Journal of Asthma, 30(6), 451–457 (1993)

Cryopreservation of Human Bronchi

E. Müller-Schweinitzer,[1] J. Hasse,[2] and L. Swoboda[2]

[1]*Preclinical Research*
Sandoz Pharma Ltd.
CH-4002 Basel, Switzerland
[2]*Klinikum der Albert-Ludwigs-Universität Freiburg*
D-79106 Freiburg, Germany

ABSTRACT

Human bronchi have been investigated in vitro without or after storage at −196°C in different media containing 1.8 M dimethyl sulfoxide and 0.1 M sucrose as cryoprotectants, dissolved in either fetal calf serum (FCS), Krebs-Henseleit solution (KH), or 50% FCS in KH as vehicles. As assessed by the post-thaw responses to both carbachol and histamine, optimal preservation of contractile responsiveness was obtained with bronchi that had been frozen in a medium containing KH solution as the vehicle. With each group of cryopreserved bronchi, maximal responses to relaxant agonists such as isoprenaline, papaverine, and the potassium channel activator bimakalim were attenuated by up to 50%, and the passive resting tension after maximal pharmacological relaxation was considerably higher than in the unfrozen tissues. The evidence suggests that, despite some reduction in elasticity, cryopreservation of human bronchi at −196°C preserves both contractile and relaxant mechanisms and offers clear potential for storing human airway smooth muscle for subsequent pharmacological experiments.

Address correspondence to: E. Müller-Schweinitzer, Preclinical Research, Sandoz Pharma Ltd., CH-4002 Basel, Switzerland.

INTRODUCTION

Cryopreservation has been shown to be a useful technique to store isolated organs for subsequent pharmacological experiments (1). Most of these investigations, however, have been performed on vascular smooth muscle, and little is known about the postthaw functional recovery of airway smooth muscle. Histological examinations of cryopreserved segments of canine trachea have shown normal smooth muscle cells, epithelium with mucus production, and well-retained ciliary function (2). Normal ciliary activity after cryopreservation in dimethyl sulfoxide (DMSO)-containing media has also been observed in human embryonic trachea (3) and nasal mucosa (4). Comparative pharmacological studies, however, on functional changes following storage at subzero temperatures of airway smooth muscles have not been performed up to now. The purpose of the present study was, therefore, to assess how well the function of airway smooth muscle was preserved after cryostorage in liquid nitrogen and whether this technique can be used to store human airway smooth muscle for subsequent pharmacological experiments. The postthaw recovery of human bronchi was investigated using three different media, namely fetal calf serum (FCS), Krebs-Henseleit solution (KH), and 50% FCS in KH as vehicles for the cryoprotecting agents DMSO and sucrose during storage at −196°C in liquid nitrogen.

MATERIALS AND METHODS

Tissue Preparation and Storage Method

Samples of human lungs, obtained from surgery for cancer (19 patients, mean age 61 years), were placed in Krebs-Henseleit (KH) solution (composition mM: NaCl 118, KCl 4.7, $MgSO_2$ 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. Small bronchi (inner diameter ≈ 2–4 mm) were carefully excised, cut into rings, and distributed well balanced into several groups. A first series of experiments consisted of two groups of human bronchi. The tissues of group 1, "unfrozen bronchi," were used after storage in KH solution overnight at 4°C for organ bath studies. The tissues of group 2, "cryopreserved bronchi," were placed in 2 ml Liquid Nitrogen Storage Ampoules (Gibco AG, Basel, Switzerland) filled with fetal calf serum (FCS) containing 1.8 M dimethyl sulfoxide (DMSO) and 0.1 M sucrose as the cryoprotecting agents, and were frozen as described below. In a second series of experiments three groups of cryopreserved human bronchi were investigated. In these experiments bronchial rings were placed into 2 ml Liquid Nitrogen Storage Ampoules containing either FCS, KH solution, or 50% FCS in KH (FCS + KH) as vehicles for the cryoprotecting agents DMSO (1.8 M) and sucrose (0.1 M). Following an equilibration time of about 30–60 min at room temperature, the ampoules were placed in a polystyrol box (11 × 11 × 22 cm) and slowly frozen at a mean cooling rate of about 0.6°c/min in a freezer maintained at −70°C. After 3–15 hr the ampoules were transferred into liquid nitrogen (−196°C), where they were stored for 3–4 weeks until use. Before use, the tissues were exposed for 10–20 min to −70°C and then thawed within 2.5 min by placing the ampoules in a 37°C waterbath. Thereafter the bronchial segments were rinsed in a dish containing KH solution at 37°C and suspended in 10-ml organ baths for isometric tension recording.

