British Journal of Pharmacology (1995) 114, 259-261

SPECIAL REPORT

Prevention by the tachykinin NK_2 receptor antagonist, SR 48968, of antigen-induced airway hyperresponsiveness in sensitized guinea-pigs

Elisabeth Boichot, Noella Germain, Vincent Lagente & [1]*Charles Advenier

Laboratoire de Pharmacodynamie et de Pharmacologie Moléculaire, Faculté des Sciences Pharmaceutiques et Biologiques, Université de Rennes 1, Rennes, France and *Laboratoire de Pharmacologie Respiratoire, Faculté de Médecine Paris-Ouest, Université Paris V, 15, rue de l'ecole de Medecine, 75006 Paris, France

The involvement of tachykinins in antigen-induced airway hyperresponsiveness (AHR) was characterized pharmacologically in guinea-pigs sensitized to ovalbumin with antagonists of tachykinin NK_1 and NK_2 receptors, namely SR 140333 and SR 48968, respectively. AHR was illustrated by increased sensitivity to bronchoconstriction provoked by aerosolized acetylcholine in anaesthetized, ventilated animals, administrated 48 h after ovalbumin aerosol challenge. SR 48968 (1 mg kg^{-1}, i.p.), when given once 30 min before the antigen challenge, prevented AHR, whereas SR 140333 did not. These findings suggest that the tachykinin NK_2 receptor antagonist, SR 48968, may be useful for investigating mechanisms of tachykinins in the development of airway hyperresponsiveness.

Keywords: Tachykinin NK_1 receptor; tachykinin NK_2 receptor; SR 140333; SR 48968; ovalbumin; aerosol; guinea-pig airway hyperresponsiveness.

Introduction Airway hyperresponsiveness (AHR) is a functional consequence of airway inflammation and is a main feature of asthma. Among the different inflammatory mediators involved, several lines of evidence suggest that tachykinins, such as substance P and neurokinin A might be involved in the pathogenesis of AHR. Indeed, recent studies have reported that exposure of guinea-pigs to an aerosol of either capsaicin, a substance releasing endogenous sensory neuropeptides, or of substance P elicited AHR to exogenous bronchoconstrictor agents (Hsuie *et al.*, 1992; Boichot *et al.*, 1993). Conversely, chronic treatment with high doses (i.p.) of capsaicin which depletes tachykinins from non-adrenergic non-cholinergic nerves, eliminates AHR induced by acute capsaicin or ovalbumin (Matsuse *et al.*, 1991; Hsuie *et al.*, 1992).

The aim of the present study was to investigate the role of tachykinins in the development of AHR and to determine which type of receptor is involved. We have analysed the effects on antigen-induced airway hyperresponsiveness in the sensitized guinea-pig of SR 140333 (Emonds-Alt *et al.*, 1993) and SR 48968 (Emonds-Alt *et al.*, 1992) two non-peptide selective antagonists of tachykinin NK_1 and NK_2 receptors, respectively.

Methods Specific pathogen-free male Hartley guinea-pigs (Charles River, Saint-Aubin-lès-Elboeuf, France) were exposed twice for 30 min to an aerosol (ULTRA-NEB 99, Devilbiss Medical) of ovalbumin (OA) 2 mg ml^{-1} in saline (NaCl, 0.9%), at a 48 h interval in a plexiglass chamber (30 × 50 × 30 cm). Fifteen to 20 days after the initial sensitization procedure, the guinea-pigs were challenged by exposure to five successive solutions of OA of respectively 10 μg ml^{-1}, 100 μg ml^{-1}, 1 mg ml^{-1}, 5 mg ml^{-1} and 10 mg ml^{-1} for 15 min each. The bronchopulmonary reactivity of anaesthetized (urethane, 1.2 g kg^{-1}, i.p.) and ventilated (1 ml laboratory air for 100 g body weight, 60 breaths min^{-1}) guinea-pigs was assessed 48 h after exposure to either OA challenge or saline. Spontaneous breathing was abolished with pancuronium bromide (Pavulon, Organon, France; 2 mg kg^{-1}, i.v.). Airway inflation pressure was evaluated with a pressure transducer (Gould PE 10, Cleveland, Ohio, USA) connected to a lateral port of the ventilator circuit. After a 10 min stabilization period, four successive administrations of ACh aerosol (50, 100, 200 and 500 μg ml^{-1}), generated by a Devilbiss Pulmosonic ultrasonic nebulizer, were given for 1 min each at 10 min intervals. The bronchopulmonary response was monitored constantly and expressed as percentage change over the 100% obtained by clamping the tracheal cannula at the end of the experiment. The following drugs were used: ovalbumin (OA, chicken egg, grade V) and acetylcholine chloride (ACh) (Sigma, St. Louis, MO, U.S.A.). SR 48968 {(S)-*N*-methyl-*N*[4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl) butyl]benzamide} and SR 140333 ((S)1-{2-[3-(3,4-dichlorophenyl)-1-(3-iso-propoxyphenylacetyl) piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2.2.2] octane, chloride) (Sanofi Recherche, Montpellier, France). SR 48968 and SR 140333 were dissolved in saline and injected i.p. at the dose of 1 mg kg^{-1}, 30 min before the ovalbumin challenge.

