Inhibition of bradykinin-induced bronchoconstriction in the guinea-pig by a synthetic B₂ receptor antagonist

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- 1 Intravenous bradykinin (Bk) elicited bronchoconstriction in the anaesthetized ventilated guinea-pig which was not mimicked by the B₁ receptor agonist, des-Arg⁹-Bk.
- 2 Bradykinin-induced bronchoconstriction was inhibited by the B₂ receptor antagonist B4881, but not by the B₁ receptor antagonist des-Arg⁹-Leu⁸-Bk. The effect of B4881 was short-lived.
- 3 The B₂ receptor antagonist as B4881 was selective for bradykinin as B4881 did not significantly inhibit bronchoconstriction induced by i.v. bombesin, platelet activating factor, acetylcholine, histamine or vagal stimulation.
- 4 These results suggest that bradykinin-induced bronchoconstriction in the guinea-pig is via activation of a B₂ receptor population and that B4881 is a selective B₂ antagonist that may be useful for investigating the involvement of bradykinin in the lung.

Introduction

Bradykinin has long been known to induce bronchoconstriction in a number of mammalian species including man (Collier *et al.*, 1960; Bhoola *et al.*, 1962; Herxheimer & Stresemann, 1963). However, the receptor type which mediates this effect has not been characterized. Recent research has shown that bradykinin can act via two distinct receptor populations, designated as B₁ and B₂ (Regoli & Barabe, 1980) which has led to the development of a number of antagonists for the B₂ receptor population (Vavrek & Stewart, 1985) based on synthetic peptide analogues. These B₂ receptor antagonists have been used to antagonize the activity of bradykinin in a number of tissues including blood vessels (Schachter *et al.*, 1987; Whalley, 1987), and smooth muscles of intestine, bladder and uterus (Vavrek & Stewart, 1985; Regoli *et al.*, 1986; Griesbacher & Lembeck, 1987). Additionally, these B₂ antagonists have been used to antagonize the pain producing activity of bradykinin on the human blister base (Whalley *et al.*, 1987; Steranka *et al.*, 1988), the ability of bradykinin to induce mucosal electrolyte transport (Kachur *et al.*, 1987) and to demonstrate the involvement of bradykinin in endotoxin-induced hypotension (Weipert *et al.*, 1988). In the present study, we have evaluated one of the more potent B₂ antagonists (B4881) D-Arg-[Hyp³-Thi^{5,8}-D-Phe⁷]-

Bk, on bradykinin-induced bronchoconstriction in the anaesthetized, ventilated guinea-pig in comparison with the B₁ antagonist des-Arg⁹-Leu⁸-Bk. Furthermore, we have examined the ability of the B₁ receptor agonist, des-Arg⁹-Bk, to mimic the bronchoconstrictor effect of bradykinin and have investigated the selectivity of the B₂ antagonist B4881 by evaluating the effect of this drug on bronchoconstriction induced by acetylcholine, histamine, bombesin, platelet activating factor (Paf) and vagal stimulation.

Methods

Male Dunkin-Hartley guinea-pigs (body weight 250–350 g) were used throughout this study. Animals were anaesthetized with urethane (25% w/v i.p., 7 ml kg⁻¹). The trachea was cannulated for measurement of airways obstruction and the polyethylene cannula was connected to a Harvard small animal ventilator. Animals were ventilated with room air (1 ml 100 g⁻¹ body weight) at 70 strokes min⁻¹. Air overflow was measured at the side arm of the tracheal cannula by means of a pressure transducer (Ormed Engineering) and expressed as pulmonary inflation pressure (PIP) in mmHg as an index of bronchoconstriction. Arterial blood pressure was recorded via a cannula in the carotid artery and

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Figure 1 Representative trace showing the increase in pulmonary inflation pressure (PIP) as an index of bronchoconstriction (a) or blood pressure (b) in response to i.v. bradykinin (Bk $1 \mu\text{g kg}^{-1}$) alone or in the presence of the B_1 antagonist des-Arg⁹-Leu⁸-Bk or the B_2 antagonist B4881. Responses to i.v. des-Arg⁹-Bk are shown for comparison. PIP and BP are expressed as mmHg. Bradykinin was administered at 10 min intervals.

intravenous injections were made via a cannulated jugular vein.

