Effects of vasoactive intestinal polypeptide on antigen-induced bronchoconstriction and thromboxane release in guinea-pig lung

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1 Exogenous vasoactive intestinal polypeptide (VIP) infused into the pulmonary artery of isolated and ventilated lungs of guinea-pigs decreased, in a dose-dependent fashion (1.0–10.0 nmol), airway resistance and thromboxane B₂ (TXB₂, the stable hydrolysis product of TXA₂) release in the perfusion medium. Prostacyclin (PGI₂) synthesis, as reflected by the release of its stable hydrolysis product 6-oxo-PGF_{1α}, was unaffected. Pretreatment with the 5-lipoxygenase inhibitor BWA4c (3.5×10^{-5} M) did not modify the bronchodilatory effect of VIP or its inhibitory action on TXB₂ release.

2 Basal release of immunoreactive VIP from perfused lungs decreased from an initial value of 0.96 ± 0.10 ng min⁻¹ (mean $\pm$ s.e.mean) in the first 2 min to an average of 0.58 ± 0.10 ng min⁻¹ in the following 15–20 min.

3 Antigen challenge with ovalbumin (0.1%) in sensitized lungs caused an anaphylactic reaction in 45% of tested lungs, concomitant with a 5 fold increase in both VIP and TXB₂ release. Tetrodotoxin pretreatment (10^{-6} M) reduced basal VIP release by >80% and abolished the VIP increase observed during anaphylaxis, without modifying TXB₂ release or the bronchoconstrictor response.

4 Indomethacin (10^{-6} M) inhibited TXB₂ synthesis and release by >90%, delayed the bronchoconstrictor response and blunted the increased VIP release during lung anaphylaxis, without influencing basal VIP release.

5 The 5-lipoxygenase inhibitor BWA4c (3.5×10^{-5} M) blunted the increase of TXB₂ and VIP release from guinea-pig lung and attenuated the bronchoconstrictor response following ovalbumin challenge.

6 The administration of exogenous VIP as a continuous infusion (10^{-8} M) attenuated the bronchoconstriction and the release of cyclo-oxygenase metabolites following antigen challenge.

7 Acetylcholine (10^{-6} – 10^{-5} M) infused into the pulmonary artery induced a dose-dependent bronchoconstriction not associated with enhanced VIP or TXB₂ release.

8 The TXA₂ mimetic U-46619 (0.1–1.0 nmol) caused dose-dependent increases in airway resistance, concomitant with an up to 10 fold increase in VIP release. VIP inhibited arachidonate-induced *in vitro* aggregation of washed rabbit platelets in a dose-dependent manner over a dose range 10^{-8} – 10^{-6} M. Despite the antiaggregatory effect of VIP, TXB₂ and PGE₂ synthesis was reduced only to a minor extent, and there was no redirection of arachidonate metabolism from TXA₂ to PGE₂, indicating that VIP does not act as a TX synthase inhibitor *in vitro*.

9 We conclude that VIP may play a role in regulating bronchial smooth muscle reactivity in lung anaphylaxis by inhibiting the synthesis and release of TXA₂, a potent vasoactive and bronchoconstrictor agent. TXA₂, on the other hand, strongly enhances neuronal VIP release.

Keywords: Vasoactive intestinal polypeptide; thromboxane A₂; lung anaphylaxis; guinea-pig isolated lung; BWA4c

Introduction

Vasoactive intestinal polypeptide (VIP), a 28-amino acid peptide, is the neuropeptide found in the highest concentrations in the lung of several mammalian species, including man (see Said, 1991a and Barnes, 1991 for reviews). Immunohistochemical studies have localized immunoreactive VIP in nerve fibres within airway smooth muscle, surrounding tracheobronchial mucous and serous glands, in the walls of airway blood vessels and in microganglia within the lungs (Dey *et al.*, 1981; Laitinen *et al.*, 1985; Lundberg *et al.*, 1984; Polak *et al.*, 1986; Said, 1988). Moreover, specific, high affinity receptors for VIP have been identified in membrane preparations of rat, mouse, guinea-pig and human lungs (Said, 1987) and on airway glands, epithelium and vascular smooth muscle of human and guinea-pig lung (Carstairs & Barnes, 1986; Uddman & Sundler, 1987).

VIP is the most favoured candidate for the role of neurotransmitter in non-adrenergic non-cholinergic (NANC) inhibitory nerves because, in animal airways, it mimics the electrophysiological changes produced by NANC nerve

stimulation (Cameron *et al.*, 1983). It is released when these nerves are stimulated electrically (Matsuzaki *et al.*, 1980), and, when tolerance is induced by exposure to high concentrations of VIP, the NANC inhibitory nerve effects are selectively reduced (Ito & Takeda, 1982).

