Effects of a potassium channel opener (SDZ PCO 400) on guinea-pig and human pulmonary airways

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1 SDZ PCO 400 evoked dose-related relaxation of isolated airway smooth muscle. For human bronchus precontracted by endogenous tone or addition of carbachol (10^{-5} M), IC_{50} values were $1.74 \mu\text{M}$ and $1.82 \mu\text{M}$ respectively. With guinea-pig trachea contracted by endogenous tone, a comparable IC_{50} ($1.79 \mu\text{M}$) was observed, but no IC_{50} ($<100 \mu\text{M}$) could be determined following contraction by carbachol (10^{-6} M).

2 Airway obstruction induced by intravenous bombesin in the anaesthetized ventilated guinea-pig was diminished by intravenous injection of SDZ PCO 400 (ID_{50} $54 \mu\text{g kg}^{-1}$) or by introduction into the duodenum (ID_{50} 1.0 mg kg^{-1}). Inhalation of nebulized SDZ PCO 400 (0.1 mg kg^{-1}) diminished airway obstruction due to intravenous injection of histamine (3.2 – $5.6 \mu\text{g kg}^{-1}$) for up to 20 min.

3 Increased bronchoconstrictor responses to bombesin (180 – 240 ng kg^{-1}) following intravenous infusion of platelet activating factor (PAF) or ($\pm$)-isoprenaline, or to histamine (1.0 – $3.2 \mu\text{g kg}^{-1}$) following intravenous injections of immune complexes, were suppressed following concomitant intravenous infusion of SDZ PCO 400 (ID_{50} $0.3 \text{ mg kg}^{-1} \text{ h}^{-1}$, $1.0 \text{ mg kg}^{-1} \text{ h}^{-1}$ and $0.1 \text{ mg kg}^{-1} \text{ h}^{-1}$ respectively).

4 Intravenous injection of SDZ PCO 400 (0.1 mg kg^{-1}) effected transient (<10 min) inhibition of histamine-induced bronchospasm, yet diminished, for prolonged periods [up to 40 min] the enhanced bronchoconstrictor responses to histamine that followed intravenous injections of immune complexes. The capacity of SDZ PCO 400 to resolve such established airway hyperreactivity was prevented by prior intraduodenal instillation of a potassium channel antagonist, glibenclamide (30 mg kg^{-1}).

5 In sensitized guinea-pigs, SDZ PCO 400 inhaled as a dry powder (5.7 mg kg^{-1}) suppressed development of allergic airway hyperreactivity to histamine (1.8 – $3.2 \mu\text{g kg}^{-1}$, i.v.), but failed to diminish accumulation of eosinophils or other inflammatory cells within the airway lumen 24 h after inhalation of ovalbumin.

6 Preincubation (30 min) of isolated sensitized trachea of guinea-pig with SDZ PCO 400 (10^{-5} – 10^{-4} M) did not influence contractile responses to ovalbumin. However in anaesthetized sensitized guinea-pigs, insufflation of SDZ PCO 400 (1.25 mg) as a powder substantially diminished airway obstruction that followed inhalation of ovalbumin. This effect was prevented by prior vagal section.

7 It is concluded that SDZ PCO 400 reduces airway obstruction not only through direct actions on airway smooth muscle but also by impairing the expression of airway hyperreactivity, without directly influencing inflammatory events in the airways.

Keywords: SDZ PCO 400; potassium channel opener; guinea-pig airways; human airways; hyperreactivity; eosinophils; antigen; vagus nerve; asthma

Introduction

SDZ PCO 400 ((-)-(3S,4R)-3,4-dihydro-3-hydroxy-2,2-dimethyl-4-(3-oxo-cyclopent-1-enyloxy)-2H-1-benzopyran-6-carbonitrile) opens channels in the sarcolemma of smooth muscle that are selective for potassium ions (Quast *et al.*, 1990). Drugs which open potassium channels may thereby relax smooth muscle, including airway smooth muscle, raising the prospect of potential anti-asthma efficacy. In this context, cromakalim has been shown to diminish histamine-induced airway obstruction in normal subjects (Baird *et al.*, 1988) and to provide symptomatic relief in nocturnal asthma (Owen *et al.*, 1990). In order to ascertain which pharmacological properties of SDZ PCO 400 might contribute to potential anti-asthma efficacy, we have examined the relaxant effects of this compound on isolated airway smooth muscle of guinea-pig and man as well as determining the capacity of SDZ PCO 400 to prevent or reverse established airways

