Available online at www.sciencedirect.com
ScienceDirect
Pulmonary Pharmacology & Therapeutics 21 (2008) 67–72
PULMONARY PHARMACOLOGY & THERAPEUTICS
www.elsevier.com/locate/ypupt

Increased blocking activity of combined tachykinin NK_1- and NK_2-receptor antagonists on hyperventilation-induced bronchoconstriction in the guinea pig

M.R. Corboz*, X. Fernandez, John A. Hey

Pulmonary and Peripheral Neurobiology, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

Received 10 March 2006; received in revised form 27 October 2006; accepted 29 November 2006

Abstract

In vivo anesthetized guinea pigs were used to investigate the effect of tachykinin NK_1- and NK_2-receptor antagonists, as a single dose or in combination, against hyperventilation-induced bronchoconstriction (HIB). Guinea pigs were ventilated with a rodent ventilator and placed in a whole-body plethysmograph. Hyperventilation was induced by increasing the respiratory rate from 50 to 185 breaths/min for 10 min that produced a $177 \pm 45\%$ increase in pulmonary resistance (R_L) and a $68 \pm 7\%$ decrease in lung compliance (C_{Dyn}). Intravenous (0.03–0.3 mg/kg) and oral (0.3–10 mg/kg) pretreatments with the tachykinin NK_2-antagonist SR 48968 produced a dose-dependent inhibition of HIB whereas pretreatments with the tachykinin NK_1-antagonist CP 99994 (1 mg/kg intravenously and 30 mg/kg orally) had no effect on HIB. Intravenous and oral combinations of inactive and low doses of CP 99994 and SR 48968 produced a greater inhibition of HIB than SR 48968 alone. Also, the tachykinin NK_3-antagonist SB 223412 (1–3 mg/kg intravenously and 30 mg/kg orally) did not significantly reduce HIB although a trend was observed at the highest dose tested intravenously (3 mg/kg). We conclude that HIB in the guinea pig is mostly mediated by the tachykinin NK_2-receptors and to a lesser extent by the tachykinin NK_1-receptors. Because the hyperventilation response in guinea pigs may be a surrogate for exercise-induced obstructive airway disease in human, these results suggest that combined use of dual tachykinin NK_1- and NK_2-receptor antagonists may provide greater benefit than treatment with single activity tachykinin NK-receptor antagonist.

Keywords: Hyperventilation; Bronchoconstriction; Tachykinin NK-receptor; CP 99994; SR 48968; SB 223412; Guinea pig

1. Introduction

A critical event for the development of obstruction in exercise-induced asthma is an increase in ventilation [1]. Hyperventilation from exercise and voluntary hyperventilation in asthmatics, when the ventilatory rate and ambient conditions from the two stimuli are matched, result in a similar degree of bronchoconstriction [2–4] suggesting that hyperventilation of exercise and voluntary hyperventilation are identical stimuli [4].

Because of the involvement of tachykinins in guinea pig hyperventilation-induced bronchoconstriction (HIB) [5,6] and the similarities between hyperpnea-induced bronchoconstriction in asthmatics and HIB in guinea pigs, such as time course of onset [2,4,7,8], inhibition of HIB with humidification of inspired air [7,8], mechanism not mediated by parasympathetic neurotransmitter or vagal reflex [8,9], it has been speculated that HIB in humans is also mediated by tachykinins. Moreover, in vivo bronchoconstrictor responses to inhaled tachykinin NK_1-receptor agonist SP [10] and tachykinin NK_2-receptor agonist NKA [11] have been reported in asthmatic patients and tachykinin aerosol induces bronchoconstriction in asthmatics with a similar airflow obstruction that obtained after cold dry gas hyperpnea [11].

Contribution of endogenous tachykinins released from airway sensory nerves in mediating HIB was first supported by indirect evidence in guinea pigs [5]. Tachykinin depletion in sensory C-fibers with chronic capsaicin

*Corresponding author. Tel.: +1 908 740 7238; fax: +1 908 740 7175.
E-mail address: michel.corboz@spcorp.com (M.R. Corboz).

1094-5539/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pupt.2006.11.007

pretreatment diminishes the bronchoconstrictor response and inhibition of neutral endopeptidase with phosphoramidon potentiates this response by reducing the degradation of endogenous tachykinins released during or after hyperventilation [5]. The pharmacological evidence that tachykinin receptors mediate guinea pig HIB was then provided by Solway et al. [6]. Intravenous administration of the tachykinin NK_1-receptor antagonist CP 96345 and the tachykinin NK_2-receptor antagonist SR 48968 greatly inhibited HIB, with SR 48968 being more effective than CP 96345 [6], confirming the participation of both tachykinin NK_1- and NK_2-receptors in this response as suggested by the study of Ray et al. [5]. However, due to the cardiovascular effects observed with CP 96345, no combined treatment was evaluated in their study [6].