VIP is known to inhibit the bronchoconstrictor effects of histamine, prostaglandin F_{2α} (PGF_{2α}), the sulphidopeptide leukotrienes (LT) C₄ and D₄, neurokinins A and B, and endothelin (see Said, 1991a for review). It has been reported that VIP can inhibit the release of LTC₄ from rat and guinea-pig lungs incubated in the presence of platelet-activating factor (PAF) and calcium ionophore (Beaubien *et al.*, 1984; Di Marzo *et al.*, 1989). Moreover, VIP selectively inhibits the contraction induced by LTD₄ in the small airways of guinea-pig lung (Samhoun *et al.*, 1987) and considerably reduces the release of cyclo-oxygenase products induced by LTD₄ in strips of guinea-pig lung parenchyma (Samhoun *et al.*, 1987; Conroy *et al.*, 1988a,b) and from guinea-pig perfused lung (Conroy *et al.*, 1988a,b; 1992).

Previous studies have characterized the release of thromboxane and leukotrienes from anaphylactic guinea-pig lungs

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and found that TXB₂ (the stable hydrolysis product of TXA₂) is the most abundant cyclo-oxygenase product of arachidonate metabolism in anaphylactic lung perfusates (Robinson *et al.*, 1984). We have previously reported that exposure to toxicants that irritate the respiratory tract selectively enhances TXB₂ release from isolated, perfused and ventilated lung of guinea-pigs (Preziosi & Ciabattini, 1986; 1987). The aim of this study was to characterize the release of VIP from guinea-pig sensitized isolated lungs undergoing anaphylactic reactions after an antigen challenge and to explore the hypothesis that VIP and arachidonate metabolites interact in regulating bronchial smooth muscle reactivity.

A preliminary report of this study was presented at the Fifth International Symposium on VIP and Related Peptides, held on November 12–15, 1991, at Shizuoka, Japan, and published in abstract form (*Biomed. Res.*, 12, S1:34, 1991).

Methods

Isolated lungs from male Dunkin-Hartley guinea-pigs (400–600 g) were perfused according to the procedure originally described by Delaunois *et al.* (1956) and Preziosi *et al.* (1959). Each lung was removed from the chest of the animal, the pulmonary artery was cannulated and most of the heart cut away to allow perfusion. The lungs were perfused at 5 ml min⁻¹ in a thermostatic Perspex chamber with Krebs Ringer bicarbonate solution at 37°C, gassed with 5% CO₂/95% O₂ and containing bacitracin (30 mg l⁻¹) and aprotinin (100,000 iu l⁻¹). A pressure transducer connected to the trachea allowed measurement of pressure-volume variations in the lung system on a Beckman Dynograph. A pump, which aspirated air from the closed chamber during one half of a cycle and pushed the same air back during the other half, enabled lung ventilation in sequence. The lung perfusion solution was removed from the bottom of the chamber by a peristaltic pump connected to a fraction collector. The solution was chilled on ice, centrifuged at 4°C and stored at -25°C until assayed.

Sensitization of guinea-pigs

Guinea-pigs were sensitized with a 1% ovalbumin solution; 20 mg being given intraperitoneally and 20 mg subcutaneously after 10 min. Animals were used after 15 days.

VIP infusion

Exogenous VIP was given as an infusion over 1 min through the pulmonary artery in control non-sensitized lungs or after pretreatment with the lipoxygenase inhibitor BWA4c (N-(3-phenoxycinnamyl)-acetoxyhydroxamic acid; Payne *et al.*, 1988). The latter compound was dissolved in the perfusion medium at a concentration 3.5×10^{-5} M and the lungs were perfused with this solution throughout the experiment. Pressure-volume variations in the lung were recorded under basal conditions and during and after exposure to three different doses of VIP (1, 5 and 10 nmol) given in a sequence at intervals of 6 min. For this purpose 5 ml of a VIP solution at concentrations ranging between 2×10^{-7} and 2×10^{-6} M was injected into the pulmonary artery within 1 min, during which the flow of the perfusion medium from the reservoir was stopped. The perfusate was recovered at intervals of 2 min for measurement of TXB₂ and 6-oxo-PGF_{1α}, the stable hydrolysis product of prostacyclin (PGI₂; see below).

Release experiments

Ovalbumin-sensitized guinea-pig lungs were perfused, allowed to equilibrate for at least 10 min and challenged with a 0.1% ovalbumin solution given through the pulmonary artery. In 25 of all the lungs tested in this study ($n = 59$) a bronchoconstriction followed antigen challenge within a few minutes.