Results Exposure of sensitized and saline challenged guinea-pigs to successive aerosols of ACh (50– 500 μg ml^{-1}) induced a dose-related bronchopulmonary response which was not modified by either SR 48968 or SR 140333 (1 mg kg^{-1}). In sensitized guinea-pigs that had been challenged with an aerosol of allergen, responses to ACh given 48 h later were augmented compared to those in saline-exposed animals. Hence, the dose-response curve to ACh was significantly shifted to the left ($P < 0.001$), suggesting the development of AHR (Figure 1a and b). Pretreatment of the sensitized guinea-pigs with a single dose of SR 48968 (1 mg kg^{-1}) 30 min before allergen challenge significantly prevented the AHR (Figure 1a). In contrast, pretreatment with SR 140333 (1 mg kg^{-1}) did not modify the significant leftward shift of the dose-response curve to Ach, observed after allergen challenge (Figure 1b).

Discussion Stimulation of NK_2 receptor is involved in bronchoconstriction induced by tachykinins, by substances which release tachykinins from sensory nerves, and by antigen in

[1] Author for correspondence.

sensitized guinea-pigs (Advenier *et al.*, 1992; Bertrand *et al.*, 1993). It has also been suggested that tachykinins are involved in the development of AHR (Hsuie *et al.*, 1992; Boichot *et al.*, 1993). The present finding that the antagonist SR 48968 prevented antigen-induced AHR in sensitized guinea-pigs, an effect which could not be attributed to functional antagonism of acetylcholine-induced bronchoconstriction (Advenier *et al.*, 1992), provides evidence that NK_2 receptor stimulation is involved in the development of AHR. Since the NK_1 receptor antagonist, SR 140333, failed to inhibit the antigen-induced AHR, NK_1 receptor stimulation appears not to be involved. The lack of effect of SR 140333 cannot be attributed to a difference in pharmacokinetic profile since SR 48968 and SR 140333 have comparable time-related dose-effect relationship *in vivo* in the guinea-pig (Emonds-Alt *et al.*, 1992; 1993).

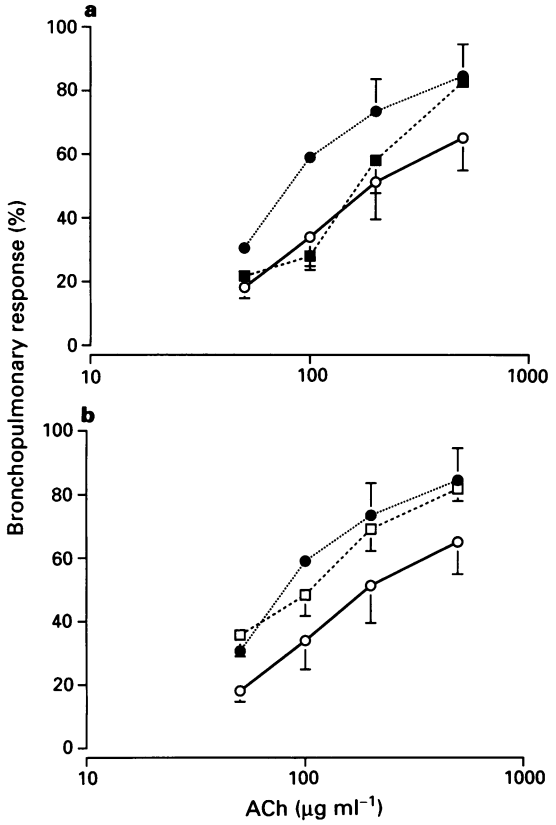

Figure 1 Effect of SR 48968 (a) and SR 140333 (b) on antigen-induced airway hyperresponsiveness to acetylcholine (ACh), 48 h after ovalbumin (OA) challenge. Sensitized guinea-pigs were treated i.p., 30 min before OA exposure, with 1 mg kg^{-1} of SR 48968 or of SR 140333 and the bronchopulmonary responses were assessed 48 h after. The data were compared by two way analysis of variance in order to analyse the whole dose-response curve for each group of guinea pigs. The results are expressed as mean (± s.e. mean) percentages of the maximal bronchopulmonary response obtained by total clamping of the tracheal cannula. Significant differences between saline (○, $n=5$) and OA (●, $n=8$): $P<0.001$. Significant differences between OA (●) and SR 48968 + OA (■, $n=6$): $P<0.01$. No significant differences between SR 48968 + OA (■) and saline (○) or between OA (●) and SR 140333 + OA (□, $n=6$). Significant differences between SR 140333 + OA (□) and saline (○): $P<0.01$.

