British Journal of Pharmacology (1997) 120, 1241–1248

SCA 40: studies of the relaxant effects on cryopreserved human airway and vascular smooth muscle

*E. Müller-Schweinitzer & [1]J.R. Fozard

Research Department, Novarh's Pharma Inc., CH 4002 Basel and *Division of Clinical Pharmacology, Department of Internal Medicine, University Hospital, CH-4031 Basel, Switzerland

1 6-Bromo-8-methylaminoimidazol[1,2-a]pyrazine-2carbonitrile (SCA 40) has been claimed to induce relaxation in guinea-pig trachea by opening high conductance, calcium-activated potassium (BK_{Ca}) channels. The mechanism of action of SCA 40 has now been further investigated in ring preparations from cryopreserved human airway and vascular smooth muscle preparations *in vitro*.

2 Human bronchi with spontaneous tone relaxed in response to SCA 40 in a biphasic way. A high affinity component (pD_2 8.61 ± 0.21; mean ± s.e.mean) accounted for 30% of the response and a low affinity component (pD_2 6.53 ± 0.14) for the remaining 70%. In contrast, in bronchi contracted with carbachol, 1 μM, the concentration-response curve to SCA 40 was monophasic and yielded a pD_2 of 6.31 ± 0.29.

3 SCA 40 relaxed pulmonary and mesenteric arteries and peripheral veins which had been precontracted by 10 nM U46619 nearly completely and in a monophasic way; the pD_2 values were 6.37 ± 0.08, 6.17 ± 0.15 and 5.45 ± 0.25, respectively.

4 Lemakalim, an opener of ATP-dependent potassium (K_{ATP}) channels, also relaxed human bronchi under spontaneous tone and the vascular tissues. NS 1619, a recognised opener of BK_{ca} channels, was inactive up to 10 μM on bronchial and vascular tissues.

5 The SCA 40-induced relaxation of human bronchi was reduced concentration-dependently in the presence of high potassium chloride (20 and 80 mM). However, in the presence of 80 mM KCl and nifedipine, 30 nM, SCA 40 fully relaxed the remaining contractile response with pD_2 values of 8.08 ± 0.13 and 5.27 ± 0.13 for the high and low affinity component, respectively.

6 Relaxation responses to SCA 40 in human bronchi were resistant to blockade by glibenclamide at concentrations up to 10 μM (which blocked the relaxant response to lemakalim), quinine (30 μM), apamin (100 nM), tetraethylammonium (0.1–1 mM) and charybdotoxin (10–100 nM), thus excluding the involvement of a variety of K^+ channels including K_{ATP} and K_{Ca} channels.

7 In bronchi contracted with carbachol, 1 μM, the nature of the interaction between SCA 40 and the β_2-adrenoceptor agonist, salbutamol, was synergistic.

8 These experiments establish that SCA 40 is a potent relaxant of human bronchial smooth muscle manifesting spontaneous tone. A low affinity relaxant component has its counterpart in the relaxation seen in both human arterial and venous smooth muscle. The consensus of the evidence suggests that K^+ channel opening is not the basis of the relaxant response to SCA 40. Furthermore, BK_{Ca} channels appear to be of minor importance in the regulation of human airway smooth muscle tone. Our data suggest that inhibition of an adenosine 3′:5′-cyclic monophosphate phosphodiesterase may contribute, at least to the low affinity relaxant component of SCA 40. However, the exact mechanism mediating the SCA 40-induced relaxation of human airways remains to be defined.

Keywords: SCA 40; lemakalim; potassium channels; human bronchi; cryopreservation

Introduction

Various types of K^+ channels have been identified and shown to play an important role in the regulation of smooth muscle tone; activation of such channels increases K^+ conductance which causes hyperpolarization of the cell membrane and smooth muscle relaxation. The therapeutic potential of K^+ channel openers for the treatment of diseases associated with airway obstruction, e.g., bronchial asthma, has been pointed out (Black & Barnes, 1990; Morley, 1994). Indeed, K^+ channel openers acting on ATP-dependent channels (K_{ATP}), such as cromakalim, Hoe 234, lemakalim and bimakalim, have been shown to inhibit human airway smooth muscle contraction both *in vivo* and *in vitro* (Black *et al*., 1990; Williams *et al*., 1990; Miura *et al*., 1992; 1993; Müller-Schweinitzer *et al*., 1993). However, concomitant vasodilator effects may limit the therapeutic usefulness of these drugs in airway diseases (Black *et al*., 1990; Williams *et al*., 1990). Therefore, more selective K^+ channel openers in terms of airway smooth muscle relaxation still need to be developed.

In airway smooth muscle one of the most prevalent K^+ channels is the large conductance, calcium-activated K^+ channel (BK_{Ca}), a channel which in animal tissues has been shown to be selectively inhibited by charybdotoxin (Gimenez-Gallego *et al*., 1988) and also by the non-selective blocker, tetraethylammonium chloride (Huang *et al*., 1993). 6-Bromo-8-methylaminoimidazol[1,2-a]pyrazine-2carbonitrile (SCA 40), has been claimed to be an opener of charybdotoxin-sensitive potassium channels in guinea-pig isolated trachea (Laurent *et al*., 1993; Michel *et al*., 1993; Naline *et al*., 1996). However, more recent studies, in which both electrophysiological and functional techniques were employed, have suggested that on both guinea-pig trachea and bovine tracheal smooth muscle cells in culture the opening of BK_{Ca} channels with consequent voltage-dependent inhibition of Ca^{2+} influx through L-type channels is not important for the relaxant activity of SCA 40 (Cook *et al*., 1995; Macmillan *et al*., 1995). The present studies were undertaken to clarify the mechanism of action of SCA 40 in human isolated airway tissue.

[1] Author for correspondence.