Pressure-volume variations in the lung were monitored throughout the experiment and the perfusate was recovered at intervals of 2 min. Some of the sensitized lungs were pretreated with tetrodotoxin (TTX) 10^{-6} M, or indomethacin 10^{-6} M, or BWA4c (3.5×10^{-5} M) for at least 20 min before exposure to ovalbumin. These compounds were dissolved in the perfusing medium and the lungs were perfused with the appropriate solution throughout the experiment.

A total of 12 sensitized lungs were challenged with ovalbumin during administration of exogenous VIP. For this purpose, the lungs were perfused with a medium containing VIP 10^{-8} M, i.e. a concentration that has been shown to inhibit the release of cyclo-oxygenase products from guinea-pig isolated perfused lung (Conroy *et al.*, 1988a,b; 1992). After an equilibration period of 20 min they were exposed to ovalbumin; 5 of these underwent bronchoconstriction. Pressure-volume variations in the lung were recorded and the perfusate was collected at 2 min intervals.

Acetylcholine infusion

Acetylcholine was infused over 1 min through the pulmonary artery of non-sensitized lungs at concentrations ranging from 10^{-6} to 10^{-5} M (flow rate: 5 ml min⁻¹) to decrease the tracing recording the pressure-volume variations in the lung to an extent comparable to that observed in the lungs undergoing anaphylactic reaction after antigen challenge. The perfusate from these lungs was recovered at 2 min intervals.

Thromboxane-mimetic infusion

The stable thromboxane A₂ analogue U-46619 (15 S-hydroxy-11α, 9α-epoxymethanoprostano-5 Z, 13 E-dienoic acid) was infused over 1 min through the pulmonary artery of non-sensitized lungs. Three different doses (0.1, 0.5 and 1.0 nmol) were given in sequence at 6 min intervals. For this purpose 5 ml of a U-46619 solution at concentrations ranging between 2×10^{-8} and 2×10^{-7} M was injected into the pulmonary artery within 1 min, during which the flow of the perfusion medium from the reservoir was stopped. Pressure-volume variations in the lung were recorded, and the perfusate was recovered as above.

Washed-platelet experiments

Washed rabbit platelets were prepared according to the method originally described by Ardlie *et al.* (1970) with some modifications. Blood was drawn from New Zealand rabbits by cardiac puncture in plastic tubes with sodium citrate 3.8% in the ratio 9:1 (blood:anticoagulant), and centrifuged at 120 g for 15 min. The resulting platelet-rich plasma was aspirated and centrifuged at 1,100 g for 15 min at room temperature. The pellet was dissolved in 10 ml of washing solution (modified Tyrode solution, containing (mM): NaCl 129, KCl 2.6, Na₂PO₄ 0.39, NaHCO₃ 12, glucose 5.5, MgCl₂ 2.0 and 0.35% bovine serum albumin) and kept for 15 min before centrifugation. The procedure was repeated twice and finally platelets were suspended in a HEPES-buffered Tyrode solution (the same solution described above, containing 1.8 mM CaCl₂, 5 mM HEPES, 30 mg l⁻¹ bacitracin and 100,000 iu l⁻¹ aprotinin). Platelets were stirred at 1,000 r.p.m. at 37°C in an automated aggregometer (Elvi-Logos) and incubated for 60 s in the presence of VIP; thereafter, sodium arachidonate was added at final concentrations ranging from $5-15 \times 10^{-6}$ M. Dose-dependent aggregation curves were repeated in the absence and in the presence of VIP at final concentrations ranging from 10^{-8} to 10^{-6} M.

Analytical methods

TXB₂, 6-oxo-PGF_{1α} and PGE₂ were measured by specific radioimmunoassay (RIA) systems developed in our laboratory. Details of these RIA techniques and validation of

the measurements by comparison with other independent assay methods are described elsewhere (Ciabattoni *et al.*, 1979; Patrono *et al.*, 1982; 1985).

LTC₄-like immunoreactivity (LTC₄-LI) was measured by RIA with anti-LTC₄ serum AS1 (Preziosi & Ciabattoni, 1986). This antiserum was a gift from the Merck Frosst Canada. It showed a 43% cross-reactivity with LTD₄, 6% with LTE₄ and less than 1% with other prostanoids (Hayes *et al.*, 1983).

VIP-like immunoreactivity (VIP-LI) was measured with a recently developed RIA system, employing a specific polyclonal rabbit antiserum and ¹²⁵I-labelled VIP. The entire procedure has been described in detail by D'Amato *et al.* (1992).

Materials

The following compounds were used: VIP (porcine, Sigma), aprotinin (Kir, Richter), bacitracin (Lusofarmaco), ovalbumin (Merck), TTX (Sigma), indomethacin (Merck Sharp & Dohme), BWA4c (Wellcome), acetylcholine (Merck), U-46619 (The Upjohn Company), arachidonic acid (Fluka), [³H]-TXB₂ and [³H]-PGE₂ (New England Nuclear), [³H]-6-oxo-PGF_{1α}, [³H]-LTC₄ and [¹²⁵I]-VIP (Amersham).

