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Effects of CGS 21680, a selective adenosine A_{2A} receptor agonist, on allergic airways inflammation in the rat

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Abstract

We have investigated the effect of 2(4-((2-carboxymethyl)phenyl)ethylamino)-5′ -*N*-ethylcarboxamidoadenosine (CGS 21680), a potent and selective agonist at adenosine A_{2A} receptors, on pulmonary inflammation induced by allergen challenge in the ovalbumin-sensitised, Brown Norway rat. Aerosol administration of ovalbumin (5 mg ml^{-1} for 60 min; calculated dose 0.4 mg kg^{-1}) induced increases in bronchoalveolar lavage fluid leukocyte numbers, protein content and myeloperoxidase and eosinophil peroxidase activities measured 24 h post challenge. CGS 21680 (10 and 100 μg kg^{-1} given intratracheally (i.t.) 30 min before and 3 h after allergen challenge) inhibited dose-dependently all the parameters of inflammation. Qualitatively similar results were obtained with the glucocorticosteroid, budesonide (0.1, 1 and 10 mg kg^{-1} given 3 h prior to ovalbumin challenge). CGS 21680 given i.t. reduced blood pressure in anaesthetised rats at similar doses to those at which anti-inflammatory effects were manifested. Both the anti-inflammatory and hypotensive responses to CGS 21680 were blocked by pretreatment with the selective adenosine A_{2A} receptor antagonist, 4-(2-(7-amino-2-(2-furyl)(1,2,4)triazolo(2,3-a(1,3,5)triazin-5-yl amino)ethyl)phenol (ZM 241385), 3 mg kg^{-1} p.o., 1 h prior to the agonist. Thus, CGS 21680 manifests broad-spectrum anti-inflammatory activity in a model of allergic asthma in the Brown Norway rat through activation of adenosine A_{2A} receptors. The striking similarity to budesonide, a clinically used anti-inflammatory agent, suggests that adenosine A_{2A} receptor agonists may be useful alternatives to glucocorticosteroids in the treatment of asthma.

Keywords: Allergic pulmonary inflammation; Brown Norway rat; Ovalbumin; Adenosine A_{2A} receptor; CGS 21680; ZM 243185

1. Introduction

The purine nucleoside, adenosine, is formed during ischaemia and inflammation and plays a protective role in the development of the inflammatory response through activation of adenosine receptor subtypes (Cronstein, 1994; Fozard and Hannon, 1999; Shanley and Bshesh, 2000; Linden, 2001). Of the four cloned adenosine receptors, it is the A_{2A} subtype that mediates the powerful suppressant effects of adenosine on immuno-inflammatory cells in vitro and the protective effects seen in a number of paradigms of experimental inflammation in vivo (Daval et al., 1996; Sullivan, 1998; Sullivan and Linden, 1998; Linden, 2001).

An effect to suppress inflammatory responses of the airways through adenosine A_{2A} receptor activation has been postulated (Fozard and Hannon, 1999; Marx et al., 2001) but, as yet, there have been no data from respiratory disease models in animals that would support the concept. We report here the effects of CGS 21680, a potent and selective adenosine A_{2A} receptor agonist (Klotz, 2000; Alexander and Peters, 2001), on the pulmonary inflammation induced by allergen challenge in an animal model of allergic asthma, the ovalbumin-sensitised Brown Norway rat (Chung, 1997).

2. Methods

2.1. Animals

Male Brown Norway rats weighing 200–300 g were supplied by Biological Research Laboratories (Füllinsdorf, Switzerland). They were kept at an ambient temperature of 22 ± 2 °C under a 12-h normal phase light–dark cycle and fed on NAFAG® pellets supplied by Nahr und Futtermittel, Gossau, Switzerland. Drinking water was freely available. All experiments were carried out with the approval of the Veterinary Authority of the City of Basel (Kantonales Veterinaeramt, Basel-Stadt).

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2.2. Sensitisation procedure

Ovalbumin (20 μg ml^{-1}) was mixed (30 min on ice) in a blender (Polytron, Kinematica) with aluminium hydroxide (20 mg ml^{-1}) and injected s.c. (0.5 ml per animal). The injection was repeated 14 and 21 days later. Sensitised animals were used in experiments between days 28 and 35.