Experimental Procedure

The rings 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. The tension of the rings was recorded isometrically under a resting tension of 1 *g* with electromechanical transducers (Statham model UC 3) and a potentiometric recorder. The tissues were allowed to equilibrate for about 2–3 hr in the bathing medium. During this time the preparations were challenged once with car-

bachol (1 μM) and the baseline tension of the rings was readjusted to 1 *g* if required. Concentration-response curves for agonists were determined by cumulative additions, each concentration being added when the maximum effect had been produced by the previous concentration. Relaxant responses were investigated under resting tone. At the end of these concentration-response curves, papaverine (300 μM) was added to elicit complete relaxation of the bronchial rings.

Drugs

The following pharmacological agents were used: carbamylcholine chloride, histamine dihydrochloride, isoprenaline sulfate, papaverine hydrochloride (Sigma, Munich, Germany), bimakalim (Merck, Darmstadt, Germany). Bimakalim was dissolved in ethanol to give a 1-mM solution containing 10% ethanol. All compounds were dissolved just before use.

Data Analysis

Concentration-response curves were analyzed with a linear computer program and E_{max} (maximal effects), and pD_2 values (negative logarithm of the molar concentration of the agonist producing 50% of E_{max}) were derived from this analysis. Data are presented as means ± SEM. Statistical analysis was performed using the unpaired *t*-test, with a *p*-value < 0.05 considered to be significant.

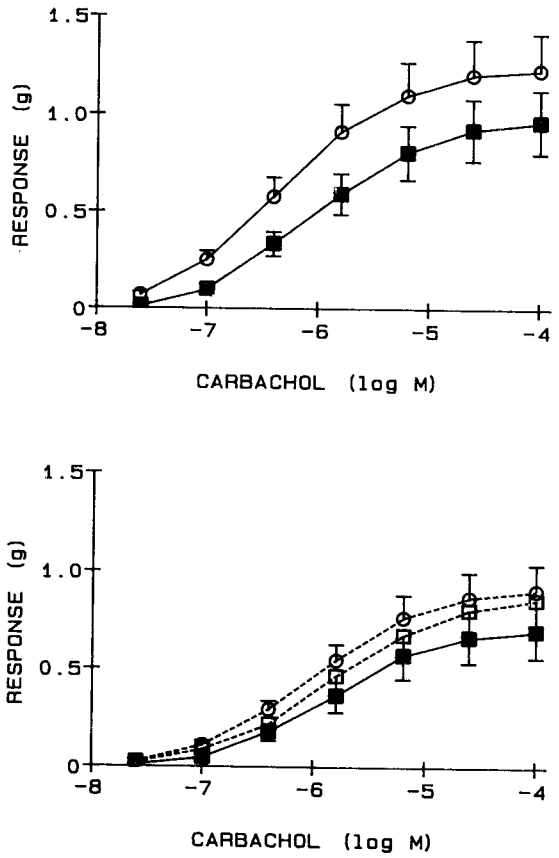

Figure 1. Cumulative concentration-response curves to carbachol in rings from human bronchi. (Top) Unfrozen (○——○) and after cryopreservation in FCS containing 1.8M DMSO and 0.1 M sucrose (■——■), for each point $n = 11$. (Bottom) After cryopreservation in 1.8 M DMSO and 0.1 M sucrose dissolved in FCS (■——■), KH (○——○), and 50% FCS in KH (□- - -□), for each point $n = 9$. The effects are expressed in *g*, the bars represent ± SEM.

RESULTS

Contractile Responses

After cryopreservation in a medium containing FCS as vehicle for the cryoprotecting agents DMSO and sucrose, the maximal contractile responses in *g* of human bronchi to carbachol (Fig. 1, upper traces) and histamine (Fig. 2, upper traces) were reduced by about 25% compared to that produced by unfrozen tissues. Moreover, the pD_2 values of both carbachol and histamine were 2 times lower ($p < 0.001$) than in the unfrozen airway smooth muscle preparations (Table 1). In a second series of experiments, bronchial rings were suspended in cryomedia containing three different vehicles, namely FCS, KH, or 50% FCS in KH, during the freezing/thawing process. Human bronchi that had been frozen in a cryomedium containing KH as the vehicle produced considerably higher contractile responses to both carbachol (Fig. 1, lower traces) and histamine (Fig. 2, lower traces) as compared to that elicited by tissues that had been frozen in cryomedium containing FCS as the vehicle. In these tissues the maximal contractile responses to both ago-

