Effects of selective phosphodiesterase inhibitors on platelet-activating factor- and antigen-induced airway hyperreactivity, eosinophil accumulation, and microvascular leakage in guinea pigs

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Abstract There is currently interest in the potential use of selective inhibitors of cyclic nucleotide phosphodiesterases (PDE) in the treatment of asthma. In this study we examined the effects of three selective PDE inhibitors, milrinone (PDE III), rolipram (PDE IV) and zaprinast (PDE V), on the bronchoconstriction produced by antigen and histamine, the airway hyperreactivity and microvascular leakage after aerosol exposure to platelet-activating factor (PAF) and antigen, and the antigen-induced eosinophil infiltration in guinea-pig lung. Inhaled rolipram (0.01–10 mg ml⁻¹) inhibited dose dependently the bronchospasm produced by aerosol antigen (5 mg ml⁻¹) in anaesthetized, ventilated guinea-pigs. Rolipram (10 mg ml⁻¹) produced maximal inhibition of antigen-induced bronchoconstriction but only partial inhibition of the response to aerosol histamine (1 mg ml⁻¹). Milrinone and zaprinast (each 10 mg ml⁻¹) showed weak, or no, inhibitory effects against bronchoconstriction produced by aerosol antigen or histamine. Pretreatment with rolipram (10 mg kg⁻¹, i.p.) prevented airway hyperreactivity to histamine which develops 24 h after exposure of conscious guinea-pigs to aerosol PAF (500 µg ml⁻¹) or antigen (5 mg ml⁻¹). The pulmonary eosinophil infiltration obtained with 24 h of antigen-exposure was inhibited by rolipram. In contrast, milrinone and zaprinast (each 10 mg kg⁻¹, i.p.) failed to reduce either the airway hyperreactivity of the eosinophil accumulation in these animals. Rolipram (1–10 mg ml⁻¹) reduced the extravasation of Evans blue after aerosol PAF (500 µg ml⁻¹) at all airway levels while a lower dose (0.1 mg ml⁻¹) was only effective at intrapulmonary airways. Rolipram (0.01–1 mg ml⁻¹) markedly reduced airway extravasation produced by inhaled antigen (5 mg ml⁻¹). Zaprinast (1–10 mg ml⁻¹) was also effective against airway microvascular leakage produced by aerosol PAF or antigen while milrinone (10 mg ml⁻¹) had no antiexudative effect. These data support previous suggestions that pharmacological inhibition of PDE IV results in anti-spasmogenic and anti-inflammatory effects in the airways and may be useful in the treatment of asthma.

Key words Sensitized guinea-pig • Cyclic nucleotide phosphodiesterase isoenzymes • Selective PDE inhibitors • Airways hyperreactivity • Airway eosinophil infiltration • Airways microvascular leakage

Introduction

Asthma is a multifaceted condition involving non-specific airways hyperreactivity, inflammatory cell infiltration and airways oedema resulting from plasma extravasation. There is currently interest in the potential use of selective inhibitors of cyclic nucleotide phosphodiesterase (PDEs) in the treatment of asthma (Torphy and Undem 1991; Giembycz and Dent 1992; Dent et al. 1994; Nicholson and Shahid 1994). There are known to be at least five distinct families of PDE isoenzymes, PDEs I–V, which differ in their substrate specificity and affinity as well as in their regulatory properties and tissue distribution (Beavo and Reifsnyder 1990). The presence of all these families of PDE isoenzymes has been demonstrated in human isolated bronchus (de Boer et al. 1992; Cortijo et al. 1993).

Numerous reports have appeared recently covering different aspects of the pulmonary antiinflammatory effects of isoenzyme-selective PDE inhibitors in guinea-pig models (Howell et al. 1993; Raeburn and Karlsson 1993; Underwood et al. 1993, 1994; Raeburn et al. 1994; Lagente et al. 1994, 1995; Banner and Page 1995). In the present investigation we have examined systematically the effects of three selective PDE inhibitors, milrinone (PDE III), rolipram (PDE IV) and zaprinast (PDE V), on the bronchoconstriction produced by antigen and histamine, the airway hyperreactivity and microvascular leakage after aerosol exposure to platelet-activating factor (PAF) and antigen and...
the antigen-induced eosinophil infiltration in guinea-pig lung. Part of this work has been presented to the British Pharmacological Society (Cortijo et al. 1994).

