_2 is the most important relaxant prostaglandin in airways [26], we assayed its release in the organ bath fluid following incubation with PAR2-AP. As expected, PGE_2 levels in the organ bath fluid were significantly increased following tissue incubation with PAR2-AP. Furthermore, the immunohistochemistry analysis confirmed the COX-2 localization on bronchial epithelium of LPS-treated animals.

There is evidence that inflammatory mediators regulate the release of neuropeptides and that several features of LPS-treated animals are dependent upon neuropeptide release in response to circulating cytokines [18,27]. An association of PAR2 with sensory neurons, has already been demonstrated. Indeed, it has been shown that PAR2 is expressed on sensory nerves and its activation causes an intracellular Ca^{2+} signal and the following release of CGRP and substance P (SP) by nerve endings. Following this evidence it has been suggested that proinflammatory effects due to PAR2 activation might involve these neuropetides [19,28]. CGRP is a neuropeptide that colocalizes with SP in sensory C-fibre afferents in the airways [29,30]. CGRP is produced in the vicinity of bronchial smooth muscle cells, suggesting that it could modulate tracheobronchial tone. However, the role of CGRP in airways has often been controversial; it has been reported to be a potent bronchoconstrictor [31] involved in the bronchoconstriction induced by PAR2 agonist in guinea pigs [9]. However, there is also much evidence that it can be protective in rat, human, mouse and guinea pig airways [32,33]. On the basis of this knowledge, we sought to investigate whether, under our

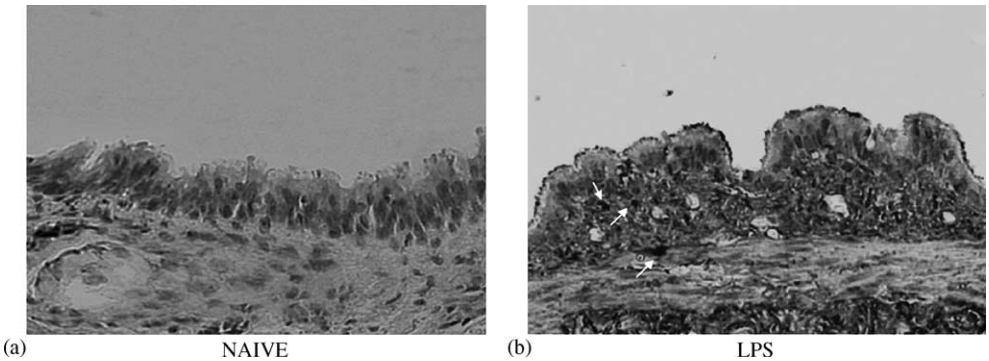

Fig. 6 – Representative photomicrographs of sections of rat bronchi labelling for COX-2 (original magnification: ×100). Results illustrated are from a single experiment and are representative of three separate experiments: (a) naïve rat; (b) LPS (−20 h)-treated rat.

experimental conditions, these neuropeptides, CGRP and SP, were involved in bronchorelaxation induced by PAR2 agonist. Experiments performed using the selective antagonist, rat-$CGRP_{[8–37]}$, show the partial involvement of CGRP in the effect of PAR2-agonist. On the contrary, NK1R antagonist did not have any effect, ruling out the involvement of SP. On the basis of results obtained in the functional studies, we assayed tissue CGRP content after incubation with PAR2-AP. It is worth noting that CGRP content in bronchi from animals treated with LPS was increased in comparison with tissue CGRP content of control tissue, according to data demonstrating that LPS and cytokines influence the peripheral activity of primary sensory afferent fibres by sensitizing the terminals and facilitating the release of CGRP [18]. Interestingly, our data show that stimulation with PAR2-AP causes a further increase in the tissue content of CGRP of LPS-treated animals.

In conclusion, our data show a bronchorelaxant effect of PAR2-AP in rat that is further increased by an in vivo LPS treatment and involves both CGRP and PGE_2. The increased tissue sensitivity to PAR2 agonist correlates with an increased tissue receptor proteic expression and the COX-2 localization on bronchial epithelium.