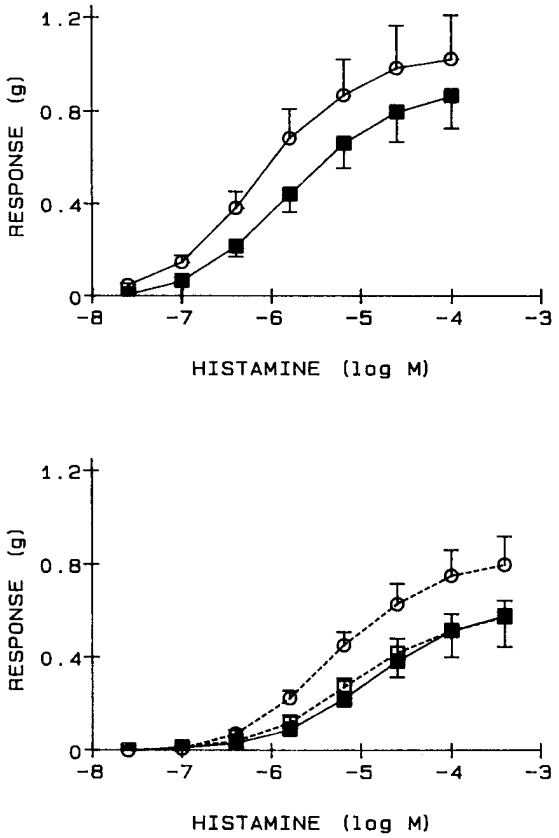

Figure 2. Cumulative concentration-response curves to histamine in rings from human bronchi. (Top) Unfrozen (○——○) and after cryopreservation in FCS containing 1.8 M DMSO and 0.1 M sucrose (■——■), for each point $n = 11$. (Bottom) After cryopreservation in 1.8 M DMSO and 0.1 M sucrose dissolved in FCS (■——■), KH (○- - -○), and 50% FCS in KH (□- - -□), for each point $n = 9$. The effects are expressed in g, the bars represent ± SEM.

nists were enhanced by 30% and the pD_2 values of both agonists were increased by a factor 2 (Table 2).

Relaxant Responses

Responses to relaxant agonists were investigated in both unfrozen and cryopreserved human bronchi under resting tone. In unfrozen bronchi, relaxant agonists such as isoprenaline, papaverine, and the potassium channel activator bimakalim (5) eliminated about 75% of the preexisting tone. In contrast, after cryopreservation in a medium containing FCS as the vehicle, the bronchial rings relaxed only by about 50% in response to these agonists (Figs. 3 and 4, upper traces). After maximal relaxation of cryopreserved bronchi by the combined action of papaverine and either isoprenaline or bimakalim, there was still a passive tension of 0.49 ± 0.04 g compared to a passive resting tension of 0.29 ± 0.04 g in unfrozen rings, the difference being statistically significant ($p < 0.001$, $n = 21$). The diminished elasticity of cryopreserved human bronchi was confirmed in the second series of experiments, where in each group of cryopreserved bronchi maximal relaxation by papaverine eliminated only about 50–60% of the preexisting tone. The use of different cryomedia did not modify the relaxant responses to either isoprenaline, bimakalim, or papaverine of frozen/thawed human bronchi (Figs. 3 and 4, lower traces; Table 2).

DISCUSSION

The present data extend and confirm the usefulness of cryopreservation for storing isolated organs for subsequent pharmacological studies. Recently it has been shown that the postthaw recovery of isolated blood vessels was considerably improved when 0.1 M sucrose had been added to the cryomedium consisting of 1.8 M DMSO in FCS (6). As demonstrated by our first series of experiments, human bronchi that had been frozen in FCS containing DMSO and sucrose as cryoprotectants retained about 75% of their initial contractility in response to carbachol and histamine. The degree of postthaw contractility of these bronchi was similar to that observed with cryopreserved human intrapulmonary arteries, which retain about 76% of their contractile force (7). In a second series of experiments, the FCS in the cryomedium was replaced by KH, which resulted in a considerable improvement of the contractile responsiveness to both carbachol and histamine, suggesting that a cryomedium consisting of KH as the vehicle for DMSO and sucrose may provide optimal postthaw recovery of human airway smooth muscle.