Materials and methods

Animal preparation and assessment of the anti-spasmodenic responses of selective PDE inhibitors. Male, tricoloured guinea-pigs (300–500 g) were used in this study. Animals were anaesthetised with urethane (1.5–2 g kg⁻¹, i.p.). The jugular vein was cannulated for intravenous drug administration. Animals were mechanically ventilated with room air by means of a Harvard pump at a rate of 60 breaths min⁻¹ with a stroke volume of 1 ml 100 g⁻¹ body weight. Pulmonary inflation pressure was recorded at the side arm of the tracheal cannula by means of a pressure transducer. Airway reactivity was determined from dose response curves to inhaled histamine. After a 10-min stabilization period, five successive administrations of histamine aerosol (0.01, 0.03, 0.1, 0.3, and 1 mg ml⁻¹), generated via DeVilbiss Pulmosonic ultrasonic nebuliser (Somerset, PA, USA) were given for 30 s each at 10 min intervals. The bronchopulmonary response was expressed as the percentage change from baseline. The histamine dose response curve was obtained 30 min after inhaled milrinone, rolipram or zaprinast (each at 10 mg ml⁻¹, 60 s; ≥700 µg, see below). Animals pretreated with the corresponding drug vehicles served as controls. The output from the nebuliser for 3 ml of saline in each chamber and with airflow of 0.3 l min⁻¹ was 70.0±4.3 µl min⁻¹ (n=6). This value is consistent with that reported by Sakamoto et al. (1993) and was used to calculate the inhaled dose of PDE inhibitors. In a separate group of experiments histamine (5 µg kg⁻¹, i.v.) was administered 30 min or 24 h after rolipram (10 mg kg⁻¹, i.p.) to test for residual inhibitory activity of this compound against histamine-induced bronchoconstriction.

Assessment of the effects of selective PDE inhibitors on PAF-induced airways hyperreactivity. Guinea-pigs were exposed for 5 min to an aerosol of PAF (500 µg ml⁻¹) or vehicle [0.25% bovine serum albumin (BSA) in 0.9% sterile saline] as previously outlined by Underwood et al. (1992). The aerosols were generated by a DeVilbiss ultrasonic nebuliser and delivered into a 4-l exposure chamber. Some 24 h after exposure to the aerosol, guinea-pigs were anaesthetised and prepared as above. Airways reactivity was assessed by constructing a dose response curve to histamine (2–50 µg kg⁻¹, i.v.). Five groups of animals were tested in this part of the study. Negative control group consisted of untreated animals exposed to PAF vehicle (BSA 0.25% in saline) aerosol. Positive control group consisted of animals exposed to PAF aerosol after receiving drug vehicles. The treated groups were the animals exposed to PAF and treated with milrinone, rolipram or zaprinast (each at 10 mg kg⁻¹, i.p.) 30 min before PAF challenge.

Assessment of the effects of selective PDE inhibitors on antigen-induced bronchoconstriction, airway hyperreactivity and eosinophil infiltration. Guinea-pigs were actively sensitized as reported by Hui et al. (1991). In brief, animals received two intraperitoneal injections of 0.5 ml of sterile 0.9% (w/v) sodium chloride in distilled water (saline) containing 20 µg ovalbumin (OA) and 100 mg Al(OH)₃, given 24 h apart. Experiments were carried out 28–31 days after sensitization. A group of experiments was addressed to the evaluation of the effects of selective PDE inhibitors on antigen-induced bronchoconstriction. Sensitized guinea-pigs were anaesthetised and instrumented as indicated above. After a 10-min stabilization, the animals received inhaled milrinone (10 mg ml⁻¹, 60 s; ≥700 µg), rolipram (0.01, 0.1 or 10 mg ml⁻¹, 60 s; ≥0.7, 7, 70 or 700 µg), or zaprinast (1 or 10 mg ml⁻¹, 60 s; ≥70 or 700 µg) and 30 min later were challenged with inhaled antigen (5 mg ml⁻¹, 30 s). Control animals were equally treated but received inhaled drug vehicles instead of drug treatments. A separate group of sensitized, conscious guinea-pigs were exposed to antigen aerosol as described by Farmer et al. (1992). Animals were placed in a 4-l exposure chamber connected to the output of a DeVilbiss ultrasonic nebuliser. The nebuliser chamber was filled with an OA (0.1% in saline) or saline solution. Nebuliser output was approximately 8–10 ml h⁻¹. The duration of the antigen challenge was 60 min. Some 24 h after exposure to the aerosol, airways reactivity was determined (histamine 2–50 µg kg⁻¹, i.v.) and the animals subsequently killed by an overdose of urethane. The lungs were lavaged with six aliquots of 10 ml saline with heparin 10 IU ml⁻¹. Total fluid recovery exceeded 85%. Cell suspensions were concentrated by low speed centrifugation and the cell pellet resuspended. Total cell counts were made in a haemocytometer. Differential counts were made from cytospin preparations stained with May-Grünwald-Giemsa. Five groups of animals were tested in this part of the study. The negative control group consisted of sensitized animals exposed to aerosol saline, the positive control group of sensitized animals subsequently exposed to inhaled antigen and treated with milrinone, rolipram or zaprinast (each at 10 mg kg⁻¹, i.p.) 30 min before antigen challenge.