2.3. Bronchoalveolar lavage fluid collection and analysis

Animals were killed with sodium pentobarbital (250 mg kg^{-1} i.p.). The lungs were lavaged using three aliquots (4 ml) of Hanks' balanced salt solution ((HBSS, × 10) 100 ml; ethylenediaminetetraacetic acid (EDTA), 100 mM, 100 ml; 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) 1 M, 10 ml; dd.H_2O 790 ml); the recovered solution was pooled (mean recovery 11.3 ± 0.1 ml, $n = 55$) and the total volume of recovered fluid was adjusted to 12 ml by addition of HBSS.

The methods for the determination of total leukocyte numbers and differential cell counts, eosinophil peroxidase and myeloperoxidase activities and protein concentration in the bronchoalveolar lavage fluid have been described in detail recently (Beckmann et al., 2001). In brief, leukocyte numbers and differential cell counts were obtained using an automatic cell analysing system (Cobas Helios 5Diff, Hoffmann-La Roche, Axon Lab, Switzerland). Myeloperoxidase activity was measured in a photometric assay based on the oxidation of *O*-dianiside dihydrochloride by myeloperoxidase in the presence of hydrogen peroxide. Eosinophil peroxidase activity was measured in a photometric assay based on the oxidation of *O*-phenylenediamine by eosinophil peroxidase in the presence of hydrogen peroxide. Protein concentrations were measured in a photometric assay based