obstruction in the anaesthetized guinea-pig. In addition, we have investigated the effects of SDZ PCO 400 upon both the development and expression of airway hyperreactivity and determined whether altered kinetics of inflammatory cell migration might account for inhibition. A preliminary account of part of this work has been presented to the British Pharmacological Society (Chapman *et al.*, 1991a) and at an IUPHAR satellite meeting (Chapman *et al.*, 1991b).

Methods

Animals

Male guinea-pigs (Dunkin-Hartley circa 350–550 g) were used throughout. Food and water were available *ad libitum*.

Sensitization to ovalbumin

Guinea-pigs (350–400 g) were pretreated with cyclophosphamide (100 mg kg^{-1} , i.p.); 24 h later, ovalbumin ($10 \mu\text{g ml}^{-1}$) was added to aluminium hydroxide (10 mg ml^{-1}) and,

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after mixing with a magnetic stirrer (1 h, room temp), injected intraperitoneally (1.0 ml) coincidentally with *Bordetella pertussis* vaccine (0.25 ml). Injection of ovalbumin, together with adjuvants, was repeated at 21 and 42 days (Andersson, 1980). Hypersensitivity was detected by intracutaneous injection of ovalbumin (1 µg in 0.1 ml) and only animals with positive (>10 mm diameter) skin reactions were used for experimental studies.

Antiserum to bovine gamma globulin

A solution of bovine gamma globulin in normal saline (1.0 mg ml⁻¹) and an equal volume of Freund's complete adjuvant were homogenized for 3 min in a high speed blender to form an oil-in-water emulsion. This mixture was injected intraperitoneally (1.0 ml per animal) and the procedure repeated two weeks later. After a further week, guinea-pigs were anaesthetized with ether and bled by cardiac puncture. Clotted blood was stored overnight at 4°C; after clot retraction, serum was separated for storage (-20°C) as aliquots (1 ml).

Relaxation of airway smooth muscle in vitro

Guinea-pigs (350–500 g) were killed by an intraperitoneal injection of sodium pentobarbitone (100 mg kg⁻¹). A section of trachea (2–3 cm) was excised and transected at alternate cartilage plates to give rings of tissue 3–5 mm in depth. The proximal and distal rings were discarded. Individual rings of tissue were mounted on stainless steel supports in a tissue bath (10 ml) under a load of 1 g. Tissue was bathed in Krebs solution at 37°C, aerated with a mixture of oxygen and carbon dioxide (95:5 v/v) and isometric tension (Strain gauge UC3, Gould and Statham, USA) was recorded on a chart recorder (W. & W. Electronic Ltd., Switzerland). SDZ PCO 400 was dissolved in ethanol (100 ml) and polyethyleneglycol (3 ml) and further diluted in saline (0.9%) such that the final concentration of solvent in the organ bath did not exceed 1% at the highest concentration of SDZ PCO 400 used. This concentration of solvent was without effect upon either endogenous tone or tissue precontracted with carbachol (data not shown). Increasing quantities of SDZ PCO 400 were added at intervals (10 min) to define a cumulative concentration-effect curve upon tissue contracted either by endogenous tone (tension generated = 381 ± 50 mg, *n* = 10) or by addition of a spasmogen (carbachol 10⁻⁶ M; EC₈₀, tension generated = 1088 ± 137 mg, *n* = 10). Tissue from sensitized animals was contracted by addition of ovalbumin (100 µg ml⁻¹) with, or without, prior (30 min) incubation with SDZ PCO 400 (10⁻⁴ or 10⁻⁵ M).

Macroscopically normal rings of human bronchus (circa 5 mm diameter), dissected from lung that had been resected for carcinoma, were used immediately, or were immersed in foetal calf serum containing DMSO (1.8 M), frozen (-70°C) and stored in liquid nitrogen (-190°C) (Mueller-Schweinitzer, 1988). Freshly collected, or thawed (30 min room temperature, 3 min at 37°C), bronchial rings were mounted in a tissue bath as described for guinea-pig trachea. Such preparations produced tone spontaneously (tension generated = 778 ± 184 mg, *n* = 5) or were precontracted with carbachol (10⁻⁵ M; EC₈₀, tension generated = 702 ± 78 mg, *n* = 10).