Although the site of action of SR 48968 is not clear, it is possible that SR 48968 acts to regulate responses of target-cells involved in bronchoconstriction and airway inflammation or on nerve transmission. One of the most important cells involved in the inflammatory process of allergic reactions may be the alveolar macrophage. Indeed, we have previously reported that AHR observed after exposure of guinea-pigs to substance P is associated with an increase in alveolar macrophage activity (Boichot *et al.*, 1993) and an increased responsiveness to NK_2 receptor stimulation of alveolar macrophages from sensitized guinea-pigs has been demonstrated (Brunelleschi *et al.*, 1992). Regarding an effect on nerve transmission, Fischer *et al.* (1994) have recently demonstrated that sensitization of guinea-pigs to ovalbumin induced a 1–5 fold increase of neuropeptide-concentration in lung tissue and also increased two fold the substance P/neurokinin A-immunoreactive neurones in the nodose ganglion 24 h after allergen challenge. Furthermore, in vagal sensory ganglia, these authors observed that the expression of preprotachykinin A mRNA was increased in allergic animals, 12–24 h after allergen challenge.

Finally, Adcock & Garland (1993) have suggested that tachykinins might decrease the threshold for stimulation of sensory nerves endings in the lung as in other inflamed tissues involved in hyperalgesia. In this respect, repeated exposure to sub-threshold doses of neurokinin A leads to an increase in the peripheral hyperalgesia in the rat paw, lasting for more than 4 h and is ten fold more potent than substance P (Nakamura-Craig *et al.*, 1990). Hence, it is possible that SR 48968 may reduce this putative effect on sensory nerves in the airways.

In conclusion, the present data demonstrate the importance of NK_2 receptor stimulation in the development of antigen-induced AHR in sensitized guinea-pigs and suggest that NK_2 receptor antagonists may be useful for investigating mechanisms of bronchopulmonary alterations of allergic reaction and possibly reducing AHR in asthmatic patients.

References

ADCOCK, J.J. & GARLAND, L.G. (1993). The contribution of sensory reflexes and 'hyperalgesia' to airway hyperresponsiveness. In *Airway Hyperresponsiveness: It is really important for Asthma?* ed. Page C.P. & Gardiner, P.J. pp. 234–255. Oxford: Blackwell.

ADVENIER, C., NALINE, E., TOTY, L., BAKDACH, H., EMONDS-ALT, X., VILAIN, P., BRELIÈRE, J.C. & LE FUR, G. (1992). Effects on the isolated human bronchus of SR 48968, a potent and selective non peptide antagonist of the neurokinin A (NK_2) receptors. *Am. Rev. Respir. Dis.*, **146**, 1177–1181.

BERTRAND, C., GEPPETI, P., GRAF, P.D., FORESI, A. & NADEL, J.A. (1993). Involvement of neurogenic inflammation in antigen-induced bronchoconstriction in guinea-pigs. *Am. J. Physiol.*, **265**, L507–L511.

BOICHOT, E., LAGENTE, V., PAUBERT-BRAQUET, M. & FROSSARD, N. (1993). Inhaled substance P induces activation of alveolar macrophages and increases airway responses in the guinea-pig. *Neuropeptides*, **25**, 307–313.

BRUNELLESCHI, S., PARENTI, A., CENI, E., GIOTTI, A. & FANTOZZI, R. (1992). Enhanced responsiveness of ovalbumin-sensitized guinea-pig alveolar macrophages to tachykinins. *Br. J. Pharmacol.*, **107**, 964–969.

EMONDS-ALT, X., DOUTREMEPUICH, J.D., HEAULME, M., NELIAT, G., SANTUCCI, V., STEINBERG, R., VILAIN, P., BICHON, D., DUCOUX, J.P., PROIETTO, V., VAN BROECK, D., SOUBRIÉ, P., LE FUR, G. & BRELIÈRE, J.C. (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**, 403–413.

EMONDS-ALT, X., VILAIN, P., GOULAOUIC, P., PROIETTO, V., VAN BROECK, D., ADVENIER, C., NALINE, E., NELIAT, G., LEFUR, G. & BRELIÈRE, J.C. (1992). A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor. *Life Sci.*, **50**, PL101–PL106.

FISCHER, A., PHILIPPIN, B., SARIA, A., MCGREGOR, G. & KUMMER, W. (1994). Neuronal plasticity in sensitized and challenged guinea pigs: neuropeptides and neuropeptide gene expression. *Am. J. Respir. Crit. Care Med.*, **149,** A890.

HSUIE, T., GARLAND, A., RAY, D.W., HERSHENSON, M.B., LEFF, A.R. & SOLWAY, J. (1992). Endogenous sensory neuropeptide release enhances nonspecific airway responsiveness in guinea pigs. *Am. Rev. Respir. Dis.*, **146,** 148–153.

MATSUSE, T., THOMSON, R.J., CHEN, X.R., SALARI, H. & SCHELLENBERG, R.R. (1991). Capsaicin inhibits airway hyperresponsiveness but not lipoxygenase activity or eosinophilia after repeated aerosolized antigen in guinea pigs. *Am. Rev. Respir. Dis.*, **144,** 366–372.

NAKAMURA-CRAIG, M. & GILL, B.K. (1990). Possible involvement of neurokinin A, substance P and calcitonin gene-related peptide in peripheral hyperalgesia in the rat. *Br. J. Pharmacol.*, **99,** 288P.

(Received September 23, 1994
Accepted September 28, 1994)