Methods

Storage methods

Small bronchi and pulmonary arteries (inner diameter ≈ 2 to 5 mm) were excised from macroscopically normal samples of human lungs obtained during surgery for cancer. Peripheral veins (V. saphena magna, V. subcutanea, and V. perforans) were obtained from patients undergoing surgical removal of varicose veins and mesenteric arteries from organ donors. In all cases, permission was obtained from the local ethical committee. The preparations were placed in Krebs-Henseleit (KH) solution (composition mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, EDTA 0.03) and stored at 4°C until transported to the laboratory within 3 days after removal for cryopreservation as described previously in detail (Müller-Schweinitzer *et al*., 1993; Müller-Schweinitzer, 1994; Schilling *et al*., 1995). Briefly, the preparations were cleaned of loose connective tissue and placed in 2 ml liquid nitrogen storage ampoules (Gibco AG, Basel, Switzerland) filled with KH solution (bronchi and saphenous veins) or with 50% foetal calf serum in KH solution (pulmonary and mesenteric arteries) as vehicles for the cryoprotecting agents dimethyl sulphoxide (DMSO, 1.8 M and sucrose (0.1 M). Within 10 min the ampoules were placed in a polystyrene box ($11 \times 11 \times 22$ cm) and slowly frozen at a mean cooling rate of about 1°C min^{-1} in a freezer maintained at −70°C. After 3–20 h the ampoules were transferred into liquid nitrogen (−196°C) where they were stored until use. Before use the tissues were thawed within 3 min by placing the ampoules in a 40°C water bath. Thereafter the preparations were rinsed in a dish containing, KH solution at room temperature, cut into rings and suspended in 10 ml organ baths for isometric tension recording.

In some experiments, fresh lung tissue, i.e. bronchi which had not been cryopreserved, were used to allow comparison between the effects of SCA 40 on fresh or cryopreserved tissues.

Organ bath studies

The rings (about 2 mm in length) were mounted between two hooks of stainless steel wire (diameter 0.15 mm) and suspended in 10 ml organ baths containing KH solution at 37°C, gassed continuously with 5% CO_2 in oxygen. Contraction was recorded isometrically under a resting tension of 1 g with electromechanical transducers (Statham model UC 3) and a potentiometric recorder. The preparations were allowed to equilibrate for about 3 h in the bathing medium. During this time the vascular and bronchial preparations were challenged once with noradrenaline (1 μM) or carbachol (1 μM), respectively, and the baseline tension of the rings was readjusted to 1 g if necessary. Vascular tissues were then contracted with 10 nM U46619 which induced a stable contraction of the tissue to about 50% of its maximum. Concentration-response curves to relaxant agonists were established by cumulative addition of drug, the concentration being increased when the maximum effect had been produced by the previous concentration. Antagonists were added 20 min before the first administration of the agonist. After the concentration-response curves had been completed papaverine (300 μM) was added to induce complete relaxation of the rings. This effect was taken as 100% relaxation.

Drugs

The following compounds were used: apamin, glibenclamide, nifedipine, salbutamol, papaverine hydrochloride, U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$), propranolol hydrochloride, quinine hydrochloride (Sigma, Munich, F.R.G.), charybdotoxin (Latoxan, Rosans, France), tetraethylammonium chloride (Janssen, Beerse, Belgium), lemakalim (Smith Kline Beecham, Surrey, U.K.), SCA 40 (6-bromo-8-methylaminoimidazol[1,2-a]pyrazine-2carbonitrile, University of Montpellier), NS1619 (1-(2′-hydroxy-5′-trifluoromethyl-phenyl)-5-trifluoromethyl-2(3H) benzimidazolone, NeuroSearch A/S, Glostrup, Denmark). Lyophilized charybdotoxin was reconstituted in 150 mM NaCl. Lemakalim, NS1619, SCA 40 and U46619 were dissolved in ethanol and diluted to give 1 mM solutions containing 60% ethanol. Further dilutions were performed in 5% glucose or normal saline. Samples of charybdotoxin (10 μM) and U46619 (1 μM) were stored at −20°C until use. All other compounds were dissolved in saline just before use.

Data analysis

Monophasic concentration-response curves were analysed with a computer programme in RS/1 (BBN Software Products Corporation, Cambridge, Mass., U.S.A.) and E_{max} (maximal effects) and pD_2 values (negative logarithm of the molar concentrations of the agonists producing 50% of E_{max}) were derived from this analysis. Biphasic concentration-response curves were analysed by the procedure Fit Function in RS/1 according to the equation $f(x) = (A/(1 + B/X)) + (C/(1 + D/X))$ where f(x) is the fraction of receptors activated by the concentration X, A and C are the maximal responses and B and D represent the EC_{50} values (concentrations of the agonists producing 50% of E_{max}) respectively. The nature of the interaction between salbutamol and SCA 40 was determined by the algebraic method described by Berenbaum (1977) with the equation $(A/A_e) + (B/B_e)$ where A and B are concentrations of two agonists producing in combination a given effect and A_e and B_e are concentrations of the respective agonists producing the same effects when administered alone. If the relation of both drugs is synergistic the sum of the fractions in the above equation is less than 1 and will be equal to 1 in case of an additive effect. Data are presented as means ± s.e.mean. One-way analysis of variance (ANOVA) was performed, followed by the Bonferroni-corrected *t* test to assign differences to individual between-group comparisons when overall significance ($P < 0.05$) was attained.

Results

Relaxant activity in isolated bronchi and blood vessels

When tested in cryopreserved human bronchi with spontaneous tone, SCA 40 produced a biphasic relaxation concentration-response curve with maximal effects of about 30% and 70% and pD_2 values of 8.61 ± 0.21 and 6.53 ± 0.14 (mean ± s.e.mean, $n = 20$) for the high and low affinity components, respectively (Figure 1a; Table 1). SCA 40 induced similar effects on fresh, unfrozen bronchi. The pD_2 values were 8.68 ± 0.28 and 6.39 ± 0.31 and the maximal effects were 48 ± 12 and 40 ± 8% ($n = 5$) for the high and low affinity phases, respectively. In contrast, SCA 40 relaxed human vascular tissues which had been contracted by 10 nM U46619 (a concentration eliciting about 50% of the maximum response) in a monophasic manner and nearly completely (Figure 1b – d). As indicated by the pD_2 values (Table 1), SCA 40 was about 10 and 5 times less potent in relaxing peripheral veins than pulmonary and mesenteric arteries, respectively.

The K_{ATP} channel opener, lemakalim, relaxed both airway and vascular smooth muscle preparations by 65% to 90% in a monophasic way (Figure 1a – d) and within a broadly similar concentration range (Table 1). In human bronchi under spontaneous tone the K_{ATP} channel blocker, glibenclamide, antagonized responses to lemakalim producing progressive decreases in the pD_2 values and a marked reduction of the maximal relaxant responses to lemakalim (Table 2). NS1619, a recognised opener of BK_{Ca} channels (Olesen *et al*., 1994), proved to be the weakest agonist, relaxing human bronchi with spontaneous tone by only about 10% at 1 μM while the effect of 10 μM was similar to the solvent control effect (Figure 1a).