Measurement of lung function

Guinea-pigs (circa 500 g) were anaesthetized by intraperitoneal injection of sodium phenobarbitone (100 mg kg⁻¹) supplemented with sodium pentobarbitone (30 mg kg⁻¹) and were then paralysed by intramuscular injection of gallamine (10 mg kg⁻¹). Animals, maintained at 37°C with a heated pad controlled by a rectal thermistor, were ventilated (8 ml kg⁻¹, 1 Hz) via a tracheal cannula with a mixture of air and oxygen (50:50, v/v). Ventilation was monitored at the trachea

by a pneumotachograph (Fleisch 0000, Zabona, Switzerland) 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, USA); blood pressure and heart rate were recorded from the carotid artery with a pressure transducer (P23Dd, Gould, USA).

From measurements of air-flow and transpulmonary pressure, airway resistance (R_L, cmH₂O l⁻¹ s⁻¹) was calculated after each respiratory cycle by use of a digital electronic pulmonary monitoring system (PMS, Mumed, London, U.K.) which displayed blood pressure, intrathoracic pressure and airflow and computed R_L in real time for display on a visual display unit (model AT3, IBM, USA); experimental data were stored electronically and experimental traces or processed data were plotted on a laser printer (LaserJet Series II, Hewlett Packard, USA) as required.

Bronchodilator action

In the anaesthetized ventilated guinea-pig intravenous injection of bombesin (300–600 ng kg⁻¹) caused sustained bronchospasm. When the response was maximal (circa 2 min), SDZ PCO 400 was injected into the jugular vein. Airway resistance was measured at 3 min and reduction has been expressed as a percentile of maximal R_L observed following injection of bombesin.

During an intravenous infusion of bombesin (50–100 ng kg⁻¹ min⁻¹) to induce sustained airway obstruction, SDZ PCO 400 was introduced into the gastric end of the transected duodenum via a 12 gauge metal needle fixed by a purse string ligature. Bronchodilator responses were estimated as the reduction of maximal R_L and expressed as a percentile of maximal R_L prior to introduction of SDZ PCO 400.

SDZ PCO 400 (1 mg ml⁻¹) or vehicle were nebulized for 10 min in a De Villbis nebulizer, (Hospitak, USA) in-line with the inspiratory flow so that aerosols were introduced directly into the trachea of anaesthetized, ventilated animals. Sensitivity of the airways to intravenous injection of histamine (3.2–5.6 µg kg⁻¹) was determined at regular (10 min) intervals and reduction of responses (maximal R_L) have been expressed as a percentile of the pretreatment response to histamine.

Allergic bronchospasm

Either saline or SDZ PCO 400 (1.25 mg powder), were insufflated directly into the trachea of anaesthetized sensitized guinea-pigs (Schiantarelli *et al.*, 1982), with or without prior bilateral vagal section, 15 min prior to inhalation of ovalbumin in saline (1.0 mg ml⁻¹ for 5 min) from a De Villbis nebulizer.

Airway hyperreactivity

Intravenous injections of bombesin (180–240 ng kg⁻¹) were used to establish sensitivity of the airways in anaesthetized ventilated guinea-pigs; 20 min after intravenous infusion of PAF (600 ng kg⁻¹ h⁻¹) administered in a graded fashion: 30 ng kg⁻¹ for 10 min, followed by 120 ng kg⁻¹ for 20 min, followed by 450 ng kg⁻¹ for 30 min) or (±)-isoprenaline as free base (100 µg kg⁻¹ h⁻¹), sensitivity to bombesin was redefined. Airway hyperreactivity has been expressed as the paired difference between the maximal value for R_L to bombesin before and after exposure to PAF. Alternatively, an allergic reaction was initiated by intravenous injections of preformed immune complexes (prepared by adding 30 µg of bovine gamma globulin in 0.05 ml of saline to 0.05 ml of guinea-pig anti-bovine gamma globulin anti-serum, with a titre of at least 1 in 1000) at regular (10 min) intervals for 50 min. Intravenous injections of histamine (1.0–3.2 µg kg⁻¹ at 10 min intervals) were used to define the sensitivity of the

