ELSEVIER

European Journal of Pharmacology 308 (1996) 325–328

ejp

Short communication

SR 140333 prevents potentiation by citric acid of plasma exudation induced by histamine in airways

Keltoum Biyah [a], Mathieu Molimard [a], Xavier Emonds-Alt [b], Charles Advenier [a,*]

[a] *Laboratoire de Pharmacologie, Faculté de Médecine Paris-Ouest, 15 Rue de l'Ecole de Médecine, F-75006 Paris, France*
[b] *Sanofi Recherche, 371 Avenue du Pr Blayac, F-34184 Montpellier Cedex, France*

Received 29 January 1996; revised 25 April 1996; accepted 30 April 1996

Abstract

We here report a model of potentiation by citric acid of airway microvascular leakage induced by histamine and its modification by the tachykinin NK_1 and NK_2 receptor antagonists, SR 140333 ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl)piperidin-3-yl]ethyl}-phenyl-1-azoniabicyclo[2.2.2]octane,chloride) and SR 48968 (*S*)-*N*-methyl-*N*-[4-(4-acetyl-amino-4-phenylpiperidino)-2-(3,4-dichlorophenyl-butyl]benzamide. Guinea-pigs exposed to an aerosol of citric acid 0.4 M for 1 h developed 24 h later a hyperresponsiveness to histamine-induced microvascular leakage measured by Evans blue dye extravasation. SR 140333, but not SR 48968 (1 mg kg^{-1} given each once 30 min before citric acid exposure), prevented this potentiation. These results provide further evidence of the role of tachykinin and tachykinin NK_1 receptor stimulation on airway hyperresponsiveness and its neurogenic inflammatory component.

Keywords: Tachykinin receptor antagonist; Microvascular leakage; (Guinea-pig)

1. Introduction

Several lines of evidence suggest that tachykinins are involved in airway hyperresponsiveness to spasmogens. Indeed, tachykinins, such as substance P or neurokinin A, or capsaicin, a drug known to induce tachykinin release from sensory nerve endings, can induce airway hyperresponsiveness in guinea-pigs (Omini et al., 1989; Umeno et al., 1992; Boichot et al., 1993; Tocker et al., 1995) whereas high doses of capsaicin, which induce tachykinin depletion and sensory nerve degeneration, prevent ovalbumin-induced hyperresponsiveness in sensitized animals (Matsuse et al., 1991). Recently, Girard et al. (1995) have demonstrated in guinea-pigs that aerosolized citric acid, a substance known to induce tachykinin release (Forsberg et al., 1988), induces 24 h later airway inflation pressure hyperresponsiveness to acetylcholine, an effect that is prevented by the tachykinin NK_2 receptor antagonist SR 48968. Since airway inflammation is a deciding factor of airway hyperresponsiveness and since increased microvascular leakage is itself an important component of inflammation, the aim of this study was to investigate whether tachykinins are able to induce a potentiation of histamine-induced airway microvascular leakage. We therefore studied the effect of citric acid on histamine-induced airway microvascular leakage in guinea-pig and the effects of the specific tachykinin NK_1 and NK_2 receptor antagonists, SR 140333 (Emonds-Alt et al., 1993) and SR 48968 (Emonds-Alt et al., 1992), respectively, on the development of this hyperreactivity.

2. Materials and methods

2.1. Citric acid aerosol exposure

Tricolored unanaesthetized, unrestrained male or female guinea-pigs, 250–400 g, were placed individually in a body plethysmograph and exposed to a nebulized aqueous solution of citric acid (0.4 M) or saline for 60 min. The aerosols were produced by an ultrasonic nebulizer (De-Vilbiss, Somerset, PA, USA) and had an aerodynamic mass median of 1.8 μm (manufacturer's indications). About 0.3 ml of the solution was nebulized per minute.

* Corresponding author. Tel.: (33-1) 43.26.09.52; fax: (33-1) 44.07.13.52.