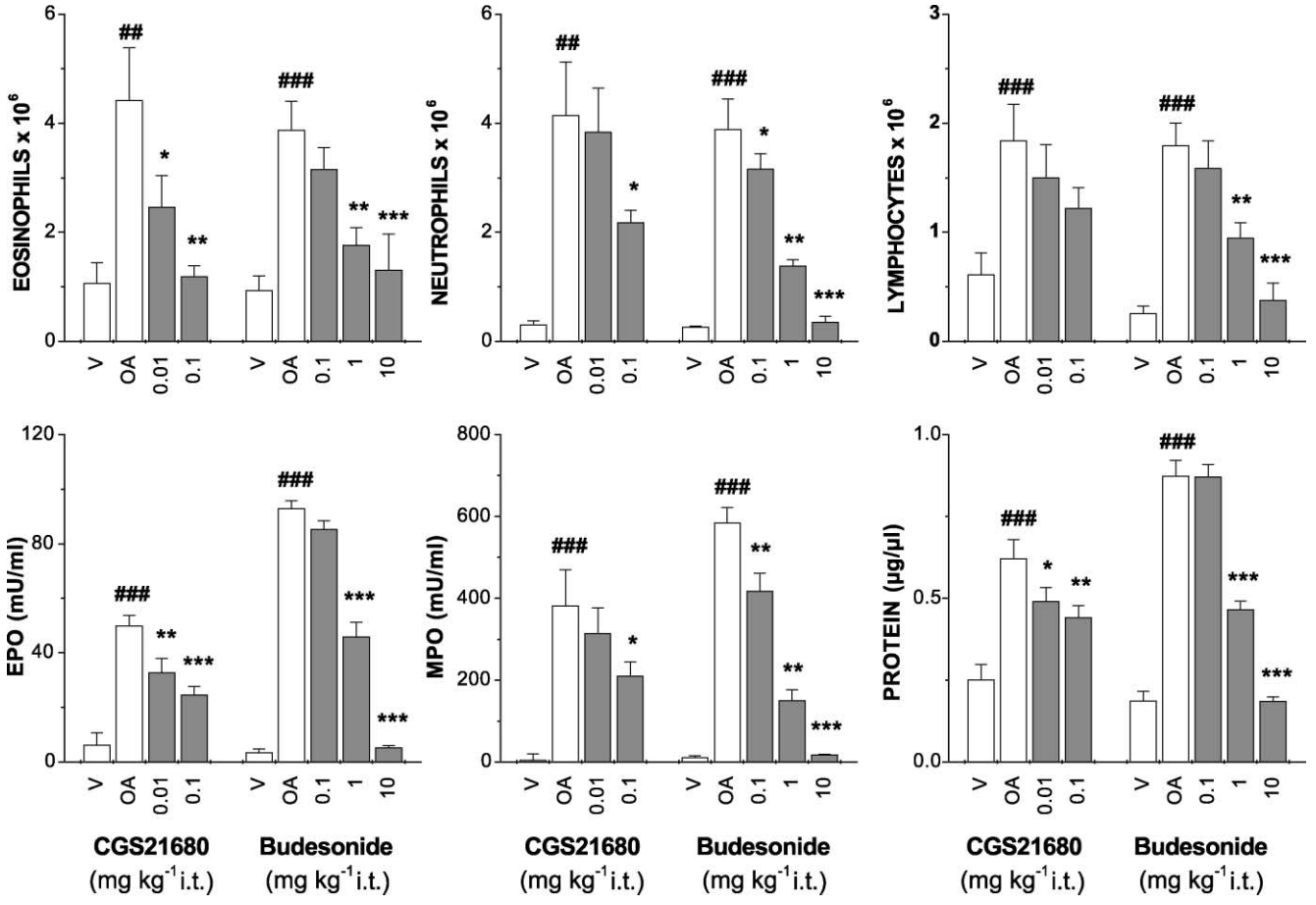

Fig. 1. Inflammatory cell infiltration and activation in the lungs of Brown Norway rats induced by ovalbumin challenge: effect of pretreatment with CGS 21680 or budesonide. The data (means ± S.E.M.; $n = 6–12$) show the effects of CGS 21680 or budesonide on the changes in the numbers of eosinophils, neutrophils, lymphocytes, the eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activities and the protein concentration measured in bronchoalveolar lavage fluid of actively sensitised Brown Norway rats 24 h following aerosol challenge with ovalbumin (OA; 5 mg ml^{-1} for 60 min). CGS 21680 was given intratracheally (i.t.) at the doses indicated 30 min before and 3 h after the ovalbumin challenge. Budesonide was given i.t. at the doses indicated 3 h prior to the ovalbumin challenge. ##$P < 0.01$, ###$P < 0.001$ indicate significant difference from the animals challenged with vehicle (V). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ indicate significant difference from animals challenged with ovalbumin without CGS 21680 or budesonide treatment.

on the reaction of protein with an alkaline copper tartrate solution and Folin reagent.

2.4. Measurement of lung function and cardiovascular effects in anaesthetised animals

Animals were anaesthetised with sodium pentothal (70 mg kg^{-1} i.p.) and a tracheotomy performed. A heparinised polyethylene catheter was inserted into the left carotid artery for recording mean arterial blood pressure. To suppress spontaneous respiration, animals were given an intramuscular injection of vecuronium bromide (12 mg kg^{-1}). No experiment lasted longer than 90 min, during which time surgical anaesthesia was maintained without the need for supplementary anaesthesia. Body temperature was maintained at 37 °C with a heated pad controlled by a rectal thermistor.

Animals were ventilated (7 ml kg^{-1}, 1 Hz) via the tracheal cannula with a mixture of air and oxygen (50:50, v v^{-1}). Ventilation was monitored at the trachea by a pneumotachograph (Fleisch 0000, Zabona, Switzerland) in line with the respiratory pump and connected to a differential pressure transducer (MP 4514871, Validyne, USA). Coincident pressure changes within the thorax were measured via an intrathoracic cannula, using a differential pressure transducer (MP 4524, Validyne). From measurements of airflow and transpulmonary pressure, airway resistance (R_L, cm H_2O l^{-1} s^{-1}) was calculated after each respiratory cycle by use of a digital electronic pulmonary monitoring system (PMS, Mumed, London, UK). Mean arterial blood pressure (and heart rate by derivation) was recorded from the carotid artery by means of a pressure transducer (P23Dd, Gould, USA).