airways prior to, and following exposure to immune complexes. Airway hyperreactivity has been expressed as the paired difference for the maximal value of R_L in response to histamine before, and after, repeated injection of immune complexes. SDZ PCO 400 was administered as an intravenous infusion ($0.1\text{--}1.0\text{ }\mu\text{g kg}^{-1}$) prior to (15 min) and concomitantly with either PAF, ($\pm$)-isoprenaline or immune complex injections. In other experiments, SDZ PCO 400 ($0.1\text{ }\mu\text{g kg}^{-1}$) was injected intravenously immediately following induction of airway hyperreactivity to histamine by successive intravenous injections of immune complexes.

Glibenclamide (30 mg kg^{-1} , bolus) was injected into the duodenum before (10 min) injection of SDZ PCO 400 in normal and hyperreactive animals or in normal animals during intravenous injection of histamine.

In actively sensitized guinea-pigs, SDZ PCO 400 was administered either as a dry powder aerosol by nose only inhalation (RCC Geneva, Switzerland) for 60 min (estimated doses 5.7 mg kg^{-1}) in conscious animals. Animals were prepared for measurement of pulmonary function 24 h later and sensitivity to histamine ($1.8\text{--}3.2\text{ }\mu\text{g kg}^{-1}$, i.v.) was determined.

Eosinophilia

Sensitized guinea-pigs were exposed for 1 h to an aerosol of ovalbumin (10 ml, 0.1% w/v) within 1 h of exposure to an aerosol of SDZ PCO 400 (estimated dose 5.7 mg kg^{-1}).

Animals were killed with pentobarbitone (100 mg kg^{-1} , i.p.) and the lungs washed with six aliquots (10 ml) of HBSS (total recovery $57.6 \pm 0.4\text{ ml}$, $n = 10$). Cell suspensions were concentrated by centrifugation (200 g for 10 min) and resuspended in HBSS (1 ml). Total cell counts were made in a haemocytometer. Differential cell counts were made from smears fixed in methanol (100%) and stained with Leishman's stain in order to differentiate cell types. A total of at least 500 cells per smear were counted at 1000 fold magnification.

Materials

The following were used: concentrated (10x) Hank's balanced salt solution (HBSS) and phosphate buffered saline (PBS), (Gibco, U.K.), bovine serum albumin (BSA), histamine dichloride, ovalbumin (Fluka, Switzerland), sodium pentobarbitone, sodium phenobarbitone (Siegfried, Switzerland), bombesin (Bachem, Switzerland), bovine gamma globulin (Miles Scientific, USA), gallamine (Davis and Geck, USA), Leishmann's stain, Turk's solution, ethanol, aluminium hydroxide, dimethylsulphoxide (DMSO), (Merck, Germany), cyclophosphamide, ($\pm$)-isoprenaline sulphate, carbachol (Sigma, USA), *Bordetella pertussis* vaccine (Swiss serum institute, Switzerland), Freund's complete adjuvant, (Difco, USA), platelet-activating factor (PAF) (Noviobiochem, Switzerland), SDZ PCO 400 (Sandoz, Switzerland).

Krebs solution (composition mM: NaHCO_3 25.0, NaCl 113, KCl 4.7, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, KH_2PO_4 1.2, CaCl_2 2.5, glucose 11.7) was prepared freshly from analytical grade reagents. PAF was dissolved in absolute ethanol and stored at -18°C as a stock solution (1 mg ml^{-1}). Dilutions of PAF in saline 0.9% containing BSA 0.25% were prepared immediately prior to use.

Statistical comparison

Data are presented as means $\pm$ s.e.mean and Student's *t* test was used for comparison between treatments. Estimates of IC_{50} or ID_{50} together with confidence limits were computed by Sigfit (Sandoz Pharma Ltd., Switzerland).