PII S0014-2999(96)00335-4

2.2. Measurement of airway microvascular leakage

Twenty four hours after aerosol exposure, vascular permeability was quantified by the extravasation of Evans blue dye, which correlates well with extravasation of radiolabelled albumin in the skin and airways (Rogers et al., 1988). Animals were anaesthetized with urethane (1.25 g kg^{-1} intraperitoneally). A jugular vein was cannulated to inject drugs. Evans blue dye (30 mg kg^{-1} i.v.) was injected. After a further 1 min, saline (1 ml kg^{-1} i.v.) or histamine (30 μg kg^{-1} i.v.) was injected, and 5 min later the thorax was opened and a blunt-ended, 13-gauge needle was passed through a left ventriculotomy into the aorta. The ventricles were cross-clamped and blood was expelled through an incision in the right atrium at 80 mm Hg pressure with about 100 ml saline (pH 5.5), in order to remove the intravascular dye from the systemic and pulmonary circulations until the perfusate was clear. The lungs were then removed. The connective tissues, vasculature, and parenchyma were gently scraped away, and the airways were divided into four components: lower part of trachea, main bronchi and proximal (the proximal 3 mm portion) and distal intrapulmonary airways (Rogers et al., 1988). The tissues were blotted dry, placed in preweighed tubes and reweighed, and their dye content was extracted in formamide at 37°C for 18 h. Dye concentration was quantified by light absorbance at 620 nm (DCP spectrophotometer, Vital, Dieren, Netherlands) and its tissue content (ng dye mg^{-1} wet weight tissue) was calculated from a standard curve of dye concentrations in the 0.5–10 μg ml^{-1} range.

The dose of histamine (30 μg kg^{-1}) was chosen from preliminary experiments and gave 30–70% of the maximal effect.

2.3. Protocols

The tachykinin receptor antagonists SR 140333 (1 mg kg^{-1}) or SR 48968 (1 mg kg^{-1}) were injected intraperitoneally 30 min before aerosol exposure.

2.4. Drugs

Drugs used were: histamine, formamide, Evans blue, substance P (Sigma, St Louis, USA), urethane, citric acid (Prolabo, Paris, France), SR 48968: (*S*)-*N*-methyl-*N*-[4-(4-acetyl-amino-4-phenylpiperidino)-2-(3,4-dichlorophen-

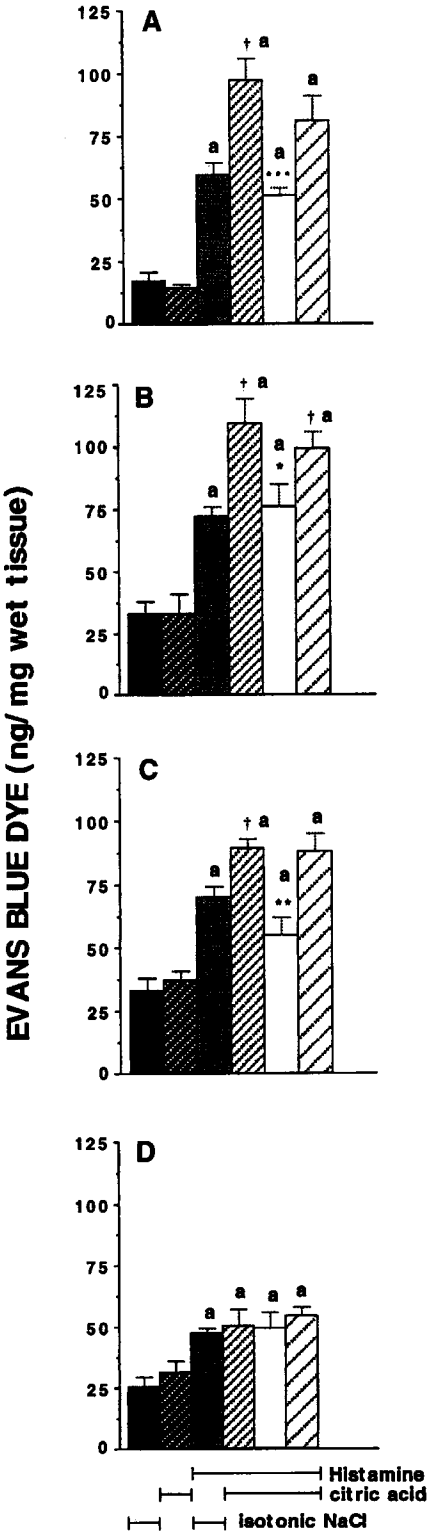

Fig. 1. Potentiation by citric acid aerosol exposure (0.4 M for 1 h) 24 h earlier of Evans blue dye extravasation induced by histamine 30 μg kg^{-1} in guinea-pig trachea (A), main bronchi (B), proximal (C) or distal (D) intrapulmonary airways and its modifications by SR 140333 and SR 48968. Columns represent basal extravasation in control animals exposed to isotonic NaCl (filled column) or exposed to citric acid aerosol (heavily hatched column, white-on-black) or extravasation induced by histamine in control animals (grey column) or after citric acid exposure in the absence (heavily hatched column) or after a pretreatment with SR 140333 (blank column) or SR 48968 (hatched column). Vascular extravasation was evaluated by measuring the amount of Evans blue dye extravasated in lung tissues after 5 min. Values are means ± S.E.M.; $n = 6$ per group. $^{a}P < 0.01$ compared with basal extravasation; $^{\dagger}P < 0.01$ compared with histamine without citric acid exposure; $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with histamine after citric acid exposure.