There is evidence that systemic inflammatory response and MODS caused by injection of LPS to experimental animals is also accompanied by bronchial hyperreactivity and inflammation [34]. Thus, on the basis of results obtained we hypothesise that the increased expression of PAR2 on bronchial tissue of LPS-treated rats might be part of a defence mechanism aimed to preserve bronchial functionality under those condition characterized by airway inflammation.

REFERENCES

[1] D'Andrea MR, Derian CK, Leturcq D, Baker SM, Brunmark A, Ling P, et al. Characterization of protease activated receptor-2 immunoreactivity in normal human tissues. J Histochem Cytochem 1998;46:157–64.

[2] Knight DA, Lim S, Scaffidi AK, Roche N, Chung KF, Stewart GA, et al. Protease-activated receptors in human airways: upregulation of PAR-2 in respiratory epithelium from patients with asthma. J Allergy Clin Immunol 2001;108:797–803.

[3] Lan RS, Stewart GA, Henry PJ. Role of protease-activated receptors in airway function: a target for therapeutic intervention? Pharmacol Ther 2002;95:239–57.

[4] Bolton SJ, McNulty CS, Thomas RJ, Hewitt CR, Wardlaw AJ. Expression of and functional responses to protease-activated receptors on human eosinophils. J Leukoc Biol 2003;74:60–8.

[5] Cocks TM, Fong B, Chow JM, Anderson GP, Frauman AG, Goldie RG, et al. A protective role for protease activated receptor in the airways. Nature 1999;398:156–60.

[6] Lan RS, Knight DA, Stewart GA, Henry PJ. Role of PGE(2) in protease-activated receptor-1, -2 and -4 mediated relaxation in the mouse isolated trachea. Br J Pharmacol 2001;132:93–100.

[7] Risse PA, Naline E, Faisy C, Huchon G, Chung KF, Kleinmann P, et al. Protease-activated receptor 2 in regulation of bronchomotor tone: effect of tobacco smoking. Life Sci 2004;75:991–1002.

[8] Cicala C, Spina D, Keir SD, Severino B, Meli R, Page CP, et al. Protective effect of a PAR2-activating peptide on histamine-induced bronchoconstriction in guinea pig. Br J Pharmacol 2001;132:1229–34.

[9] Ricciardolo FL, Steinhoff M, Amadesi S, Guerrini R, Tognetto M, Trevisani M, et al. Presence and bronchomotor activity of protease-activated receptor-2 in guinea pig airways. Am J Respir Crit Care Med 2000;161:1672–80.

[10] Chambers LS, Black JL, Poronnik P, Johnson PRA. Functional effects of protease-activated receptor-2 stimulation on human airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 2001;281:L1369–78.

[11] Barrios VE, Jarosinski MA, Wright CD. Proteinase-activated receptor-2 mediates hyperresponsiveness in isolated guinea pig bronchi. Biochem Pharmacol 2003;66:519–25.

[12] Nystedt S, Ramakrishnan V, Sundelin J. The proteinase-activated receptor 2 is induced by inflammatory mediators in human endothelial cells. Comparison with the thrombin receptor. J Biol Chem 1996;21:14010–915.

[13] Lan R, Geoffrey AS, Goldie RG, Henry PJ. Altered expression and in vivo lung function of protease-activated receptors during influenza A virus infection mice. Am J Physiol Lung Cell Mol Physiol 2004;286:L388–98.

[14] Cocks TM, Moffatt JD. Protease activated receptor-2 (PAR2) in the airways. Pulm Pharmacol Ther 2001;14:83–191.

[15] Miotto D, Hollenberg MD, Bunnett NW, Papi A, Braccioni F, Boschetto P, et al. Expression of protease activated receptor-2 (PAR-2) in central airways of smokers and non-smokers. Thorax 2002;57:46–151.

[16] Thijs LG, Groeneveld ABJ, Hack CE. Multiple organ failure in septic shock. Curr Top Microbiol Immunol 1996;216:209–37.

[17] Power C, Fanning N, Redmond HP. Cellular apoptosis and organ injury in sepsis: a review. Shock 2002;18:97–211.