Assessment of the effects of selective PDE inhibitors on microvascular leakage after PAF or antigen challenge. Preparation of animals and experimental protocols were derived from Tokuyama et al. (1991) for PAF experiments and from Hui et al. (1991) for antigen experiments. Briefly, the animals were anaesthetised and instrumented as mentioned above. Unsensitized animals were used in experiments in which PAF was the stimulus for extravasation. After a 10-min stabilization, the animals were given milrinone (10 mg ml⁻¹, 60 s; ≥700 µg), rolipram (0.01, 0.1, 1, 3 or 10 mg ml⁻¹, 60 s; ≥0.7, 7, 70, 210 or 700 µg) or zaprinast (1 or 10 mg ml⁻¹, 60 s; ≥70 or 700 µg) to inhale followed 30 min later by the injection of Evans blue dye (20 mg kg⁻¹, i.v.). PAF (500 µg ml⁻¹, 30 s) was administered 1 min later and after a further 5 min the animals were hyperinflated with twice the tidal volume by manually blocking the outflow of the ventilator and the experiment terminated. Aerosols were generated from a DeVilbiss ultrasonic nebuliser. Animals pretreated with inhaled drug vehicles and then receiving PAF or its vehicle were used as controls. The effect of drugs in animals receiving PAF vehicle was also tested. At the end of the experiments, tissues (lower portion of trachea, main bronchi, proximal and distal intrapulmonary airways), as well as samples from esophagus and bladder, were collected and the Evans blue dye extracted and quantified as previously described (Ortiz et al. 1993).

Sensitized animals were used in experiments where inhaled antigen was the stimulus for extravasation. The sensitization procedure was as outlined above. After 10 min stabilisation, the animals were given milrinone (10 mg ml⁻¹, 60 s; ≥700 µg), rolipram (0.01, 0.1 or 1 mg ml⁻¹, 60 s; ≥0.7, 7 or 70 µg) or zaprinast (1 or 10 mg ml⁻¹, 60 s; ≥70 or 700 µg) to inhale. Drug or vehicle administration was followed 30 min later by the injection of Evans blue dye (20 mg kg⁻¹, i.v.), and 1 min later, antigen was administered (5 mg ml⁻¹, 30 s). Some 5 min after antigen inhalation the animals were hyperinflated with twice the tidal volume by manually blocking the outflow of the ventilator and the animals then disconnected from the ventilator to allow collection of the tissues for measurement of airways microvascular permeability as indicated above. Animals pretreated with aerosol drug vehicles and then receiving antigen or its vehicle were used as controls. The effect of drugs in animals receiving antigen vehicle was also tested. Evans blue dye content was determined as indicated above.

Analysis of data. Data are presented as mean±SEM. Differences between groups were assessed by ANOVA or Kruskal-Wallis and when an overall significance was obtained, a further analysis was performed by Bonferroni’s t-test or Dunn’s test as appropriate. Statistical significance was assumed when P<0.05.

Drugs. BSA (endotoxin-free) Evans blue, formamide, histamine dihydrochloride, methylprednisolone, OA grade V, fatty acid-free). PAF and urethane were all purchased from Sigma (Madrid, Spain). Rolipram and milrinone were gifts from Lab. Almirall (Barcelona, Spain). Zaprinast was a gift from Rhône-Poulenc Rorer (Dagenham, UK). The stock solutions for rolipram and milrinone were prepared in