Table 1 Summary of the relaxant effects of SCA 40 and lemakalim on cryopreserved human airway and vascular tissues *in vitro*

		SCA 40			*Lemakalim*		
	Tone	*Efficacy*			*Efficacy*		
		pD_2	(%)	n	pD_2	(%)	n
Bronchi	Spontaneous	8.61 ± 0.21[a]	32 ± 4	20			
		6.53 ± 0.14[b]*	66 ± 4	20	6.76 ± 0.04	91 ± 3	7
Pulmonary artery	U46619, 10 nM	6.37 ± 0.08*	94 ± 1	6	6.64 ± 0.17	65 ± 3	4
Mesenteric artery	U46619, 10 nM	6.17 ± 0.15*	90 ± 2	5	7.22 ± 0.21	83 ± 3	4
Peripheral vein	U46619, 10 nM	5.45 ± 0.25*	93 ± 4	4	6.31 ± 0.14	71 ± 0.1	6

Data are presented as means ± s.e.mean.
[a]High and [b]low affinity components of relaxation concentration-response curve.
*Significant difference from high affinity pD_2 value in bronchi.

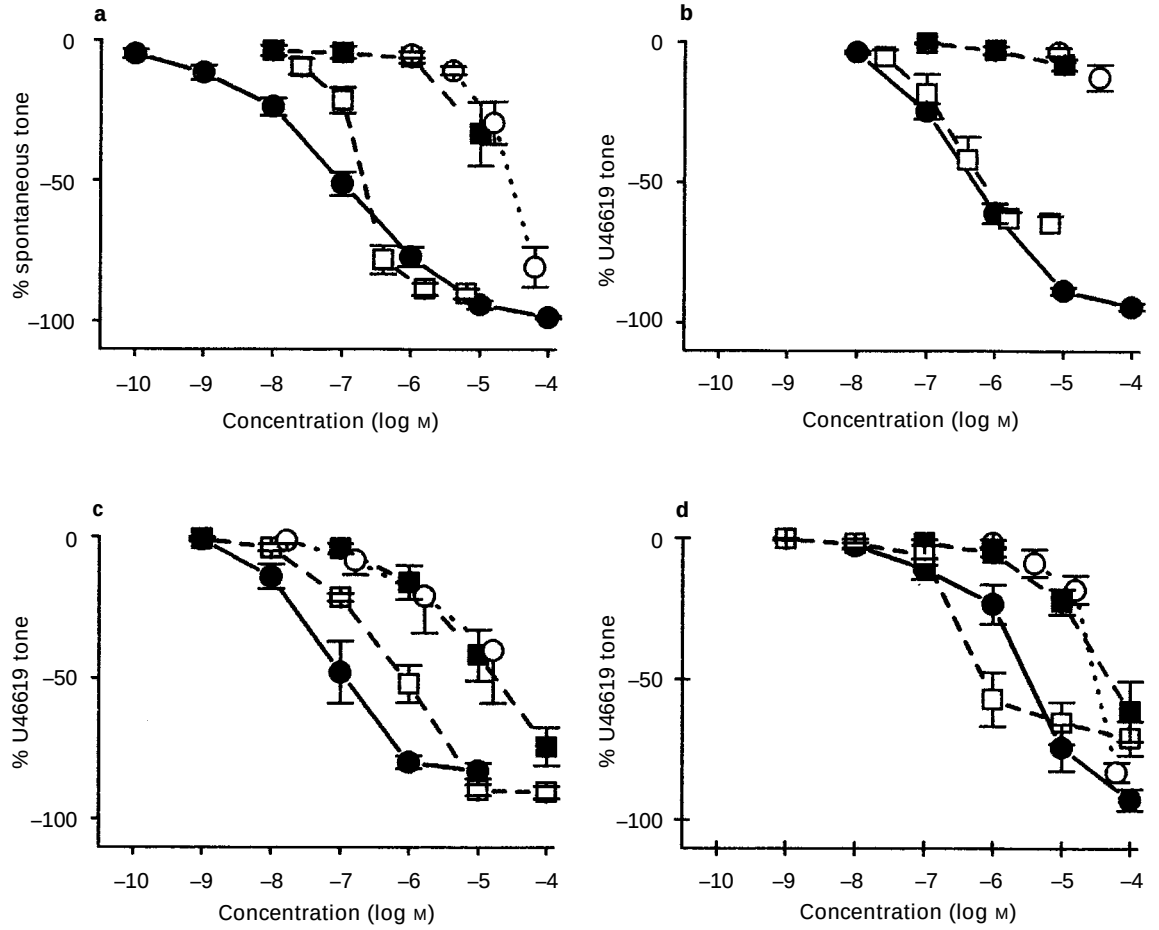

Figure 1 Cumulative concentration-response curves for SCA 40 (●), lemakalim (□), NS1619 (■) and solvent control effects (○) on rings from human bronchi with spontaneous tone (a), pulmonary arteries (b), mesenteric arteries (c) and saphenous veins (d) in each case stimulated with 10 nM U46619. The effects are expressed as percentages of the maximum relaxant responses to papaverine. Points represent mean values from 4–20 individual experiments; vertical lines show s.e.mean.

Vascular smooth muscle preparations did not respond to NS1619 unless μM concentrations were applied. However, these effects, again, were not different from those of the solvent control (Figure 1b, c and d).

Bronchorelaxant effect of SCA 40 in the presence of carbachol

SCA 40 induced concentration-dependent relaxation of human bronchi given active tone by the addition of 1 μM carbachol (a concentration inducing about 50% of the maximum response). However, in contrast to the effects seen on tissues with spon-

Table 2 Effects of glibenclamide on the relaxant effects of lemakalim on human isolated bronchi under spontaneous tone

	pD_2 value for lemakalim	*Efficacy* (%)	n
Control	6.80 ± 0.05	89 ± 2	3
Glibenclamide, 0.1 μM	6.54 ± 0.21	89 ± 3	3
Glibenclamide, 1 μM	6.31 ± 0.22	78 ± 4*	3
Glibenclamide, 10 μM	6.06 ± 0.26*	51 ± 8*	3

Data are presented as means ± s.e.mean.
*Significant difference from corresponding control values.

taneous tone, the concentration-response curve to SCA 40 in the presence of carbachol was monophasic rather than biphasic. The pD_2 value under these conditions (6.31 ± 0.29, $n=9$) was similar to that of the low affinity phase determined during spontaneous tone (6.53 ± 0.14, $n=20$) and of the same order as the pD_2 values recorded for relaxation of the vascular tissues under U46619-induced tone (Table 1). For comparison, the β_2-adrenoceptor agonist, salbutamol, was tested for its relaxant activity on human bronchi under both spontaneous and carbachol-induced tone. In each case, the curves were monophasic and similar pD_2 values were obtained (6.95 ± 0.11, $n=6$ and 7.08 ± 0.14, $n=4$, respectively).