[18] Hua XY, Chen P, Fox A, Myers RR. Involvement of cytokines in lipopolysaccharide-induced facilitation of CGRP release from capsaicin-sensitive nerves in the trachea: studies with interleukin-1beta and tumor necrosis factor-alpha. J Neurosci 1996;16:4742–8.

[19] Steinhoff M, Vergnolle N, Young SH, Tognetto M, Amadesi S, Ennes HS, et al. Agonists of proteinase activated receptor-2 induce inflammation by a neurogenic mechanism. Nat Med 2000;6:151–8.

[20] Nguyen C, Coelho AM, Grady E, Compton SJ, Wallace JL, Hollenberg MD, et al. Colitis induced by proteinase-activated receptor-2 agonists is mediated by a neurogenic mechanism. Can J Physiol Pharmacol 2003;81:920–7.

[21] Amadesi S, Nie J, Vergnolle N, Cottrell GS, Grady EF, Trevisani M, et al. Protease activated receptor 2 sensitizes the capsaicin receptor potential vanilloid receptor 1 to induce hyperalgesia. J Neurosci 2004;24:4300–12.

[22] Cicala C, Pinto A, Bucci M, Sorrentino R, Walker B, Harriot P, et al. Protease-activated receptor-2 involvement in hypotension in normal and endotexemic rats in vivo. Circulation 1999;99:2590–7.

[23] Roche N, Stirling RG, Lim S, Oliver BG, Oates T, Jazrawi E, et al. Effect of acute and chronic inflammatory stimuli on expression of protease-activated receptors 1 and 2 in alveolar macrophages. J Allergy Clin Immunol 2003;111:367–73.

[24] Saetta M, Turato G. Airway pathology in asthma. Eur Respir J Suppl 2001;34:18s–23s.

[25] Schulz C, Wolf K, Hart M, Ktatzel K, Kunz-Schughart L, Pfeifer M. Expression and release of interleukin-8 by human bronchial epithelial cells from patients with chronic obstructive pulmonary disease, smokers and never-smokers. Respiration 2003;70:254–61.

[26] Pavord ID, Tattersfield AE. Bronchoprotective role for endogenous prostaglandin E_2. Lancet 1995;345:436–8.

[27] Averbeck B, Ree PW. Interaction of inflammatory mediators stimulating release of calcitonin gene related peptide,

substance P and prostaglandin E(2) from isolated skin. Neuropharmacology 2001;40:416–23.
[28] De Garavilla L, Vergnolle N, Young SH, Ennes H, Steinhoff M, Ossovskaya VS, et al. Agonists of proteinase activated receptor-1 induce plasma extravasation by a neurogenic mechanism. Br J Pharmacol 2001;133:975–87.
[29] Cadieux A, Springall DR, Mulderry PK, Rodrigo J, Ghatei MA, Terenghi G, et al. Occurrence, distribution and ontogeny of CGRP immunoreactivity in the rat lower respiratory tract: effect of capsaicin treatment and surgical denervations. Neuroscience 1986;19:605–27.
[30] Martling CR. Sensory nerves containing tachykinins and CGRP in the lower airways. Functional implications for bronchoconstriction, vasodilatation and protein extravasation. Acta Physiol Scand Suppl 1987;563:1–57.
[31] Barnes PJ, Baraniuk JN, Belvisi MG. Neuropeptides in the respiratory tract. Part II. Am Rev Respir Dis 1991;144:1391–9.
[32] Cadieux A, Monast NP, Pomerleau F, Fournier A, Lanoue C. Bronchoprotector properties of calcitonin gene-related peptide in guinea pig and human airways. Effect of pulmonary inflammation. Am J Respir Crit Care Med 1999;159:235–43.
[33] Dakhama A, Kanehiro A, Makela MJ, Loader JE, Larsen GL, Gelfand EW. Regulation of airway responsiveness by calcitonin gene-related peptide in allergen sensitized and challenged mice. Am J Respir Crit Care Med 2002;165: 1137–44.
[34] Brigham KL, Meyrick B. Endotoxin and lung injury. Am Rev Respir Dis 1986;133:913–27.