Results

Relaxation of isolated airway smooth muscle

SDZ PCO 400 relaxed guinea-pig isolated trachea, when tension had developed spontaneously, with an IC_{50} value of 1.79 ($1.66\text{--}2.88$) μM ($n = 10$). When tracheal tone was induced by addition of carbachol (10^{-6}M), SDZ PCO 400 was not an effective relaxant ($\text{IC}_{50} > 100\text{ }\mu\text{M}$, $n = 10$). SDZ PCO 400 relaxed human isolated bronchus, whether tension was generated spontaneously, or by addition of carbachol (10^{-5}M) with IC_{50} values of 1.74 ($1.45\text{--}2.08$) ($n = 5$) and 1.82 ($1.3\text{--}2.55$) μM , respectively ($n = 10$).

Effect upon airway obstruction in vivo

In ventilated anaesthetized guinea-pigs, acute intravenous injection of SDZ PCO 400 ($0.01\text{--}1.0\text{ mg kg}^{-1}$) caused dose-related (ID_{50} $54\text{ }\mu\text{g kg}^{-1}$, $n = 5$ per dose) reduction of the airway obstruction which resulted from intravenous injection of bombesin (Figure 1). Introduction of SDZ PCO 400 ($1.0\text{--}3.2\text{ mg kg}^{-1}$) into the duodenum of anaesthetized ventilated guinea-pigs also effected a dose-related reduction of bombesin-induced bronchospasm with an ID_{50} value of 1.0 mg kg^{-1} ($n = 5$ per dose), 30 min after administration (Figure 1). Prior (10 min) inhalation of SDZ PCO 400 (1.0 mg ml^{-1}) as an aerosol (estimated dose 0.1 mg kg^{-1}) impaired the development of airway obstruction in response to intravenous injection of histamine ($3.2\text{--}5.6\text{ }\mu\text{g kg}^{-1}$) (Figure 1). Before inhalation of SDZ PCO 400, R_L ($\text{cmH}_2\text{O l}^{-1}\text{ s}^{-1}$) following intravenous injection of histamine was 800 ± 17 ; immediately after inhalation of SDZ PCO 400, maximal R_L achieved was 61 ± 11 and recovered progressively (R_L 85 ± 14 at 10 min and 600 ± 32 at 20 min, $n = 10$) (Figure 1).

Inhibition of airway hyperreactivity

Intravenous injection of SDZ PCO 400 ($0.1\text{--}1.0\text{ mg kg}^{-1}$) prior to and during intravenous infusion of PAF, ($\pm$)-isoprenaline or injections immune complexes caused dose-related suppression of airway hyperreactivity, with ID_{50} values of 0.3 mg kg^{-1} , 1.0 mg kg^{-1} and 0.1 mg kg^{-1} respectively ($n = 10$ per dose) (Figure 2). In addition, SDZ PCO 400 (0.1 mg kg^{-1}) was administered after induction of airway hyperreactivity by successive injections of immune complexes ($n = 10$). SDZ PCO 400 strongly suppressed the enhanced airway obstruction to histamine induced by immune complexes, even though SDZ PCO 400 produced only modest ($< R_L$ before, 310 ± 58 , $n = 10$; R_L after 191 ± 86 , $n = 10$; $P = 0.05$), transient (< 10 min) inhibition of responses to intravenous histamine in normal animals at this dosage (Table 1). Significant inhibition (R_L before, 654 ± 94 , $n = 10$; R_L after 218 ± 62 , $n = 5$; $P = < 0.05$) of responses to histamine in hyperreactive guinea-pigs was evident 40 min after injection of SDZ PCO 400. The capacity of SDZ PCO 400 (0.1 mg kg^{-1}) to suppress responses to intravenous injection of histamine in hyperreactive animals was prevented by prior (10 min) intraduodenal injection of glibenclamide (30 mg kg^{-1} , $n = 10$) (Figure 3). No effect of glibenclamide on responses to intravenous histamine ($3.2\text{ }\mu\text{g kg}^{-1}$) in hyperreactive animals was observed.