Table 1. Parameters for Various Contractile and Relaxant Effects on Rings from Human Bronchi Without (Unfrozen) and After Cryopreservation in Fetal Calf Serum (FCS) Containing 1.8 M DMSO and 0.1 M Sucrose

	UNFROZEN	FCS
pD_2 values		
Carbachol	6.32 ± 0.04 (11)	6.02 ± 0.05 (11)**
Histamine	6.20 ± 0.07 (11)	5.83 ± 0.06 (11)**
Isoprenaline	7.98 ± 0.07 (11)	8.33 ± 0.10 (11)*
Bimakalim	7.46 ± 0.06 (10)	6.93 ± 0.07 (10)**
E_{max} (g)		
Carbachol	1.22 ± 0.19 (11)	0.96 ± 0.17 (11)
Histamine	1.17 ± 0.18 (11)	0.86 ± 0.14 (11)
Isoprenaline	−0.91 ± 0.08 (11)	−0.46 ± 0.04 (11)**
Bimakalim	−0.65 ± 0.05 (10)	−0.44 ± 0.06 (10)*
Papaverine	−0.88 ± 0.06 (21)	−0.52 ± 0.04 (21)**

E_{max} = maximal response in g; pD_2 = negative logarithm of the molar concentration producing 50% of maximum response. Data are given as means ± SEM. Number of determinations in parentheses. Difference against values determined on unfrozen tissues significant at *$p < 0.05$; **$p < 0.001$.

Relaxant responses of cryopreserved airway smooth muscles proved to be somewhat less well preserved. Maximal relaxation in response to isoprenaline, bimakalim, or papaverine reached only about 60–70% of that obtained with unfrozen human bronchi and was similar with all three cryomedia. This attenuated relaxation of frozen/thawed human bronchi was reflected by an increased passive resting tension after maximal pharmacological dilatation, suggesting reduced elasticity of the bronchi after cryopreservation. Similar changes have also been reported for cryopreserved canine venous homografts, which displayed a reduction in compliance by about 50% as compared to fresh veins (8). From our present experiments, however, it cannot be ruled out that cold-induced damage of the

Table 2. Parameters for Various Contractile and Relaxant Effects on Rings from Human Bronchi After Cryopreservation in 1.8 M DMSO and 0.1 M Sucrose Dissolved in Fetal Calf Serum (FCS), in 50% FCS in Krebs-Henseleit solution (FCS + KH), and in Krebs-Henseleit solution (KH)

	FCS	FCS + KH	KH
pD_2 values			
Carbachol	5.83 ± 0.07 (9)	5.85 ± 0.12 (9)	6.01 ± 0.07 (9)
Histamine	5.12 ± 0.13 (9)	5.21 ± 0.16 (9)	5.42 ± 0.10 (9)*
Isoprenaline	8.01 ± 0.17 (10)	8.22 ± 0.13 (10)	8.35 ± 0.09 (10)*
Bimakalim	7.34 ± 0.03 (5)	7.29 ± 0.04 (5)	7.21 ± 0.03 (5)*
E_{max} (g)			
Carbachol	0.70 ± 0.14 (9)	0.86 ± 0.13 (9)	0.90 ± 0.13 (9)
Histamine	0.58 ± 0.14 (9)	0.57 ± 0.07 (9)	0.80 ± 0.12 (9)
Isoprenaline	−0.44 ± 0.08 (10)	−0.59 ± 0.13 (10)	−0.48 ± 0.09 (10)
Bimakalim	−0.42 ± 0.09 (5)	−0.44 ± 0.08 (5)	−0.31 ± 0.03 (5)
Papaverine	−0.50 ± 0.07 (15)	−0.60 ± 0.10 (15)	−0.47 ± 0.07 (15)

E_{max} = maximal response in g; pD_2 = negative logarithm of the molar concentration producing 50% of maximum response. Data are given as means ± SEM. Number of determinations in parentheses. Difference against values determined after cryopreservation in medium containing FCS as the vehicle significant at *$p < 0.05$.