Statistical analysis

The results were evaluated by means of a parametric analysis of variance for multiple comparisons and Student's *t* test for single comparisons. Chi-squared test was used to compare means calculated on values expressed as a percentage of the basal. All values are reported as mean ± s.e.mean. *P* ≤ 0.05 was considered statistically significant.

Results

Effects of VIP infusion

When infused into non-sensitized guinea-pig lungs, exogenous VIP caused a statistically significant, dose-dependent increase in the amplitude of the tracing of the pressure-volume variations recorded through the trachea. This bronchodilator response was associated with a dose-related decrease in thromboxane release (Figure 1). Inhibition of TXB₂ release seemed to be selective, since PGI₂ release, as reflected by its hydrolysis product 6-oxo-PGF_{1α}, was unaffected by VIP infusion (Figure 1). The lipoxygenase inhibitor, BWA4c, did not modify the bronchodilator effects of VIP nor its inhibitory effect on TXB₂ release (Figure 1).

Antigen challenge and VIP and TXB₂ release from guinea-pig lung

VIP-like immunoreactivity was identified in perfusates of guinea-pig isolated and ventilated lungs under basal conditions as well as after antigen challenge (Table 1). Basal release averaged 0.96 ± 0.10 ng min⁻¹ during the first 2 min and decreased to 0.58 ± 0.10 ng min⁻¹ within the following 6 min, thereafter remaining stable up to 15–20 min in lungs that were refractory to ovalbumin challenge, i.e. those that did not undergo an anaphylactic reaction (Table 1). In these lungs TXB₂ release averaged 3.36 ± 0.63 ng min⁻¹ (*n* = 6), and no change was observed after ovalbumin injection into the pulmonary artery (3.29 ± 0.78 ng min⁻¹). In descending order of magnitude, ovalbumin refractory lungs released TXB₂ > 6-oxo-PGF_{1α} > PGE₂. No LTC₄-LI was detected under these conditions. The corresponding values are shown in Table 2.

Ovalbumin challenge induced a bronchoconstrictor res-

Figure 1 (a,b,c) Effects of vasoactive intestinal polypeptide (VIP) infusion into the pulmonary artery of guinea-pig isolated perfused and ventilated lung: (a) percentage of the basal tracing recording the pressure-volume variations in the lung; (b) and (c) 6-oxo-prostaglandin (PG) F_{1α} and thromboxane (TX) B₂ release in the perfusing medium. (d,e,f) The effects of VIP infusion on the same parameters after pretreatment with the lipoxygenase inhibitor BWA4c. *P < 0.01 vs basal.

Table 1 Vasoactive intestinal polypeptide (VIP) release from isolated, perfused and ventilated lung of the guinea-pig after antigen challenge

	VIP (ng min ⁻¹)	
	Before	After
Bronchoconstriction absent (n = 6)	0.58 ± 0.10	0.59 ± 0.11
Bronchoconstriction present (n = 5)	0.43 ± 0.07	2.10 ± 0.50*

*P < 0.001 vs basal release of 0.96 ± 0.10 ng min⁻¹.

ponse which did not resolve within the time course studied in 5 of the 11 sensitized lungs, as reflected by an 88.6 ± 1.4% reduction in the tracing of the pressure-volume variations measured through the trachea. In these lungs the bronchoconstriction was temporally accompanied by enhanced release of VIP (Table 1 and Figure 2) and the measured cyclo-oxygenase products, i.e. TXB₂ (3 fold increase), 6-oxo-PGF_{1α} (2 fold increase) and PGE₂ (2.6 fold increase: Figure 2 and Table 2). LTC₄-LI, which was unmeasurable under basal conditions, appeared after ovalbumin challenge (Table 2). VIP release as well as the release of all the measured eicosanoids was maximal in the collections performed during the reduction of the tracing recording the pressure-volume variations in the lung.