2.5. Experimental protocols

2.5.1. The pulmonary inflammatory response to allergen

The pulmonary inflammatory response induced by allergen was quantified as the change in leukocyte numbers, myeloperoxidase and eosinophil peroxidase activities and protein concentration in the bronchoalveolar lavage fluid. Animals were exposed using a nose only exposure system to

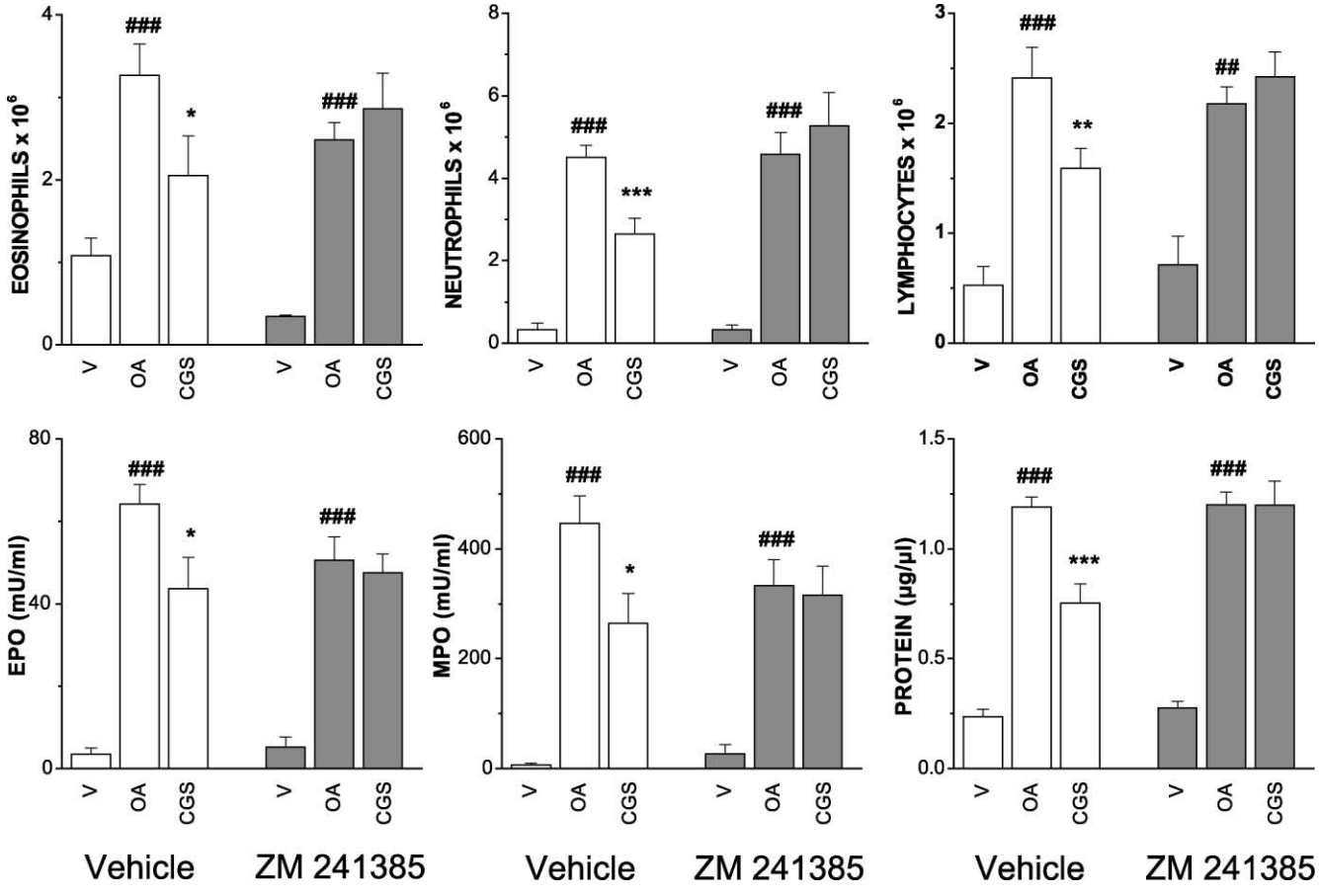

Fig. 2. Suppression by CGS 21680 of Inflammatory cell infiltration and activation in the lungs of Brown Norway rats induced by ovalbumin challenge: effect of ZM 241385. The data (means ± S.E.M.; $n = 6–12$) show the effects of CGS 21680 (CGS; 10 µg kg^{-1} given intratracheally (i.t.) 30 min before and 3 h after ovalbumin challenge) on the changes in the numbers of eosinophils, neutrophils, lymphocytes, the eosinophil peroxidase (EPO) and myeloperoxidase (MPO) activities and the protein concentration measured in bronchoalveolar lavage fluid of actively sensitised Brown Norway rats 24 h following aerosol challenge with ovalbumin (OA; 5 mg ml^{-1} for 60 min) and the effects of pretreatment with ZM 241385 (3 mg kg^{-1} given p.o. 1 h prior to CGS 21680). ##$P < 0.01$, ###$P < 0.001$ indicate significant difference from the animals challenged with vehicle (V). *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ indicate significant difference from animals challenged with ovalbumin without CGS 21680 treatment.

an aerosol of ovalbumin (5 mg ml^{-1} for 60 min) generated by a Cirrhus nebuliser (DHD Medical Products, Canastota, USA). The dose administered was approximately 0.4 mg kg^{-1}, calculated using the formula:

(f × minute volume [l] × treatment time [min]

×concentration of compound in aerosol [mg l^{-1}]

/body weight [kg]).

The factor f refers to the assumed percentage retention of inhaled material in the lung, which was taken as 0.3.