yl-butyl]benzamide, SR 140333 ((*S*)1-{2-[3-(3,4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl)piperidin-3-yl]ethyl}-phenyl-1-azoniabicyclo[2.2.2]octane,chloride) (Sanofi-Recherche, Montpellier, France). All drugs were prepared for intravenous and/or intraperitoneal injection by dilution in an isotonic NaCl solution, except for SR 48968 and SR 140333, which were dissolved in ethanol and then diluted in an isotonic NaCl solution. Moreover, in order to eliminate Evans blue deposits the solution of this dye was filtered through an antibacterial millipore filter used for peridural anaesthesia.

2.5. Statistical analysis of results

Data are expressed as means ± S.E.M. Statistical analysis of the results was performed using analysis of variance and/or Student's *t*-test. Probability values of $P < 0.05$ were considered significant.

3. Results

Fig. 1 shows that histamine significantly increased vascular permeability to Evans blue dye in pulmonary airways. This effect was significantly potentiated by citric acid aerosol exposure 24 h earlier in trachea, main bronchi and proximal airways whereas citric acid itself had no significant direct effect on microvascular leakage compared to saline. The potentiating effect of citric acid pretreatment on histamine-induced vascular permeability was abolished by SR 140333 but not by SR 48968. Neither SR 140333 nor SR 48968 had any significant effect on histamine-induced microvascular leakage (data no shown).

4. Discussion

Microvascular leakage is an important component of neurogenic airway inflammation and may contribute to the pathophysiology of asthma (Chung et al., 1990). Both pharmacological and immuno-histochemical studies indicate that tachykinin receptors are involved in neurogenic vascular extravasation. Indeed, Rogers et al. (1988) have shown that tachykinins increase vascular permeability in guinea-pig airways mainly via the tachykinin NK_1 receptor subtype, the order of potency being substance P > neurokinin A = neurokinin B. Tachykinin NK_2 receptor-mediated plasma extravasation seems to predominate in secondary bronchi and intraparenchymal airways (Tousignant et al., 1993).

Beside the ability of tachykinins to induce plasma extravasation in guinea-pig airways, our results show that tachykinins may induce a hyperreactivity phenomenon (potentiation) to other extravasation-inducing drugs. Indeed, we have shown that aerosolized citric acid induces a potentiation of histamine-induced microvascular leakage and that this potentiation is inhibited by the tachykinin NK_1 receptor specific antagonist SR 140333, but not by SR 48968. This result is in contrast with the protective effect of the tachykinin NK_2 receptor antagonist, SR 48968, on citric acid-induced airway hyperresponsiveness to acetylcholine, a mediator known to be a poor inducer of microvascular leakage (Girard et al., 1995).

Our results suggest that the role played by tachykinin NK_1 receptors is predominant in the initiation of hypersensitivity of vascular permeability as it has also been demonstrated in the digestive system by Kraneveld et al. (1995) in a model of delayed type hypersensitivity induced in the rat by dinitrofluorobenzene. In contrast, tachykinin NK_2 receptors predominate in the initiation of airway hyperresponsiveness, as explored by measuring airway inflation pressure. Indeed, in addition to the citric acid-induced hyperresponsiveness, SR 48968 but not SR 140333 prevents antigen-induced airway hyperresponsiveness in guinea-pig sensitized to ovalbumin (Boichot et al., 1995), and vagal stimulation-potentiated pulmonary anaphylaxis in sensitized and atropine-pretreated perfused guinea-pig lung (Tocker et al., 1995). Similarly, the tachykinin NK_1 receptor antagonist MEN 10,627 reduces the platelet-activating factor-induced hyperresponsiveness to histamine in the guinea-pig (Perretti et al., 1995).

We have clearly ruled out that the hyperresponsiveness we describe could only be due to the additive effect of tachykinin receptor stimulation and histamine-induced microvascular leakage since citric acid failed to significantly induce microvascular leakage 24 h later, at least in proximal airways. The target cells of tachykinins and the location of the tachykinin receptors involved in the citric acid pretreatment effect on microvascular hyperresponsiveness remain however to be determined.