Bronchorelaxant effect of SCA 40 in high potassium solutions

In these experiments concentration-response curves for SCA 40 were determined on human bronchi during exposure to KH solution containing 20 and 80 mM KCl instead of 4.7 mM. As shown in Figure 2, relaxant responses to SCA 40 were slightly reduced in the presence of 20 mM KCl, but the curve was still biphasic with pD_2 values of 9.47 ± 0.65 and 6.26 ± 0.68 ($n=3$), respectively. When the KCl concentration was increased to 80 mM, a concentration that is presumed to induce maximal depolarization of the cell membrane, responses to SCA 40 were markedly attenuated although weak concentration-related relaxation responses were evident between 0.1 and 100 μM (Figure 2). A further series of experiments was performed, with KH solution containing 80 mM KCl, and 30 nM of the calcium entry blocker, nifedipine (Figure 3). Predictably, nifedipine itself induced relaxation, eliminating completely the KCl-induced tone and about 30% of the spontaneous tone. The additional administration of SCA 40 eliminated the residual tone. The concentration-response relationship was biphasic and yielded pD_2 values of 8.08 ± 0.13 and 5.27 ± 0.13 ($n=4$) for the high and low affinity components, respectively. In control experiments, in which bronchi with spontaneous tone were used, the addition of nifedipine (100 nM) to the organ bath elicited a relaxation of about 20%, but did not modify the relaxant SCA 40 effects compared to the SCA 40 curve determined in the absence of nifedipine (data not illustrated).

Bronchorelaxant effect of SCA 40 in the presence of various potassium channel blocking agents

Various potassium channel blocking agents were tested against both the high and low affinity phases of the bronchorelaxant effects of SCA 40, manifested against spontaneous tone. Neither glibenclamide, 0.3 – 10 μM, quinine, 30 μM (a non-selec-

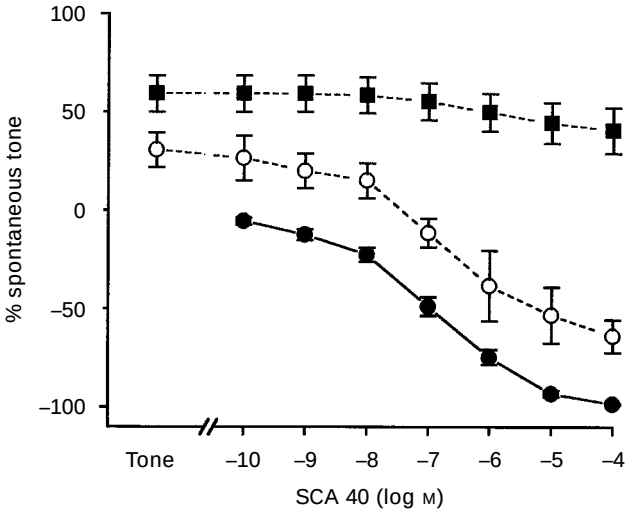

Figure 2 Cumulative concentration-response curve for SCA 40 on rings from human bronchi during spontaneous tone (●, $n=16$) and in the presence of increased potassium chloride concentrations, KCl 20 mM (○, $n=3$) and 80 mM (■, $n=6$). Points represent mean values and vertical lines show s.e.mean.

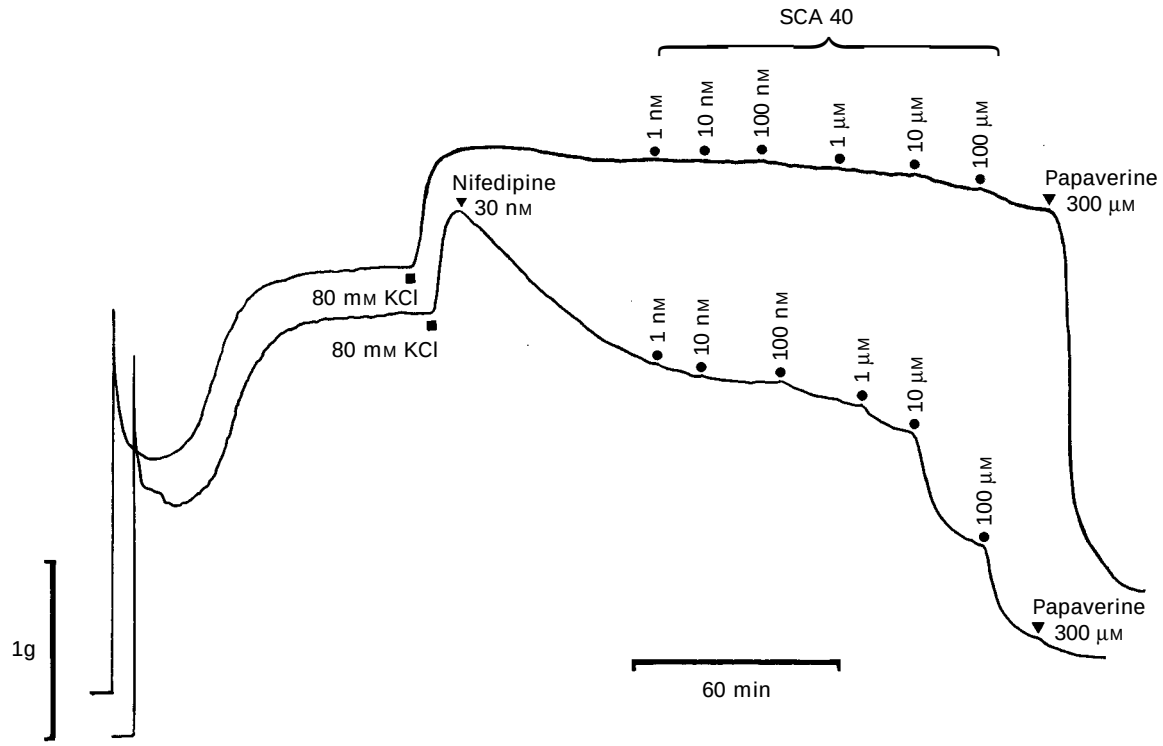

Figure 3 Representative tracing of responses to SCA 40 of rings from human bronchi during stimulation with 80 mM potassium chloride without (upper trace) and in the presence of 30 nM nifedipine (lower trace). Effects are typical of 6 and 4 similar observations, respectively.