In some experiments the cervical vagus nerve was carefully dissected free and placed on platinum electrodes. The vagus nerve was stimulated with 10 V pulses of 0.5 ms at 30 Hz for 10 s using a variable pulse stimulator (Scientific and Research Instruments Ltd.).

Bronchoconstriction was induced by i.v. administration of a spasmogen or by electrical stimulation of the vagus; 10 min later, the antagonists were injected 30 s before a second injection of spasmogen or electrical stimulation of the vagus in order to assess the

efficacy of these drugs. In order to determine the time course of action of B4881, in some experiments bradykinin was administered 5 min, 10 min and 25 min as well as 30 s after administration of B4881.

Drugs and solutions

Urethane, bovine serum albumin (BSA), histamine, acetylcholine, bombesin, bradykinin, des-Arg⁹-bradykinin and des-Arg⁹-(Leu⁸)-bradykinin were purchased from Sigma and prepared for i.v. administration in sterile 0.9% sodium chloride solution. Paf was obtained from Novabiochem (Nottingham,

Table 1 The effect of B4881 and des-Arg⁹-(Leu⁸)-Bk on bradykinin (Bk)-induced bronchoconstriction

Bk	B4881	des-Arg ⁹ -(Leu ⁸)-Bk
Before	116.7 ± 17.8 (n = 12)	103.0 ± 28.8 (n = 5)
30 s after	14.1 ± 8.3 (n = 12)**	80.2 ± 14.8 (n = 5)
5 min after	60.8 ± 15.7 (n = 5)*	ND
10 min after	96.6 ± 16.2 (n = 6)	ND
25 min after	87.9 ± 28.9 (n = 12)	ND

Bronchoconstriction induced by bradykinin ($1 \mu\text{g kg}^{-1}$) before, and at various times after pretreatment with B4881 ($400 \mu\text{g kg}^{-1}$, i.v.) or des-Arg⁹-(Leu⁸)-Bk. Results are expressed as mean ± s.e.mean of % increase in pulmonary inflation pressure at various times after treatments in comparison with responses to Bk before. Significance is indicated by * $P < 0.05$; ** $P < 0.01$. ND = not done.

England) and prepared in 0.25% BSA. The B₂ receptor antagonist, B4881, was a kind gift from Drs J.M. Stewart and R. Vavrek, Department of Biochemistry, University of Colorado School of Medicine, Denver, U.S.A.

Statistical analysis

The significance of the responses before and after treatment with either B₁ or B₂ antagonists was assessed by a paired *t* test. All results have been expressed as mean $\pm$ s.e.mean.

Results

Bradykinin ($1 \mu\text{g kg}^{-1}$, i.v.) induced a marked increase in PIP which was completely abolished by prior treatment with the B₂ antagonist B4881 ($400 \mu\text{g kg}^{-1}$, i.v.) administered immediately before the injection of bradykinin (Figure 1 and Table 1). The antagonist activity of B4881 was of short duration as the response to bradykinin had recovered 25 min after administration of the B₂ antagonist (Figure 2 and Table 1).

In contrast, no significant inhibition of bradykinin-induced bronchoconstriction was observed following pretreatment with the B₁ receptor antagonist, des-Arg⁹-(Leu⁸)-bradykinin (Figure 1 and Table 1). Occasionally, the response to bradykinin recovered to a level greater than before administration of B4881 (see Figure 1), although this was not significant in a group of animals.

Furthermore, no significant bronchoconstriction was observed following i.v. administration of des-

Table 2 The effect of B4881 on the bronchoconstrictor effect of spasmogens and vagal stimulation

	n	B4881 ($400 \mu\text{g kg}^{-1}$, i.v.)	
		Before	After
Histamine ($10 \mu\text{g kg}^{-1}$)	4	270 ± 37.1	258 ± 36.1
Acetylcholine ($10 \mu\text{g kg}^{-1}$)	4	124 ± 14.4	136 ± 11.3
Bombesin ($0.5 \mu\text{g kg}^{-1}$)	4	65.4 ± 10.3	98.9 ± 11.5
Paf (20 ng kg^{-1})	5	202 ± 18.2	233 ± 13.5
Vagal Stimulation	3	116 ± 16.3	97.3 ± 21.0

Bronchoconstriction induced by histamine, acetylcholine, bombesin, Paf and vagal stimulation before and after treatment with B4881. Results are expressed as mean $\pm$ s.e.mean of % increase in pulmonary inflation pressure.