Effect upon eosinophil accumulation

Sensitized guinea-pigs exposed to ovalbumin by inhalation develop a marked inflammatory cell accumulation in the airway lumen 24 h later, that is accompanied by increased airway reactivity to histamine and prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) (Sanjar *et al.*, 1990b). Inhalation of powdered SDZ PCO 400 (5.7 mg kg^{-1}) before inhalation of ovalbumin (0.1% w/v) precluded the expression of airway hyperreactivity to his-

Figure 1 Reduction of airway resistance by SDZ PCO 400 (arrows) following: (a) intravenous injection ($0.05 \mu\text{g kg}^{-1}$); (b) intraduodenal injection (1.0 mg kg^{-1}) and (c) inhalation of an aerosol (1.0 mg ml^{-1}) in anaesthetized ventilated guinea-pigs in which airway obstruction had been induced by intravenous injection (a) (●), or intravenous infusion of bombesin ($200\text{--}500 \text{ ng kg}^{-1}$) (b) (●), or histamine ($3.2 \mu\text{g kg}^{-1}$, ●) at 10 min intervals (c).

Figure 2 Incremental increased R_L (mean with s.e. mean shown by vertical bars, $n = 10$) following intravenous injection of bombesin (300 ng kg^{-1} , a and b) or histamine ($1.8 \mu\text{g kg}^{-1}$, c) in animals which have received intravenous infusion of saline (open columns), PAF (a), ($\pm$)-isoprenaline (b), and immune complexes (c) (solid columns) and during infusion of SDZ PCO 400 (0.1 and $1.0 \text{ mg kg}^{-1} \text{ h}^{-1}$) (cross-hatched columns) (** $P < 0.02$, *** $P < 0.001$).

tamine, yet no diminution of inflammatory cell accumulation in the airway lumen was observed ($n = 10$) (Figure 4).

Effects upon acute allergic reactions

The acute increased airway resistance that follows inhalation of an aerosol of ovalbumin (1.0 mg ml^{-1} for 5 min) was diminished markedly when powdered SDZ PCO 400 (1.25 mg) was insufflated into the trachea of sensitized anaesthetized animals before (15 min) inhalation of ovalbumin ($n = 10$) (Figure 5). This inhibitory effect of inhaled SDZ PCO 400 was not evident when bilateral vagal section, a procedure which was without detectable effect upon basal airway resistance or responses to inhaled allergen, preceded inhalation of ovalbumin ($n = 10$) (Figure 5). No inhibition of the contractile response was detected when tracheal rings from sensitized animals were exposed to ovalbumin *in vitro*, even in the presence of high concentrations of SDZ PCO 400 (10^{-4} M). Thus, increased tension following addition of ovalbumin in the absence of SDZ PCO 400 ($0.43 \pm 0.08 \text{ g}$, $n = 10$) was not significantly different from the tension generated in the presence of SDZ PCO 400 (10^{-4} M , $0.53 \pm 0.14 \text{ g}$, $n = 10$).

Discussion

SDZ PCO 400 relaxed isolated airway smooth muscle of man and guinea-pig and was bronchodilator *in vivo* in the guinea-pig. In these respects, SDZ PCO 400 resembled cromakalim, which has comparable potency in such preparations (Arch *et*

Table 1 Effect of SDZ PCO 400 upon responses to histamine in normoreactive and hyperreactive guinea-pigs

	Normoreactive				Hyperreactive			
Time	0	10	20	30	0	10	20	30
Vehicle	325 (57)	309 (51)	455 (63)	527 (55)	675 (62)	611 (52)	575 (54)	547 (50)
SDZ PCO 400	310 (58)	191 (26)	343 (45)	526 (37)	654 (94)	208*** (84)	289*** (92)	263* (95)

Responses (R_L $\text{cmH}_2\text{O l}^{-1} \text{s}^{-1} \pm \text{s.e.mean}$) to intravenous injections of histamine ($3.2 \mu\text{g kg}^{-1}$) in guinea-pigs are given immediately before (0) as well as 10, 20, and 30 min after intravenous injection of SDZ PCO 400 (0.1 mg kg^{-1}) or vehicle (0.2 ml) (mean $\pm$ s.e.mean in parentheses). Normoreactive animals (vehicle $n = 5$, SDZ PCO 400 $n = 10$) did not receive injections of immune complexes and exhibited responses to histamine that are representative for this strain of guinea-pigs. Hyperreactive animals (vehicle $n = 20$, SDZ PCO 400 $n = 10$) had received three intravenous injections of immune complexes at 10 min intervals prior to histamine exposure. Student's t test was used to assess statistical significance of differences between responses in animals treated with vehicle or SDZ PCO 400. (* $P = < 0.05$; *** $P = < 0.001$).