tive K^+ channel blocker), nor apamin, 100 nM (a blocker of small conductance, calcium-activated channels) caused any significant change in the SCA 40 responses (Table 3). Tetraethylammonium, 0.1–1 mM (a non-selective blocker of K^+ channels), produced a slight increase in tone of human bronchi during resting tone and elicited concentration-dependent relaxant responses of the bronchi when active tone was induced by 1 μM carbachol. However, neither during spontaneous tone

Table 3 Effect of potassium channel blocking agents on relaxant responses to SCA 40 on human isolated bronchi under spontaneous tone

	n	*Antagonist concentration*	*High affinity phase* pD_2	*Efficacy* (%)	*Low affinity phase* pD_2	*Efficacy* (%)
Apamin	4	0	8.08±0.42	46±7	6.38±0.20	49±5
	4	100 nM	7.60±0.06	39±15	7.07±0.49	58±15
Charybdotoxin	3	0	9.09±0.20	11±2	7.20±0.31	86±1
	3	10 nM	8.91±0.31	25±5	7.48±0.19	88±6
	3	100 nM	8.73±0.37	22±19	7.19±0.25	95±15
Glibenclamide	8	0	8.39±0.36	39±7	6.73±0.23	60±7
	3	300 nM	8.05±0.46	26±11	6.77±0.35	67±13
	3	10 μM	8.31±0.51	42±12	6.67±0.34	51±7
Quinine	4	0	8.31±0.67	38±5	6.42±0.38	61±6
	3	30 μM	8.29±0.39	43±11	6.15±0.10	43±4
Tetraethylammonium	5	0	8.41±0.55	28±5	6.41±0.42	71±6
	3	100 μM	8.33±0.22	38±17	6.06±0.07	68±15
	4	300 μM	8.22±0.38	14±4	5.98±0.37	84±4
	3	1 mM	9.07±0.28	13±18	6.89±0.69	99±30

Data are presented as means±s.e.mean.

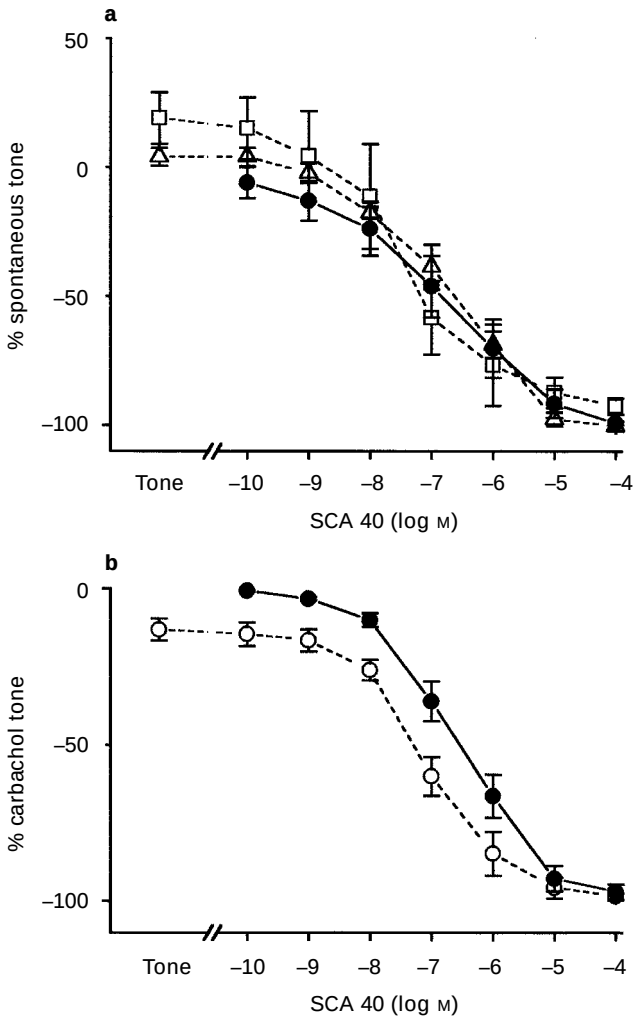

Figure 4 Cumulative concentration-response curves for SCA 40 without (●) and in the presence of tetraethylammonium (TEA) 100 μM (△) and 1 mM (□) on rings from human bronchi with spontaneous tone (a) and in the presence of TEA 300 μM (○) during stimulation with 1 μM carbachol (b). Points represent mean values from $n=3-4$ individual experiments; vertical lines show s.e.mean.

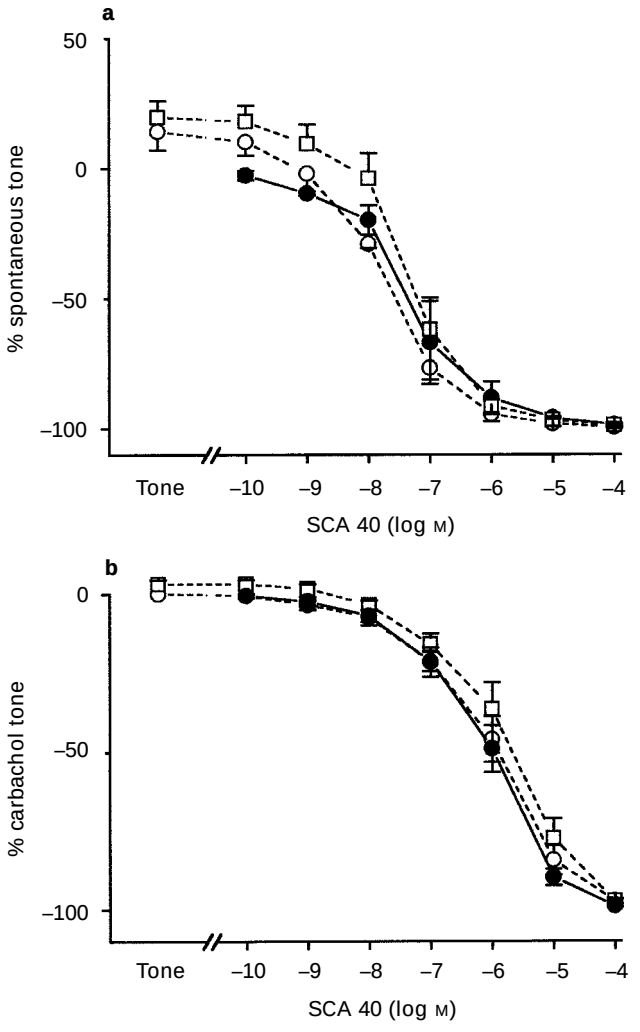

Figure 5 Cumulative concentration-response curves without (●) and in the presence of charybdotoxin 10 nM (○) and 100 nM (□) for SCA 40 on rings from human bronchi during spontaneous tone (a) and during stimulation with 1 μM carbachol (b). Points represent mean values from 3 individual experiments; vertical lines show s.e.mean.

nor during active tone (i.e., in the presence of 1 μM carbachol) did tetraethylammonium antagonize the relaxant responses to SCA 40 (Figure 4a and b). Similarly, charybdotoxin, 10–100 nM (a blocker of BK_{Ca} channels) did not modify the relaxant responses to SCA 40 either during resting tone or in the presence of 1 μM carbachol (Figure 5a and b). SCA 40-induced relaxation of human bronchi were also resistant to blockade of β-adrenoceptors by propranolol, 100 nM (data not illustrated).