Effects of tetrodotoxin and indomethacin on VIP and TXB₂ release

Figure 2 shows the effects of TTX treatment on ovalbumin-sensitized lungs that underwent anaphylactic reaction after

Figure 2 Vasoactive intestinal polypeptide (VIP) and thromboxane (TX) B₂ release in ovalbumin sensitized guinea-pig lungs undergoing anaphylactic bronchoconstriction after antigen challenge without any treatment (a) or after tetrodotoxin (TTX; b) or indomethacin (c) treatment. *P < 0.01 and **P < 0.001 vs basal of control experiments.

antigen challenge. Those lungs which were refractory to ovalbumin exposure were not considered. Basal VIP release was reduced by >80%, and the increase observed during the bronchoconstrictor response was completely abolished. However, the increase of TXB₂ release was unaffected by TTX treatment (Figure 2), and the decrease of the tracing of the pressure-volume variations in the lung observed after ovalbumin exposure was nearly identical to that observed in control experiments, as indicated by the slope and the plateau of the curve obtained by plotting the amplitude of this tracing as a function of time (Figure 3). In this graphical analysis the area under the continuous pen tracing was divided into segments of 12 s duration, and each segment was expressed as a percentage of the area covered in the same time under basal conditions, i.e. after equilibrations and before ovalbumin injection.

Indomethacin treatment almost abolished thromboxane synthesis and release under control conditions and prevented the increase observed after antigen challenge in lungs undergoing anaphylactic reaction (Figure 2). Moreover, although indomethacin did not modify basal release, it did prevent the increase in VIP observed during the bronchoconstrictor response (Figure 2). Indomethacin did not modify the maximal reduction of the pressure-volume variations induced in the lung as compared with controls (83.3 ± 5.6 vs 88.6 ± 1.4%), but it significantly (*P* < 0.01) modified the slope of the curve (Figure 3) by increasing the time required to achieve maximal bronchoconstriction from an average of 1.2 min in controls to 3.6 min after indomethacin treatment.

Effects of the 5-lipoxygenase inhibitor BWA4c on thromboxane and VIP release

Five of 12 sensitized lungs challenged with ovalbumin after BWA4c treatment underwent bronchoconstriction. In these

Figure 3 Pressure-volume variations in the perfused and ventilated guinea-pig lungs undergoing bronchoconstriction after antigen challenge: (●) control experiments (n = 5); (■) tetrodotoxin (10⁻⁶ M)-treated lungs (n = 5); (▲) indomethacin-treated lungs (10⁻⁶ M; n = 5); (○) VIP-treated lungs (10⁻⁸ M; n = 5).

Table 2 Eicosanoid release from isolated perfused and ventilated lung of guinea-pig after antigen challenge

	TXB ₂ (ng min ⁻¹)	6-oxo-PGF _{1α} (ng min ⁻¹)	PGE ₂ (ng min ⁻¹)	LTC ₄ -LI (ng min ⁻¹)
Bronchoconstriction absent (n = 6)	3.29 ± 0.78	1.95 ± 0.49	0.68 ± 0.12	< 0.3
Bronchoconstriction present (n = 5)	10.68 ± 1.33*	3.96 ± 0.85*	1.81 ± 0.31*	5.06 ± 0.45*

*P < 0.01 vs basal

Table 3 Effects of acetylcholine (ACh) infusion into the pulmonary artery of perfused and ventilated lung of the guinea-pig

	VIP (ng min ⁻¹)	TXB ₂ (ng min ⁻¹)	% reduction of the basal tracing
Basal	0.61 ± 0.10	3.30 ± 0.13	0
ACh 10 ⁻⁶ M	0.66 ± 0.05	2.88 ± 0.10	21.2 ± 3.5*
ACh 5 × 10 ⁻⁶ M	0.57 ± 0.02	2.76 ± 0.16	52.1 ± 4.4**
ACh 10 ⁻⁵ M	0.59 ± 0.04	2.89 ± 0.20	85.3 ± 5.4**

The amplitude of the pen tracing recording pressure-volume variations in the lung was measured after equilibration and at the maximal effects produced by each dose of ACh. Numbers are means ± s.e.mean of 4 experiments.

*P < 0.01 vs basal; **P < 0.001 vs basal.

lungs the 5-lipoxygenase inhibitor did not modify the basal release of TXB₂ or VIP (2.90 ± 0.43 and 0.55 ± 0.09 ng min⁻¹, respectively), but significantly (*P* < 0.01) blunted the increase of both compounds observed during the bronchoconstrictor response (4.42 ± 0.85 and 0.82 ± 0.16 ng min⁻¹, respectively). Moreover, BWA4c significantly attenuated the maximal reduction of the pressure-volume variations induced in the lung as compared with controls (72.9 ± 4.1 vs 88.6 ± 1.4%, *P* < 0.05) and increased the time required to achieve the maximal bronchoconstriction from an average of 1.2 min in controls to 3.8 min after BWA4c.