Figure 3 Following intravenous injections of immune complexes, incremental increase of R_L due to repeated (10 min intervals) intravenous injections of histamine ($1.8 \mu\text{g kg}^{-1}$) was inhibited by SDZ PCO 400 ($0.1 \mu\text{g kg}^{-1}$) (arrows) following injection of vehicle (ethanol) (a), but not following injection (b) of glibenclamide (30 mg kg^{-1} i.d. bolus, $n = 10$).

Figure 4 Left panel: incremental increase of R_L due to histamine (1.8 and $3.2 \mu\text{g kg}^{-1}$, i.v.) in sensitized guinea-pigs not exposed to ovalbumin (a) and 24 h after inhalation of ovalbumin (0.1%), without (b), or with (c), inhalation of SDZ PCO 400 as a powder (estimated dose 5.7 mg/animal) 1 h before inhalation of ovalbumin. (* $P = < 0.05$, $n = 10$ per group). Right panel: total cell numbers (open column), eosinophils (cross-hatched column) and neutrophils (vertically lined column) (mean with s.e.mean shown by vertical bars, $n = 10$ per group) in airway lavage fluid from sensitized animals not exposed to ovalbumin (a) and 24 h after exposure to ovalbumin, without (b), or with (c) inhalation of SDZ PCO 400 as a powder (estimated dose, 5.7 mg/animal), 1 h before inhalation of ovalbumin.

al., 1988; Chapman *et al.*, 1991b). The finding that spasmolytic efficacy of SDZ PCO 400 was independent of the particular contractile agencies used to increase tone of human bronchus is consistent with earlier reports for lemakalim (BRL 38227) (Black *et al.*, 1990). A reduced potency has been reported for RP 49356 when guinea-pig trachea was precontracted with carbachol (Brown *et al.*, 1989) and likewise SDZ PCO 400 was poorly effective when guinea-pig trachea was precontracted by carbachol. We obtained similar results when rabbit trachea and bronchus were used (unpublished data), so that the anomaly cannot be accounted for by differences in airway calibre. Since this difference extends to a phosphodiesterase (PDE) isoenzyme inhibitor, SDZ MKS 492 (Morley *et al.*, 1991) and to other spasmogens (Arch *et al.*, 1988), it may not be directly related to the opening of potassium channels.

In intact animals, SDZ PCO 400 reduced airway obstruction whether administered by the intravenous, oral or inhaled

Figure 5 Increased R_L (mean with s.e.mean shown by vertical bars, $n = 10$) in response to inhalation of saline (open column) or an aerosol of ovalbumin (1.0 mg ml^{-1} for 5 min) in sensitized animals without (solid column) or with (horizontally lined column) before bilateral vagotomy and without (checked column), or with (cross-hatched column) before bilateral vagotomy in animals that had previously (15 min) been administered SDZ PCO 400 powder (1.25 mg) by insufflation. Significant inhibition of the action of SDZ PCO 400 by comparison with the response to ovalbumin in untreated animals (*** $P = < 0.001$) and by comparison with the response in animals with intact vagi (* $P = 0.05$).

routes. Of particular interest was the observation that intravenous injection of SDZ PCO 400 (0.1 mg kg^{-1}) markedly inhibited the enhanced airway obstruction in response to intravenous injection of histamine in animals rendered hyper-reactive by injections of immune complexes, even though this dose of SDZ PCO 400 produced insubstantial and transient bronchodilator responses in control animals. This effect of SDZ PCO 400 on hyperreactive airways was abolished by introduction into the duodenum of glibenclamide, implying that this property of SDZ PCO 400 was dependent upon the opening of ATP-sensitive potassium channels. The capacity of SDZ PCO 400 to influence established airway hyper-reactivity may contribute to inhibition of the development of airway hyperreactivity due to immune complexes, PAF or ($\pm$)-isoprenaline.