Animals were killed for bronchoalveolar lavage fluid analysis 24 h after challenge with ovalbumin. CGS 21680 (10 or 100 μg kg^{-1}) or saline (0.2 ml) was given intratracheally (i.t.) 30 min prior to and 3 h after ovalbumin challenge. In experiments with ZM 241385, the antagonist was given at a dose of 3 mg kg^{-1} p.o., 1 h prior to the first dose of CGS 21680. Budesonide was administered i.t. at doses of 0.1, 1 or 10 mg kg^{-1}, 3 h prior to allergen challenge.

2.5.2. Effects on lung function and cardiovascular effects in the anaesthetised rat

CGS 21680 (1, 10 and 100 μg kg^{-1}; cumulative dosing) or vehicle (0.2 ml) was administered i.t. to anaesthetised rats at 15-min intervals, and the maximum changes in airways resistance, blood pressure and heart rate were recorded.

2.6. Materials

Aluminium hydroxide and EDTA were from Merck, Germany. HBSS and HEPES were obtained from Gibco BRL, UK. Pentothal sodium was obtained from Abbott, Switzerland. Ovalbumin was obtained from Fluka, Switzerland. 2(4-((2-carboxymethyl)phenyl)ethylamino)-5′ -*N*-ethylcarboxamidoadenosine (CGS 21680) and 4-(2-(7-amino-2-(2-furyl)(1,2,4)triazolo(2,3-a(1,3,5)triazin-5-yl amino)ethyl)phenol (ZM 241385) were obtained from Sigma, Switzerland. Budesonide was obtained from Sicor, Italy. Ovalbumin was dissolved in 0.9% w v^{-1} NaCl. CGS 21680 and budesonide were dissolved in 10% dimethylsulphoxide (DMSO) and diluted in 0.9% w v^{-1} NaCl for i.t. administration (0.2 ml). ZM 241385 was dissolved in 10% DMSO and diluted in Neoral™ placebo (Novartis Pharma, Swizerland) for p.o. administration (0.6 ml).

2.7. Data analysis

All data are presented as means ± S.E.M. Statistical analysis was performed on raw data by means of Student's t test for paired data or analysis of variance with post hoc pairwise multiple comparison procedures, using SigmaStat for Windows, version 2.03. A P value of <0.05 was considered significant.