Effects of exogenous VIP on the bronchoconstriction and the release of cyclo-oxygenase metabolites following ovalbumin challenge

Perfusion with exogenous VIP (10⁻⁸ M) attenuated the maximal reduction of the tracing recording the pressure-volume variations in the lung as compared with controls (63.9 ± 7.0 vs 88.6 ± 1.4%; *P* < 0.01) and reduced the slope of the curve (Figure 3) by increasing the time required to achieve maximal bronchoconstriction from an average of 1.2 min in controls to 4.5 min after VIP pretreatment. This effect was even more evident than after indomethacin or BWA4c treatment. TXB₂ release was 1.78 ± 0.27 ng min⁻¹ immediately before ovalbumin challenge and increased to 3.15 ± 0.68 ng min⁻¹ in those lungs which underwent bronchoconstriction. Both basal release and the increase after antigen challenge were significantly lower than in controls (*P* < 0.05). 6-oxo-PGF_{1α} and PGE₂ release was 1.82 ± 0.30 and 0.53 ± 0.05 ng ml⁻¹, respectively, before ovalbumin challenge. They increased to 2.56 ± 0.42 and to 0.92 ± 0.07 ng min⁻¹, respectively, during the bronchoconstrictor response. However, the percentage increase, although statistically significant (*P* < 0.05), resulted significantly lower than in untreated lungs (*P* < 0.05).

Effects of acetylcholine

When infused into the pulmonary artery of non-sensitized lungs in the concentration range 10⁻⁶–10⁻⁵ M, acetylcholine induced a dose-dependent, reversible decrease of the tracing of the pressure-volume variations in the lung (Table 3). However, despite a degree of bronchoconstriction at the highest dose, substantially identical to that observed after anaphylactic reaction, no statistically significant changes in VIP or TXB₂ release were observed (Table 3).

Effects of thromboxane analogue U-46619

The TXA₂-mimetic, U-46619, in the dose range 0.1–1.0 nmol, infused over 1 min into the pulmonary artery, caused a dose-dependent decrease of the tracing of the pressure-volume variations in the lung, together with a significant increase in VIP release. The results are detailed in Figure 4. TXB₂ levels were not determined because of the high cross reactivity of U-46619 with anti-TXB₂ serum in the RIA system.

Figure 4 Percentage of the basal tracing recording the pressure-volume variations in the lung (a), and vasoactive intestinal polypeptide (VIP) release; (b) after infusion into the pulmonary artery of the endoperoxide PGH₂ analogue U-46619 in the dose-range 0.1–1.0 nmol. *P < 0.01 vs basal.

Effects of VIP on platelet aggregation and thromboxane synthesis

VIP inhibited arachidonic acid (AA)-induced platelet aggregation in a dose-dependent manner over a dose-range 10⁻⁸–10⁻⁶ M. A 28.3 ± 2.0% inhibition of maximal aggregation was achieved at a final concentration of 10⁻⁸ M VIP and 68.3 ± 5.7% at 10⁻⁶ M. Despite the antiaggregatory effect of VIP, TXB₂ levels in the supernatant collected after platelet aggregation were only marginally reduced by 12–20% (*P* < 0.01; *n* = 12). A nearly identical reduction of PGE₂ was observed (12.0 ± 4.0%–20.6 ± 1.9% at the same VIP concentrations). However, within the range of VIP concentrations used, no clear dose-response could be established between the reduction in TXB₂ or PGE₂ levels and VIP concentrations. Moreover, no redirection of arachidonate cyclo-oxygenation from TXB₂ to PGE₂ synthesis was observed.

Discussion

VIP relaxes airway smooth muscle *in vitro* in several species, including man, and represents the most potent endogenous

bronchodilator thus far discovered (Said, 1984; Palmer *et al.*, 1986). Previous studies have reported that VIP inhibits the release of TXA₂ induced by LTD₄ and bradykinin from guinea-pig isolated perfused lung (Conroy *et al.*, 1988a,b; 1992). In addition, these studies reported that VIP also inhibited the release of PGE₂ and PGI₂. Similarly Berisha *et al.* (1990) have shown that the release of several cyclo-oxygenase metabolites induced by oxidant injury was inhibited by VIP. Our study confirms, at least in part, these findings, because we have shown that exogenous VIP infusion into the pulmonary artery of isolated, perfused and ventilated guinea-pig lung inhibits the antigen-induced release of TXB₂, 6-oxo-PGF_{1α} and PGE₂. However, our findings are not in complete agreement with those of Conroy *et al.* (1992) who showed that the basal release of the measured cyclo-oxygenase metabolites was unaffected by VIP. In contrast, we found that when infused in non-sensitized lungs VIP causes a progressive, dose-related decrease of TXB₂ release into the perfusion medium. This effect is concomitant with a dose-related relaxation of bronchial smooth muscle and seems to be selective for thromboxane, since the release of 6-oxo-PGF_{1α} was unmodified. Similarly, pre-treatment with VIP before ovalbumin challenge of sensitized lungs decreased the basal release of TXB₂, but not that of 6-oxo-PGF_{1α} or PGE₂. Discrepancies with the data of Conroy *et al.* (1992) might be explained on the basis of differences between the two experimental models. In our study, lung ventilation represents a mechanical stimulus which could increase to some extent the basal release of TXB₂.