Inhalation of SDZ PCO 400 pre-empted the acute airway obstruction which followed inhalation of ovalbumin. Inhibition was greater than might be anticipated from studies using spasmogens such as histamine or PAF in normal animals (data not shown), raising the possibility that SDZ PCO 400 might impair release or generation of allergic mediators from mast cells. However, preincubation of trachea from actively sensitized animals with high concentrations ($<10^{-4} \text{ M}$) of SDZ PCO 400 did not inhibit the reaction to allergen. This discrepancy can be accounted for since SDZ PCO 400 failed to suppress allergic bronchospasm in bilaterally vagotomized, actively sensitized animals. This finding implies an effect of SDZ PCO 400 primarily on neural tissue rather than airway smooth muscle, a conclusion which accords with evidence that cromakalim influences airway smooth muscle more effectively when electrical stimuli are used for activation (Ichinose & Barnes, 1990); such effects were attributed to inhibition of excitatory NANC-induced responses a property also evidenced by SDZ PCO 400 (Small *et al.*, unpublished observations). Additionally, or alternatively, SDZ PCO 400 may protect against allergic bronchospasm by modifying neural interaction with mast cells (Leff *et al.*, 1986). The present experiments do not distinguish between these two possibilities. It has been reported that cromakalim, pinacidil and nicorandil do not diminish mediator release in allergic reactions (Nagai *et al.*, 1991), but it cannot be concluded that protective effects of SDZ PCO 400 during allergic reactions depend necessarily upon an interaction with nerves, since SDZ PCO 400 also inhibited airway hyperreactivity induced by infusion of PAF, an effect known to be independent of vagal integrity in this species (Mazzoni *et al.*, 1985).

Prophylactic anti-asthma drugs inhibit the acute change of airway responsiveness to injected or inhaled spasmogens

which follows exposure to immune complexes (Boubekeur *et al.*, 1991) or PAF (Sanjar *et al.*, 1989). SDZ PCO 400 inhibited expression of airway hyperreactivity to an equal extent, whether given before or after exposure to immune complexes; hence, it was not possible to ascertain whether prevention contributes to the suppression of airway hyper-reactivity observed. In the absence of evidence to the contrary, it may be presumed that the capacity of SDZ PCO 400 to reduce airway hyperreactivity depends upon resolution of airway hyperreactivity, without involvement of a preventative action. Prophylactic anti-asthma drugs impair the influx of eosinophils into the airways in response to inhaled allergen in sensitized animals (Sanjar *et al.*, 1990b), in response to injection of PAF (Sanjar *et al.*, 1990a) or recombinant cytokines (Kings *et al.*, 1991) in normal animals. In the present experiments, no effects of SDZ PCO 400 upon eosinophil accumulation in response to allergen in sensitized animals were observed, nor has an inhibitory effect on eosinophilia been previously described for other potassium channel openers. However, lemakalim (BRL 38227) has been reported to inhibit the peritoneal neutrophil infiltration that follows injection of antigen without affecting other cell types (Buckle & Taylor, 1991) a finding not supported by the present study in which numbers of neutrophils and eosinophils in lavage fluid were unaffected in animals treated with SDZ PCO 400. These observations distinguish SDZ PCO 400 from preventative anti-asthma drugs such as ketotifen which selectively suppresses allergic airway eosinophilia and which pre-empts, but does not suppress airway hyperreactivity in this species (Sanjar *et al.*, 1990b).

The present study shows that SDZ PCO 400 is a modest spasmolytic agent yet is able to reduce airway obstruction most effectively in hyperreactive animals. No mechanism has been identified to account for this property. No inhibition of cellular inflammation in the airways could be detected indicating that modification of airway hyperreactivity by SDZ PCO 400 is not secondary to an anti-inflammatory action. In this context, it should be noted that hyperreactivity due to isoprenaline, which is not associated with inflammatory events (Sanjar *et al.*, 1990c), was more resistant to inhibition, but nonetheless was inhibited effectively. Drugs, with such a profile have been termed anti-hyperreactive agents (Morley, 1991), and may provide an alternative to β_2 -adrenoceptor selective sympathomimetics for maintenance symptomatic therapy in asthma. Since there is now evidence that β_2 -selective sympathomimetics may not be appropriate for routine use in asthma (Sears *et al.*, 1991), emergence of an alternative pharmacological principle may be timely.

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