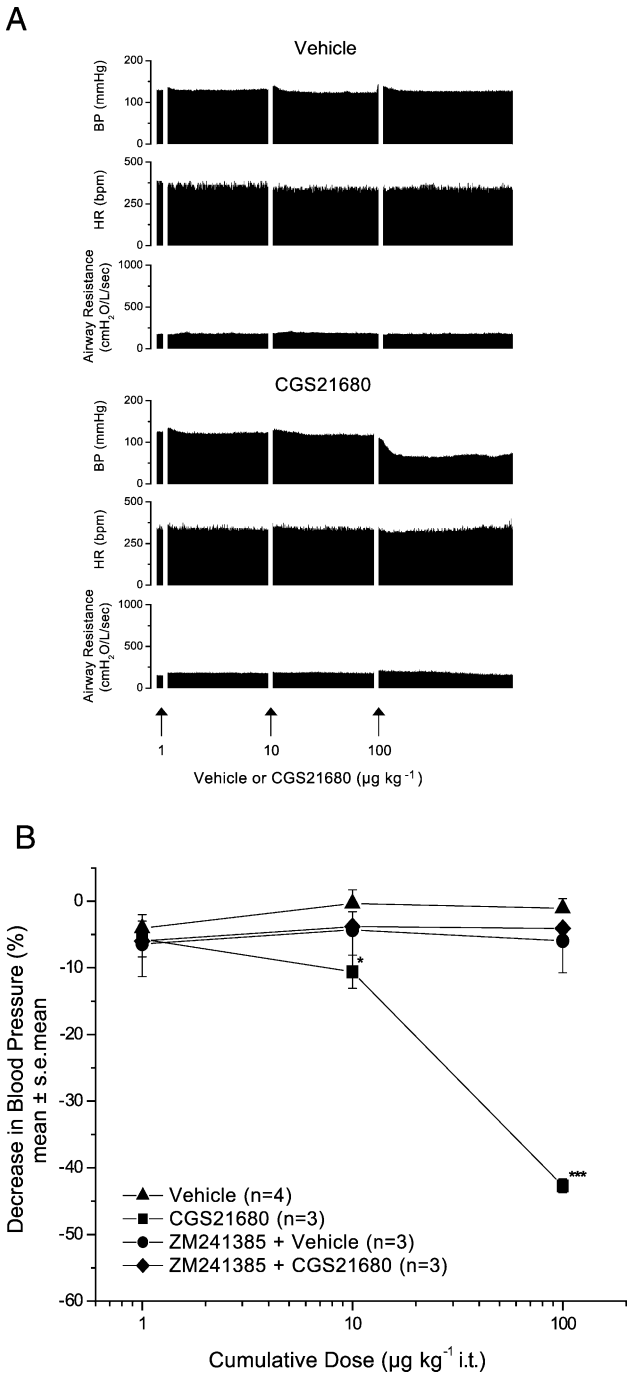

Fig. 3. Cardiovascular effects and effects on airway resistance of CGS 21680 given intratracheally to anaesthetised Brown Norway rats: effect of ZM 241385. The data show the effects of CGS 21680 (1, 10 and 100 μg kg^{-1}) given i.t. on mean arterial blood pressure (BP) and heart rate (HR) of anaesthetised Brown Norway rats. (A) Representative sample experimental records showing the effect of vehicle (upper records) and CGS 21680 (lower records). (B) Mean changes in blood pressure after CGS 21680 following pretreatment with ZM 241385 (3 mg kg^{-1} p.o. or vehicle 1 h prior to CGS 21680). Values represent means (± S.E.M) of the number of individual experiments shown in parentheses. $*P<0.05$, $***P<0.001$.

3. Results

3.1. Allergen-induced inflammatory cell infiltration and activation in the lungs of actively sensitised Brown Norway rats: effect of pretreatment with CGS 21680 or budesonide

Challenge with ovalbumin led to an inflammatory response in the airways of sensitised Brown Norway rats when assessed by changes in the bronchoalveolar lavage fluid leukocyte numbers, myeloperoxidase and eosinophil peroxidase activities and protein concentration measured 24 h after challenge (Fig. 1). Following pretreatment with CGS 21680 at doses of 10 and 100 μg kg^{-1}, given i.t. 30 min prior to and 3 h after allergen exposure, all parameters of inflammation from the bronchoalveolar lavage fluid of challenged animals were significantly reduced (Fig. 1). Qualitatively similar results were obtained with budesonide (0.1, 1 and 10 mg kg^{-1}) given i.t., 3 h prior to allergen (Fig. 1). Pretreatment with ZM 241385 3 mg kg^{-1} p.o., 1 h before CGS 21680 (10 μg kg^{-1}) had no effects per se on the response to allergen but fully blocked the suppression of the inflammatory parameters induced by CGS 21680 (Fig. 2).