Sulphidopeptide leukotrienes are known to stimulate TXA₂ synthesis in guinea-pig lung and it has recently been shown that VIP inhibits leukotriene synthesis and release from guinea-pig lung (Beaubien *et al.*, 1984; Samhoun *et al.*, 1987; Di Marzo *et al.*, 1989). Therefore, we have tested the effect of VIP infusion in the presence of the lipoxygenase inhibitor BWA4c at a dose that produces a nearly maximal inhibition of this enzyme *in vitro* (Payne *et al.*, 1988; Higgs *et al.*, 1988). Lipoxygenase inhibition did not modify the bronchodilator response nor the reduction in thromboxane release induced by VIP, suggesting that VIP directly affects TXA₂ synthesis in guinea-pig lung. This is not surprising, since the levels of LTC₄-LI found in the perfusate of lungs not exposed to ovalbumin or not undergoing anaphylactic reaction after antigen challenge were below the picogram detection limit of the assays used (Ciabattoni & Preziosi, 1985; Preziosi & Ciabattoni, 1986; 1987).

VIP has been proposed as a modulator of lung inflammation and airway constriction because of its ability to inhibit inflammatory cell function, antagonize major humoral mediators of inflammation and attenuate acute oedematous lung injury in several experimental models (Berisha *et al.*, 1990). We identified immunoreactive VIP in the perfusate of isolated, perfused and ventilated guinea-pig lung under basal conditions and found that antigen challenge causes an approximate five fold increase in VIP release in those lungs undergoing persistent bronchoconstriction after exposure to ovalbumin. Previous studies have characterized the pattern of arachidonate metabolite release by anaphylactic guinea-pig lung and identified TXB₂ as the most abundant product followed, in descending order of magnitude, by 6-oxo-PGF_{1α} and PGE₂ (Robinson *et al.*, 1984). A platelet source of TXA₂ was excluded by pretreating animals with aspirin 24 h before they were killed (Preziosi & Ciabattoni, 1986). Moreover, we have previously reported the presence of LTC₄-LI in the perfusates of anaphylactic guinea-pig lungs by means of two anti-LTC₄ sera showing a different cross-reactivity with LTD₄ and LTE₄ (Ciabattoni & Preziosi, 1985; Preziosi & Ciabattoni, 1986). The data presented in this study confirm these previous observations. However, because the cross-reactivity of our antisera with LTD₄ and LTE₄ is high, we cannot discriminate among the mixture of sulphidopeptide leukotrienes which may be present in lung perfusate.

TXA₂ plays an essential role in the bronchospasm that

accompanies the anaphylactic reaction, as demonstrated by studies with selective TX-synthase inhibitors (Preziosi & Ciabattoni, 1986) and by the effect of indomethacin pre-treatment which delays the development of bronchoconstriction. However, because indomethacin does not block the 5-lipoxygenase pathway of arachidonic acid metabolism, leukotriene synthesis is unaffected and a final bronchoconstriction results as an effect of these substances, once termed 'slow reacting substance of anaphylaxis'. In the present study we found increased VIP release that was concomitant with a massive increase in TXA₂ synthesis and release. This enhanced VIP release appears to be quite specific for an inflammatory reaction such as anaphylaxis, since acetylcholine infusion in non-sensitized lungs failed to increase either VIP or thromboxane release, despite a bronchoconstriction similar to (although reversible) that observed in anaphylactic lungs.

The neuronal origin of the VIP in lung perfusates was confirmed by TTX treatment, which inhibited by >80% the basal VIP release. TTX also abolished the increased VIP release observed during the anaphylactic reaction, without affecting thromboxane release or the bronchoconstrictor response. On the other hand, cyclo-oxygenase inhibition achieved by pretreating the lungs with indomethacin, reduced TXB₂ release by >90%, delayed the bronchoconstrictor response and blunted the increase in VIP release during anaphylaxis, without modifying basal VIP release. Failure of TTX treatment to modify the bronchoconstrictor response seems feasible, since it inhibits the release of a bronchodilator substance. The observed effects should be an increased bronchoconstriction, but because this response after ovalbumin challenge is maximal, it is hard to suppose, although theoretically possible, a shift of the curve of Figure 3 toward the left.