Arg⁹-Bk at both similar and higher concentrations to that used with bradykinin (Figure 3). However, this concentration of des-Arg⁹-Bk induced marked changes in blood pressure indicating that the material was active (Figure 3).

Intravenous administration of histamine ($10 \mu\text{g kg}^{-1}$), acetylcholine ($10 \mu\text{g kg}^{-1}$), bombesin ($0.5 \mu\text{g kg}^{-1}$) or Paf (20 ng kg^{-1}) all induced bronchoconstriction assessed as a significant increase in PIP. However, bronchoconstriction induced by these spasmogens was not significantly attenuated by prior treatment with B4881 at a concentration of

Figure 2 Representative trace showing the increase in pulmonary inflation pressure (PIP) as an index of bronchoconstriction (a) or blood pressure (BP) (b) in response to i.v. bradykinin (Bk, $1 \mu\text{g kg}^{-1}$) before and at various times after treatment with B4881 ($400 \mu\text{g kg}^{-1}$). PIP and BP are expressed in mmHg.

Figure 3 Representative trace showing the increase in pulmonary inflation pressure (PIP) as an index of bronchoconstriction (a) or blood pressure (BP) (b) in response to i.v. bradykinin (Bk $1 \mu\text{g kg}^{-1}$, i.v.). PIP and BP are expressed in mmHg. Responses to i.v. des-Arg⁹-Bk are shown for comparison.

$400 \mu\text{g kg}^{-1}$ i.v., a dose sufficient to abolish completely bradykinin-induced bronchoconstriction (Table 2). Moreover, bronchoconstriction induced by electrical stimulation of the vagus was not attenuated by prior treatment with B4881 ($400 \mu\text{g kg}^{-1}$, i.v.) (Table 2).

Discussion

There is now substantial evidence indicating that bradykinin will induce a wide array of biological activities including bronchoconstriction and that there are two distinct bradykinin receptor populations, namely B_1 and B_2 (Regoli & Barabe, 1980). Our results demonstrate that bradykinin-induced bronchoconstriction in the anaesthetized guinea-pig can be abolished by pretreatment with the selective B_2 antagonist B4881. In contrast, the suggested B_1 agonist des-Arg⁹-Bk (Regoli & Barabe, 1980) had no significant effect on bradykinin-induced bronchoconstriction. These results taken together suggest that the effect of bradykinin on airway smooth muscle is predominantly via the activation of the B_2 rather than via the B_1 receptor population. Whilst we were not formally analysing blood pressure it was noted that the B_1 agonist had a marked effect on systemic blood pressure indicating that this molecule had biological activity in our preparations. Further-

more, the B_2 antagonist did not appear to attenuate the blood pressure fall induced by i.v. bradykinin which may suggest that the activity of bradykinin on the vasculature is via the B_1 receptor.

B4881 has been described elsewhere as a selective B_2 antagonist but to date such observations have not been made in the lung. In the present study, however, B4881 had no significant effect on bronchoconstriction induced by other spasmogens, either acting on peptide receptors (bombesin), or not (histamine, acetylcholine and Paf). These results suggest that B4881 does not possess significant antagonistic activity for histamine (H_1), muscarinic or Paf receptors. Although the mechanisms underlying histamine and acetylcholine-induced bronchoconstriction are well documented, the precise mechanism of Paf and bombesin-induced bronchoconstriction are less well described. However, the present results suggest that neither Paf nor bombesin induced bronchoconstriction is via the activation of B_2 receptors. Furthermore, B4881 had no significant effect on bronchoconstriction induced by electrical stimulation of the vagus suggesting that bradykinin is not involved in this phenomenon. The present results suggest that B4881 is a selective B_2 antagonist in the guinea-pig lung and it is hoped that this compound will provide a useful tool in assessing the suggested contribution of bradykinin to respiratory diseases (Proud *et al.*, 1988).

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