In anaesthetised rats set up for recording airway resistance, blood pressure and heart rate, CGS 21680 given i.t. at 10 and 100 μg kg^{-1} induced dose-related falls in blood pressure, which were significantly different from vehicle-treated control values (Fig. 3). There were no changes in heart rate or airway resistance following either vehicle or CGS 21680 (Fig. 3A). Pretreatment with ZM 241385, 3 mg kg^{-1} p.o., 1 h before CGS 21680, fully blocked the fall in blood pressure induced by CGS 21680 (Fig. 3B).

4. Discussion

Our results demonstrate potent inhibition by CGS 21680 of the pulmonary inflammatory response to allergen in actively sensitized Brown Norway rats, manifested as suppression of leukocyte influx into bronchoalveolar lavage fluid and a decrease in the myeloperoxidase and eosinophil peroxidase activities and protein concentrations. These effects were blocked by pretreatment with ZM 241385, the selective adenosine A_{2A} receptor antagonist (Poucher et al., 1995; Keddie et al., 1996), confirming their mediation by the adenosine A_{2A} receptor subtype. The result adds allergic inflammation of the respiratory tract of the Brown Norway rat to the list of organs and tissues in which the anti-inflammatory effects of adenosine A_{2A} receptor activation has been demonstrated (see Daval et al., 1996; Sullivan, 1998; Sullivan and Linden, 1998; Linden, 2001).

A_{2A} receptors are present on the major immuno-inflammatory cells, which have been implicated in inflammatory disease of the airways (Fozard and Hannon, 1999; Gessi et al., 2000; Marx et al., 2001). Activation of these sites powerfully suppresses neutrophil adherence to the endothelium (Cronstein et al., 1992), upregulation of integrins (Wollner et al., 1993) and activation/degranulation of neutrophils (Fredholm et al., 1996; Bouma et al., 1997; Hannon et al., 1998). Similarly, adenosine A_{2A} receptor activation results in suppression of FcεRI-induced degranulation of human mast cells (Suzuki et al., 1998) and the secretion of interleukin-12, a pro-inflammatory cytokine, from human monocytes (Bouma et al., 1994). Activation of T-lymphocytes, which plays a key role in the recruitment of leukocytes to the lung in the Brown Norway rat model of allergic pulmonary inflammation (Chung, 1997) and in clinical asthma (Kemeny and O'Connor, 2000), is suppressed by adenosine A_{2A} receptor activation (Huang et al., 1997; Koshiba et al., 1997, 1999). Thus, there are a multitude of mechanisms which, alone or in combination, could be the basis of the effects to suppress ovalbumin-induced pulmonary inflammation in the present studies.

The broad spectrum of the anti-inflammatory effects of CGS 21680 is strikingly similar to that seen with budesonide, a glucocorticosteroid used clinically in the treatment of asthma (Barnes, 1997). On this basis, adenosine A_{2A} receptor agonists may provide an alternative strategy for the treatment of inflammatory diseases of the airways such as asthma. In particular, since the mechanisms of action of an adenosine A_{2A} receptor agonist and a glucocorticosteroid are fundamentally different, such compounds may provide benefit in severe asthma poorly controlled by steroids. However, adenosine A_{2A} receptors have a wide tissue distribution (Ongini and Fredholm, 1996; Ralevic and Burnstock, 1998), and mediate inhibition of platelet activation, vasorelaxation and a variety of effects on the central nervous system (Ledent et al., 1997; Ralevic and Burnstock, 1998). A selective action in the lung would therefore be mandatory to avoid unacceptable side effects. In this context, CGS 21680 would not represent a suitable candidate for development since, despite being given locally to the airways, falls in blood pressure occur at the same doses, which are needed for anti-inflammatory activity.

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