In conclusion, this study is the first demonstration that, beside the tachykinin NK_2 receptor stimulation-mediated airway inflation pressure hyperresponsiveness to acetylcholine, tachykinins can participate via tachykinin NK_1 receptor stimulation to the hyperresponsiveness of microvascular leakage induced by histamine.

References

Boichot, E., V. Lagente, M. Paubert-Braquet and N. Frossard, 1993, Inhaled substance P induces activation of alveolar macrophages and increases airway responses in the guinea-pig, Neuropeptides 25, 307.

Boichot, E., N. Germain, V. Lagente and C. Advenier, 1995, Prevention by the tachykinin NK_2 receptor antagonist, SR 48968, of antigen-induced airway hyperresponsiveness in sensitized guinea-pigs, Br. J. Pharmacol. 114, 259.

Chung, K.F., D.F. Rogers, P.J. Barnes and T.W. Evans, 1990, The role of increased airway microvascular permeability and plasma exudation in asthma, Eur. Respir. J. 3, 329.

Emonds-Alt, X., P. Vilain, P. Goulaouic, V. Proietto, D.V. Broeck, C. Advenier, G. Neliat, G. Le Fur and J.C. Brelière, 1992, A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor, Life Sci. 50, PL101.

Emonds-Alt, X., J.D. Doutremepuich, M. Heaulme, G. Neliat, V. Santucci, R. Steinberg, P. Vilain, D. Bichon, J.P. Ducoux, V. Proietto, D. Van Broeck, P. Soubrié, G. Le Fur and J.C. Brelière, 1993, In vitro and in vivo biological activities of SR 140333, a novel potent non-peptide tachykinin NK_1 receptor antagonist, Eur. J. Pharmacol. 250, 403.

Forsberg, K., J.A. Karlsson, E. Theodorsson, J.M. Lundberg and C.G.A. Persson, 1988, Cough and bronchoconstriction mediated by capsaicin-sensitive sensory neurons in the guinea-pig, Pulm. Pharmacol. 1, 33.

Girard, V., J.C. Yavo, E. Naline, X. Emonds-Alt and C. Advenier, 1995, The tachykinin NK_2 receptor antagonist SR 48968 inhibits citric acid-induced airway hyperresponsiveness in guinea pigs, Am. J. Respir. Crit. Care Med. 151, A227.

Kraneveld, A., T. Buckley, Y.V. Heuven-Holsen, Y.V. Schaick, A. Koster and F. Nijkamp, 1995, Delayed-type hypersensitivity-induced increase in vascular permeability in the mouse small intestine: inhibition by depletion of sensory neuropeptides and NK_1 receptor blockade, Br. J. Pharmacol. 114, 1483.

Matsuse, T., R. Thomson, X. Chen, H. Salari and R. Schellenberg, 1991, Capsaicin inhibits airway hyperresponsiveness but not lipoxygenase activity or eosinophilia after repeated aerosolized antigen in guinea-pigs, Am. Rev. Respir. Dis. 144, 368.

Omini, C., G. Brunelli, A. Hernandez and L. Daffonchio, 1989, Bradykinin and substance P potentiate acetylcholine-induced bronchospasm in guinea-pig, Eur. J. Pharmacol. 163, 195.

Perretti, F., L. Ballati, S. Manzini, C.A. Maggi and S. Evangelista, 1995, Antibronchospastic activity of MEN 10,627, a novel tachykinin NK_2 receptor antagonist, in guinea-pig airway, Eur. J. Pharmacol. 273, 129.

Rogers, D.F., M.G. Belvisi, B. Aursudkij, T.W. Evans and P.J. Barnes, 1988, Effects and interaction of sensory neuropeptides on airway microvascular leakage in guinea-pigs, Br. J. Pharmacol. 95, 1109.

Tocker, J.E., S.B. Gertner, A.F. Welton and W.X. Selig, 1995, Vagal stimulation augments pulmonary anaphylaxis in the guinea-pig lung, Am. J. Care Med. 151, 461.

Tousignant, C., C.-C. Chan, D. Guevremont, C. Brideau, J.J. Hale, M. MacCoss and I.W. Rodger, 1993, NK_2 receptors mediate plasma extravasation in guinea-pig lower airways, Br. J. Pharmacol. 108, 383.

Umeno, E., T. Hirose and S. Nishima, 1992, Pretreatment with aerosolized capsaicin potentiates histamine-induced bronchoconstriction in guinea-pigs, Am. Rev. Respir. Dis. 146, 159.