Bronchorelaxant effects of SCA 40 in the presence of salbutamol

In a further series of experiments concentration-response curves were determined for salbutamol alone and for SCA 40 in the absence and in the presence of salbutamol (10 and 100 nM) on human bronchi with tone induced by 1 μM carbachol (Figure 6). From the curves for each agonist alone as well as for the combined action of both compounds the concentrations producing the same effect were determined at the level of 40% and 50% relaxation. Analysis of the data according to the algebraic method described by Berenbaum (1977) gave a mean sum of fractions of 0.21 (0.15–0.37; mean with 95% confidence limits) which was significantly less than 1, indicating that the nature of the interaction between the two drugs was synergistic.

Discussion

High conductance, calcium-activated K^+ channels (BK_{Ca}) are important target proteins which are involved in agonist-induced contraction and relaxation of numerous smooth muscle cells. BK_{Ca} channels are selectively blocked by toxins such as charybdotoxin (Gimenez-Gallego *et al*., 1988; Jones *et al*., 1990), and the open-state probability of these channels is markedly increased at higher concentrations of cytosolic calcium and also during membrane depolarization (Kume *et al*., 1989). In airway smooth muscle cells the BK_{Ca} channels are regulated by muscarinic (M_2) receptors and β-adrenoceptors, i.e., inhibition of K_{Ca} channels during muscarinic contraction and activation of K_{Ca} channels during β-adrenoceptor-mediated relaxation are important regulatory mechanisms (Kotlikoff, 1993).

Experiments in animal tissues have suggested that the smooth muscle relaxant activity of SCA 40 involves opening of charybdotoxin-sensitive K^+ channels, i.e., BK_{Ca} channels (Laurent *et al*., 1993; Michel *et al*., 1993). However, data from more recent electrophysiological and pharmacological studies have not provided support for this mechanism. Thus, SCA 40 was able to abolish the spontaneous tone of guinea-pig trachea without any significant change in membrane potential, suggesting that opening of K_{Ca} channels is not the basis of the relaxant activity of SCA 40 in this tissue (Cook *et al*., 1995). Moreover, SCA 40 had no effects on the large conductance, Ca^{2+} activated K^+ channels in bovine tracheal smooth muscle cells in culture (Macmillan *et al*., 1995).

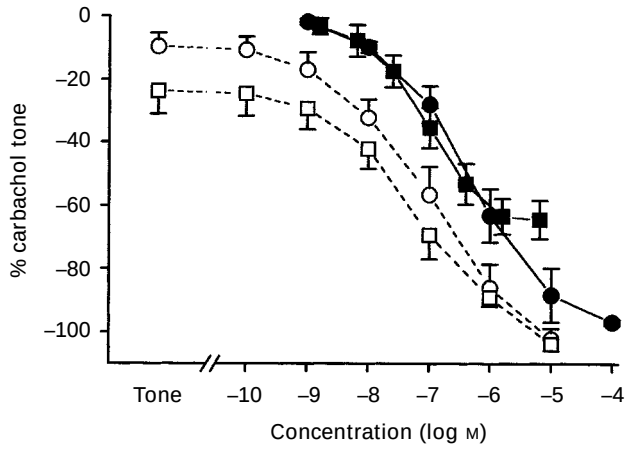

Figure 6 Cumulative concentration-response curves for salbutamol alone (■, $n=5$) and SCA 40 in the absence (●) and in the presence of salbutamol 10 nM (○) and 100 nM (□) on rings from human bronchi during stimulation with 1 μM carbachol. The effects are expressed as percentages of the maximum relaxant responses to papaverine. Points represent mean values from 6 individual experiments; vertical lines show s.e.mean.

The present results, demonstrating the potent relaxant activity in human airway smooth muscle of SCA 40, are consistent with the potent relaxant activity observed with this agent in the guinea-pig trachea (Laurent *et al*., 1993; Cook *et al*., 1995). However, in contrast to the observations in guinea-pig trachea, SCA 40 elicited a biphasic curve when tested in human bronchi under spontaneous tone. A high affinity component accounted for approximately 30% of the SCA 40 response and a low affinity component for the remaining 70%. However, as observed with unfrozen human bronchi during acetylcholine-induced tone (Naline *et al*., 1996), only a monophasic curve was obtained when SCA 40 was tested in human blood vessels during stimulation with U46619 or in human bronchi when active tone was induced by the addition of carbachol or high potassium concentrations. Hence, only in bronchi under spontaneous tone did SCA 40 elicit a biphasic dose-response curve, suggesting the involvement of more than one mechanism in the bronchorelaxant activity of SCA 40. One might argue, that the present results were influenced by the freezing/thawing process during cryopreservation of the human material. However, it has been demonstrated that various functional mechanisms in smooth muscle and epithelial cells are either well preserved or only slightly diminished after cryopreservation of human airways (Müller-Schweinitzer *et al*., 1993; Vacciana *et al*., 1994; Johnson *et al*., 1995; Sarriá *et al*., 1995). Moreover, lemakalim relaxed cryopreserved human bronchi in the present study with the same potency as has been published for unfrozen bronchi and the antagonism of lemakalim by glibenclamide was quantitatively similar (Black *et al*., 1990). Specifically in the context of the present study, there were no qualitative or quantitative differences between the reactivity of fresh and cryopreserved human bronchi to SCA 40.