The main goals of this study were to extend upon earlier studies from Solway et al. [6] on HIB and from our group [12], in which we showed in three different airway preparations (isolated bronchi, isolated lungs and in vivo in guinea pigs), the additive and/or synergistic effects of the tachykinin NK_1-receptor antagonist CP 99994 and the tachykinin NK_2-receptor antagonist SR 48968 against contractions elicited by exogenous tachykinins (using selective tachykinin NK-receptor agonists) and endogenously released tachykinins (using capsaicin). In the present study, bronchoconstriction in guinea pigs was induced by hyperventilation in the absence and the presence of CP 99994 and SR 48968 as a single treatment or in combination to determine (1) the subtype of tachykinin NK-receptors involve in this response, and (2) whether a dual tachykinin NK_1- and NK_2-receptor antagonist may provide an advantage over single subtype specific tachykinin NK-receptor antagonist. To our knowledge, the beneficial effect of a dual antagonist has never been demonstrated in HIB in guinea pig, a surrogate for exercise-induced asthma in human. Because the tachykinin NK_3-receptors have been found to be expressed in human and guinea pig airways [13,14], the tachykinin NK_3-receptor antagonist SB 223412 was also tested in this preparation to determine if the tachykinin NK_3-receptors are involved in HIB.

2. Methods

2.1. Animal preparation

Studies were performed on male Hartley guinea pigs (350–550 g) anesthetized with an intraperitoneal injection of sodium pentobarbital (35 mg/kg). The guinea pigs were surgically instrumented with tracheal, esophageal and jugular vein cannulae. The animals were mechanically ventilated with a rodent ventilator on room air ($f = 50$ breaths/min) and were placed inside a whole-body plethysmograph with catheters connected to outlet ports in the plethysmograph wall. A differential pressure transducer (model MP45-1, range ± 2 cm H_2O; Validyne, Northridge, CA, USA) measured the pressure difference across a wire mesh screen in the wall of the plethysmograph and was used to measure airflow. The airflow signal was electrically integrated to a signal proportional to volume. Transpulmonary pressure was measured with a Validyne differential pressure transducer (range ± 20 cm H_2O) as the pressure difference between the trachea and the esophagus. The volume, airflow and transpulmonary pressure signals were monitored by means of a Buxco pulmonary analyser used for the derivation of pulmonary resistance R_L (cm H_2O/ml/s) and dynamic lung compliance C_{DYN} (ml/cm H_2O) according to the method of Amdur and Mead [15]. R_L and C_{DYN} were computed for each breath and displayed every 6 s on a printer.

2.2. Experimental protocol

Hyperventilation was induced by increasing the respiratory rate from 50 to 185 breaths/min for 10 min. Tidal volume was fixed at 1.25 ml/100 g throughout the study based on the study of Mauser et al. [16]. After 10 min of hyperventilation, the respiratory rate was returned to 50 breaths/min. R_L and C_{DYN} were measured immediately before the start of the hyperventilation period and at the end of the 10 min hyperventilation period. Peak increases in pulmonary resistance due to hyperventilation normally occur within 10 min period [17]. Only one hyperventilation challenge was performed per animal.

To determine whether functional tachykinin NK_1-, NK_2- and NK_3-receptors contribute to HIB, the effects of specific tachykinin NK_1-, NK_2- and NK_3-receptor antagonists were evaluated after intravenous administration and oral dosing. When the drugs were given intravenously, the tachykinin NK-receptor antagonists were injected 5 min before hyperventilation, and when the pretreatment was performed orally, the tachykinin NK-receptor antagonists were given 2 hours before hyperventilation. The pretreatment period of the tachykinin NK-receptor antagonists is based on previous studies performed by our group using the same preparation [16,17].

The tachykinin NK_1-receptor antagonist CP 99994, the tachykinin NK_2-receptor antagonist SR 48968, the tachykinin NK_3-receptor antagonist SB 223412, and a combination of CP 99994 and SR 48968 doses were tested orally and intravenously. A combination of CP 99994, SR 48968 and SB 223412 doses was also evaluated orally.

2.3. Statistical analysis

Each animal was used to study the effects of only one treatment. ANOVA in conjunction with Dunnett's t-test was used to compare the responses between vehicle and drug treated animals, and probability (P) < 0.05 was accepted as the level of statistical significance. All results are expressed as means $\pm$ standard error of the mean (SEM).

2.4. Drugs and reagents

The tachykinin NK_1-receptor antagonist CP 99994, the tachykinin NK_2-receptor antagonist SR 48968 and the