To explore the hypothesis that thromboxane acts on NANC neurones by releasing VIP, we tested the effects of the TXA₂ mimetic U-46619 (Coleman *et al.*, 1981). This compound is a stable analogue of prostaglandin H₂, the precursor of TXA₂, and an agonist at TXA₂/PGH₂ receptor(s), which exists in many tissues (see Halushka *et al.*, 1989 for a review). As expected, U-46619 caused a dose-dependent bronchoconstrictor response. At the highest dose (1.0 nmol) the bronchoconstriction did not resolve within the time course studied and was comparable to that induced by lung anaphylaxis. Similarly, in a dose-related fashion, U-46619 increased VIP release up to ten fold. As no change in the release of this peptide was observed with comparable increases in airway resistance induced by acetylcholine, we might conclude that the enhanced VIP release is a consequence of the interaction of U-46619 with the TXA₂/PGH₂ receptor rather than a non-specific response to bronchoconstriction.

The selective inhibition of thromboxane synthesis and release induced by VIP infusion could be interpreted on the basis of an inhibition of the TX synthase, i.e. the enzyme converting PGH₂ into TXA₂. An inhibition of cyclo-oxygenase can be excluded because the synthesis and release of other prostaglandins, such as PGI₂, was unaffected. To answer this question, we investigated the effects of exogenous VIP on *in vitro* platelet TXA₂ synthesis. This model represents the most abundant source of TX-synthase, and redirection of arachidonate synthesis toward other prostanoids may be achieved with selective TX-synthase inhibitors (Patrignani *et al.*, 1984). The effects of VIP on *in vitro* platelet aggregation induced by AA are consistent with the reported ability of VIP to inhibit PAF-induced platelet aggregation and secretion (Cox *et al.*, 1984). However, despite a substantial reduction in the platelet aggregating capacity, the inhibition of TXB₂ release occurred only to a minor extent, not closely dose-related and without any evidence of a redirection of arachidonate metabolism toward other cyclo-oxygenase products. This finding can exclude an inhibitory effect of VIP on TX synthase.

Pretreatment with the 5-lipoxygenase inhibitor, BWA4c, was effective in reducing the release of TXB₂ and VIP from guinea-pig lung following ovalbumin challenge. It also delayed the development of bronchoconstriction and attenuated the extent of the final response. These data suggest a primary release of sulphidopeptide leukotrienes in lung anaphylaxis. Leukotrienes, on the other hand, enhance TXA₂ synthesis and release, which, in turn, might stimulate the release of VIP. Such a view could explain not only the partial protection of BWA4c against bronchoconstriction because of reduction in leukotriene release, but also the observed inhibition of both TXB₂ and VIP release after antigen challenge.

In recent studies on isolated, perfused lungs of rat and guinea-pigs, VIP has been shown to reduce all signs of acute lung injury caused by a series of experimental inflammatory injuries (Berisha *et al.*, 1988; 1990; Pakbaz *et al.*, 1988; 1990). Possible mechanisms by which VIP may reduce or prevent acute lung injury include the inhibition of inflammatory cell function and inhibition of the generation of inflammatory mediators, including eicosanoids (see Said, 1991a,b for reviews). In the present study the administration of exogenous VIP attenuated the bronchoconstriction following ovalbumin challenge to an extent even more evident than indomethacin or BWA4c. This effect was concomitant with the reduction in the release of the measured cyclo-oxygenase metabolites. Recently, Trotz & Said (1991) have shown that VIP can inhibit phospholipase A₂ activity, i.e. the enzyme which releases arachidonic acid from membrane phospholipids. Also from the data of Conroy *et al.* (1992) it appears

that VIP acts at a step preceding cyclo-oxygenase activation. Therefore, it is reasonable to suppose that the observed inhibitory effect of VIP on cyclo-oxygenase metabolite release as well as on antigen-induced bronchoconstriction might be the consequence of a reduced availability of arachidonic acid.

A possible explanation of the selective inhibition of thromboxane release by VIP could be represented by the cellular origin of the former. If we exclude platelets, an important source is represented by mononuclear cells, mast cells and leukocytes, i.e. cells that mediate and modulate inflammation. VIP binds to specific receptor sites on these cells and prevents the release of several inflammatory mediators (see Said, 1991a for a review). TXA₂ in guinea-pig lung might be included among these mediators.

In conclusion, these data suggest that VIP plays a complex role in regulating bronchial reactivity in lung anaphylaxis. A protective role against the activity of bronchoconstrictor mediators released by the immunological reaction is strongly suggested both by the increase in VIP release during lung anaphylaxis and by its ability to inhibit the synthesis and release of TXA₂. The latter, on the other hand, seems to enhance neuronal release of VIP. Thus, a balance might exist between these two opposite mediators in regulating bronchial smooth muscle reactivity in guinea-pig lung. In this balance, VIP might have a role as an endogenous modulator of both bronchoconstriction and pulmonary inflammation.

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