A marked reduction of the relaxant effects of SCA 40 by increasing the KCl concentrations in the organ bath to 80 mM is consistent with a role for K^+ channels in the relaxation of human airway smooth muscle by SCA 40. Similar findings have been obtained by Laurent *et al*. (1993) and Cook *et al*. (1995) for the effects of SCA 40 on guinea-pig trachea. However, both in the present studies and those of Cook *et al*. (1995) blockade of SCA 40 by elevation of K^+ could be prevented by nifedipine suggesting that functional antagonism due to voltage-dependent Ca^{2+} influx through nifedipine-sensitive Ca^{2+} channels underlies the response. Consistent with this, a variety of K^+ channel blocking agents failed to influence the relaxant response to SCA 40 on human bronchi. For example, glibenclamide was devoid of activity at concentrations known to be able to block the response to lemakalim and apamin did not affect the relaxant response to SCA 40; the involvement of K_{ATP} channels (Quast & Cook, 1989) and small conductance calcium-activated K^+ channels (Banks *et al*., 1979) in the response to SCA 40 can, therefore, be excluded. This confirms for human bronchi similar findings as in the guinea-pig trachea (Laurent *et al*., 1993; Naline *et al*., 1996). However, while in guinea-pig airways the relaxant activity of SCA 40 has been shown to be susceptible to blockade by quinine and charybdotoxin (Laurent *et al*., 1993; Cook *et al*., 1995; Naline *et al*., 1996), no such inhibitory activity could be observed in the human bronchi. Furthermore, in contrast to that observed in guinea-pig isolated main bronchi (Naline *et al*., 1996), tetraethylammonium, a K^+ channel blocker with affinity for the BK_{Ca} channel protein (Kotlikoff, 1993), failed to inhibit relaxation induced by SCA 40. Taken together, our data indicate that in the human airways BK_{Ca} channels are not involved in

the activity of SCA 40. Moreover, BK_{Ca} channels appear to be of minor significance for the regulation of human airway smooth muscle tone in general. This conclusion is supported by the negligible relaxant activity of the recognised BK_{Ca} channel activator NS1619 (Olesen *et al*., 1994) in human bronchi in the present study and by recently published experiments with patch clamp techniques on human bronchial smooth muscle cells in primary culture (Templeton *et al*., 1995).

Various mechanisms such as intrinsic production of leukotrienes, cyclo-oxygenase products of arachidonic acid metabolism and spontaneous release of acetylcholine are postulated to contribute to generation of the active basal tone in human bronchial tissue (Ito *et al*., 1989) and it is possible that selective attentuation of one or more of these locally tissue-derived mediators contributes to the bronchorelaxant effect of SCA 40. Since propranolol at concentrations up to 100 nM failed to antagonize the effects of SCA 40 in human bronchi, the involvement of β-adrenoceptors can be excluded.

In bronchi contracted with carbachol, 1 μM, the combined action of salbutamol and SCA 40 was synergistic. Since β_2-adrenoceptor stimulation-induced relaxation of smooth muscle is associated with enhanced formation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) via activation of adenylyl cyclase (see Small *et al*., 1993), the synergy between the two drugs suggests that enhanced accumulation of cyclic AMP via inhibition of cyclic AMP-phosphodiesterase might contribute to the relaxant activity of SCA 40. In support of this, direct evidence for an inhibitory effect of SCA 40 on human type III phosphodiesterase at concentrations similar to those inducing relaxation of human bronchi in the present study has recently appeared (Cook *et al*., 1995; Pocock & Small, 1996; Cortijo *et al*., 1996).

In conclusion, our experiments establish that SCA 40 is a potent relaxant of human bronchial smooth muscle under spontaneous tone. A low affinity component to the relaxant concentration-response curve has its counterpart in the relaxation seen in both human arterial and venous smooth muscle. The consensus of the evidence suggests that K^+ channel opening is not the basis of the major part of the relaxant response to SCA 40. In particular, BK_{Ca} channels are not involved in the response and appear to be of minor importance in the regulation of human airway smooth muscle tone. Whilst inhibition of cyclic AMP-phosphodiesterase activity may contribute at least to the low affinity relaxant response to SCA 40, the exact mechanism(s) mediating the SCA 40-induced relaxation of human airways remain(s) to be defined.

The authors express their appreciation to Prof. Dr E. Glusa at the Institute for Experimental Angiology and Haemostasis in Erfurt, to Prof. Dr J. Hasse and the Staff of the Department of Cardiothoracic Surgery of the Albert-Ludwigs University Freiburg, to Dr M. Schilling and the Staff of the Visceral and Transplantation Surgery Department of the University Hospital Bern, for the supply of human vascular and lung tissues and wish to thank Mrs M. Bretz for her valuable technical assistance.

References

BANKS, B.E.C., BROWN, C., BURGESS, G.M., BURNSTOCK, G., CLARET, M., COCKS, T.M. & JENKINSON, D.H. (1979). Apamin blocks certain transmitter-induced increase in potassium permeability. *Nature*, **282,** 415–417.

BERENBAUM, M.C. (1977). Synergy, additivism and antagonism in immunosuppression. A critical review. *Clin. Exp. Immunol*., **28,** 1–18.

BLACK, J., ARMOUR, C.L., JOHNSON, P.R.A., ALOUAN, L.A. & BARNES, P.J. (1990). The action of a potassium channel activator, BRL 38227 (Lemakalim), on human airway smooth muscle. *Am. Rev. Respir. Dis*., **142,** 1384–1389.

BLACK, J.L. & BARNES, P.J. (1990). Potassium channels and airway function: new therapeutic prospects. *Thorax*, **45,** 213–218.

COOKS, S.J., ARCHER, K., MARTIN, A., BUCHHEIT, K.H., FOZARD, J.R., MÜLLER, T., MILLER, A.J., ELLIOT, K.R.F., FOSTER, R.W. & SMALL, R.C. (1995). Further analysis of the mechanisms underlying the tracheal relaxant actions of SCA 40. *Br. J. Pharmacol*., **114,** 143–151.

CORTIJO, J., VILLAGRASA, V., NAVARRETE, C., SANZ, C., BERTO, L., MICHEL, A., BONNET, P.A. & MORCILLO, E.J. (1996). Effects of SCA40 on human isolated bronchus and human polymorhonuclear leukocytes: comparison with rolipram. SKF94120 and levromakalim. *Br. J. Pharmacol*., **119,** 99–106.

GIMENEZ-GALLEGO, G., NAVIA, M.A., REUBEN, J.P., KATZ, G.M., KACZOROWSKY, G.J. & GARCIA, M.L. (1988). Purification, sequence, and model structure of charybdotoxin, a potent selective inhibitor of calcium-activated potassium channels. *Proc. Natl. Acad. Sci. U.S.A.,* **85,** 3329–3333.

HUANG, J.-C., GARCIA, M.L., REUBEN, J.P. & KACSOROWSKI, G.J. (1993). Inhibition of β-adrenoceptor agonist relaxation of airway smooth muscle by Ca^{2+}-activated K^+ channel blockers. *Eur. J. Pharmacol*., **235,** 37–43.

ITO, Y., SUZUKI, H., AIZAWA, H., HAKODA, H. & HIROSE, T. (1989). The spontaneous electrical and mechanical activity of human bronchial smooth muscle: its modulation by drugs. *Br. J. Pharmacol*., **98,** 1249–1260.

JOHNSON, P.R.A., MCKAY, K.O., ARMOUR, C.L. & BLACK, J.L. (1995). The maintenance of functional activity in human isolated bronchus after cryopreservation. *Pulmon. Pharmacol*., **8,** 43–47.

JONES, T.R., CHARETTE, L., GARCIA, M.L. & KACSOROWSKI, G.J. (1990). Selective inhibition of relaxation of guinea-pig trachea by charybdotoxin, a potent Ca^{2+}-activated K^+ channel inhibitor. *J. Pharmacol. Exp. Ther*., **255,** 697–706.

KOTLIKOFF, M.I. (1993). Potassium channels in airway smooth muscle: a tale of two channels. *Pharmacol. Ther*., **58,** 1–12.

KUME, H., TAKAI, A., TOKUNO, H. & TOMITA, T. (1989). Regulation of Ca^{2+}-dependent K^+-channel activity in tracheal myocytes by phosphorylation. *Nature*, **341,** 152–154.

LAURENT, F., MICHEL, A., BONNET, P.A., CHAPAT, J.P. & BOUCARD, M. (1993). Evaluation of the relaxant effects of SCA40, a novel charybdotoxin-sensitive potassium channel opener, in guinea-pig isolated trachealis. *Br. J. Pharmacol*., **108,** 622–626.

MACMILLAN, S., SHERIDAN, R.D., CHILVERS, E.R. & PATMORE, L. (1995). A comparison of the effects of SCA40, NS 004 and NS 1619 on large conductance Ca^{2+}-activated K^+ channels in bovine tracheal smooth muscle cells in culture. *Br. J. Pharmacol*., **116,** 1656–1660.

MICHEL, A., LAURENT, F., BOMPART, J., HADJ-KADDOUR, K., CHAPAT, J.P., BOUCARD, M. & BONNET, P.A. (1993). Cardiovascular effects of SCA 40, a novel potassium channel opener, in rats. *Br. J. Pharmacol*., **110,** 1031–1036.

MIURA, M., BELVISI, M.G., STRETTON, C.D., YACOUB, M.H. & BARNES, P.J. (1992). Role of potassium channels in bronchodilator responses in human airways. *Am. Rev. Respir. Dis*., **146,** 132–136.

MIURA, M., BELVISI, M.G., WARD, J.K., TADJKARIMI, S., YACOUB, M.H. & BARNES, P.J. (1993). Bronchodilating effects of the novel potassium channel opener HOE 234 in human airways in vitro. *Br. J. Pharmacol*., **35,** 318–320.

MORLEY, J. (1994). K^+ channel openers and suppression of airway hyperreactivity. *Trends Pharmacol. Sci*., **15,** 463–468.

MÜLLER-SCHWEINITZER, E. (1994). Arterial smooth muscle function after prolonged exposure to a dimethyl sulfoxide (Me_2SO) containing medium and storage at −196°C. *Cryobiology*, **31,** 330–335.

MÜLLER-SCHWEINITZER, E., HASSE, J. & SWOBODA, L. (1993). Cryopreservation of human bronchi. *J. Asthma*, **30,** 451–457.

NALINE, E., CUI, Y.Y., MICHEL, A., BONNET, P.A., BAKDACH, H. & ADVENIER, C. (1996). Effects of SCA40 on human bronchi and on guinea pig main bronchi in vitro. Comparison with cromakalim. *Fundam. Clin. Pharmacol*., **10,** 368–378.

OLESEN, S.-P., MUNCH, E., MOLDTH, P. & DREJER, J. (1994). Selective activation of Ca^{2+}-dependent K^+ channels by novel benzimidazolone. *Eur. J. Pharmacol*., **251,** 53–59.

POCOCK, T.M. & SMALL, R.C. (1996). Studies of the effects of SCA 40 in relaxing bovine trachealis muscle and in inhibiting isoenzymes of cyclic nucleotide phosphodiesterase. *Br. J. Pharmacol.*, **117,** 209P.

QUAST, U. & COOK, N.S. (1989). Moving together: K^+ channel openers and ATP-sensitive K^+ channels. *Trends Pharmacol. Sci.*, **10,** 431–435.

SARRIÁ, B., NALINE, E., CORTIJO, J., MOREAU, J., CERDÁ, J.M., MORCILLO, E.J. & ADVENIER, C. (1995). Functional, biochemical, and morphological studies on human bronchi after cryopreservation. *Br. J. Pharmacol.*, **116,** 2569–2574.

SCHILLING, A., GLUSA, E. & MÜLLER-SCHWEINITZER, E. (1995). Nature of the medium for cryopreservation of human peripheral veins. *Cryobiology*, **32,** 109–113.

SMALL, R.C., CHIU, P., COOK, S.J., FOSTER, R.A. & ISAAK, L. (1993). β-Adrenoceptor agonists in bronchial asthma: role of K^+-channel opening in mediating their bronchodilator effects. *Clin. Exp. Allergy*, **23,** 802–811.

TEMPLETON, A.G.B., MACLENNAN, S.J., PATE, M.A., MACMILLAN, S., CHILVERS, E.R. & HICKS, P.E. (1995). Is NS1619 a BK_{Ca} channel opener in human bronchus? *Br. J. Pharmacol.*, **115,** 119P.

VACCIANA, A., EBEIGBE, A.B., HASSE, J., SWOBODA, L. & MÜLLER-SCHWEINITZER, E. (1994). Evidence for preservation of epithelial function in cryopreserved porcine and human bronchi. *Exp. Physiol.*, **79,** 409–415.

WILLIAMS, A.J., LEE, T.H., COCHRANE, G.M., HOPKIRK, A., VYSE, T., CHIEW, F., LAVENDER, E., RICHARDS, D.H., OWEN, S., STONE, P., CHURCH, S. & WOODCOCK, A.A. (1990). Attenuation of nocturnal asthma by cromakalim. *Lancet*, **336,** 334–336.

(Received October 29, 1996
Accepted December 23, 1996)