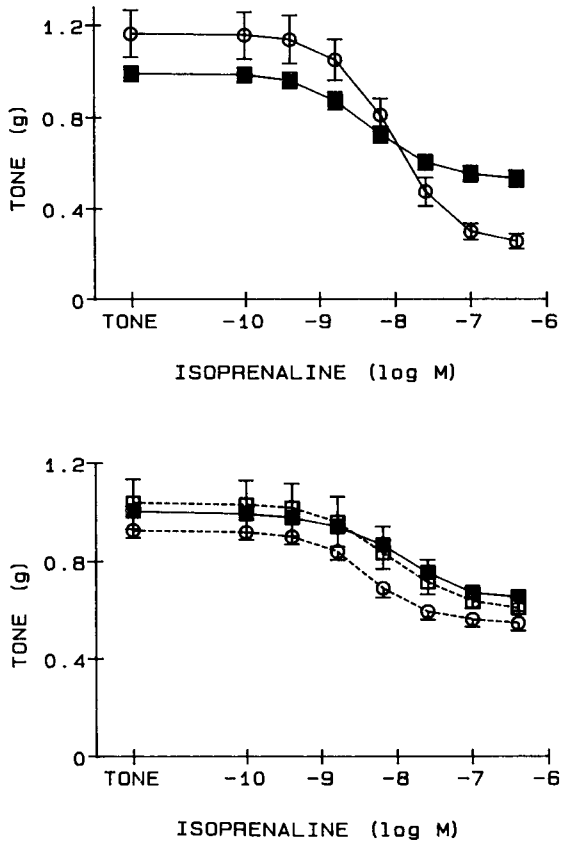

Figure 3. Cumulative concentration-response curves to isoprenaline in rings from human bronchi during spontaneous tone. (Top) Unfrozen (O——O) and after cryopreservation in FCS containing 1.8 M DMSO and 0.1 M sucrose (■——■), for each point $n = 11$. (Bottom) After cryopreservation in 1.8 M DMSO and 0.1 M sucrose dissolved in FCS (■——■), KH (O- - -O), and 50% FCS in KH (□- - -□), for each point $n = 10$. The effects are expressed in g, the bars represent ± SEM.

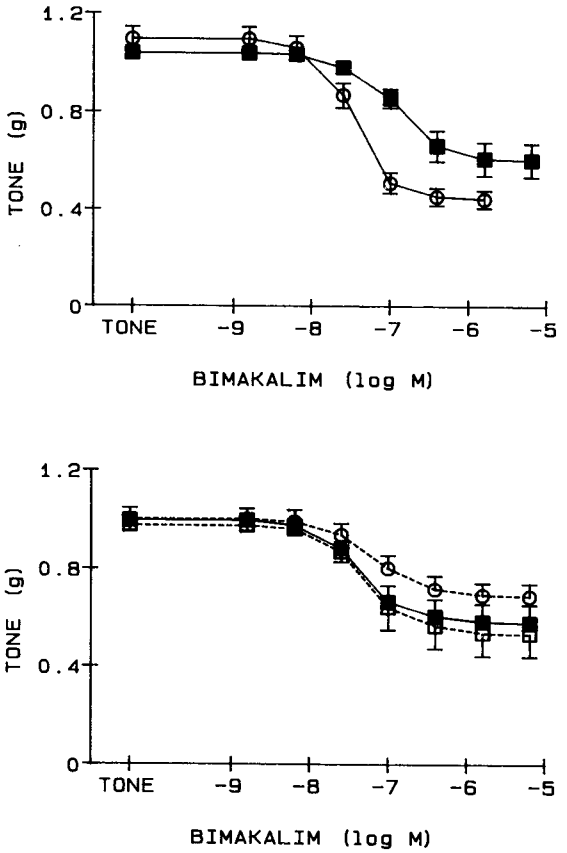

Figure 4. Cumulative concentration-response curves to bimakalim in rings from human bronchi during spontaneous tone. (Top) Unfrozen (O——O) and after cryopreservation in FCS containing 1.8 M DMSO and 0.1 M sucrose (■——■), for each point $n = 10$. (Bottom) After cryopreservation in 1.8 M DMSO and 0.1 M sucrose dissolved in FCS (■——■), KH (O- - -O), and 50% FCS in KH (□- - -□), for each point $n = 10$. The effects are expressed in g, the bars represent ± SEM.

hyaline cartilage (2) also contributed to the enhanced rigidity of cryopreserved bronchi.

There is increasing evidence that in both human and animal bronchi the epithelium modulates the reactivity to various agents by the release of a nonprostanoid inhibitory factor (EpDIF) and/or by acting as a site of loss (i.e., site of metabolism) of drugs from the biophase (9). From the present data, damage of the bronchial epithelium during the freezing/thawing process cannot be excluded. However, histological examinations have demonstrated that cryopreservation in DSMO-containing media of airway tissues from dog and human preserves normal epithelium with mucus production and well-retained ciliary activity (2–4). It seems rather unlikely, therefore, that the observed post-thaw functional changes were due to epithelial damage. Furthermore, in the present experiments responses of cryopreserved human bronchi to both contractile and relaxant agents were generally reduced, whereas removal of the epithelium increases the contractile sensitivity in response to histamine but does not modify relaxant responses to isoprenaline (10,11). Nevertheless, further experiments are required to clarify whether

the pharmacological function of the epithelium is maintained after cryopreservation of human bronchi.

In conclusion, the present experiments suggest that despite some reduction in the elasticity, cryopreservation of human bronchi in KH containing 1.8 M DMSO and 0.1 M sucrose preserves both contractile and relaxant mechanisms and offers clear potential for storing human airway smooth muscle for subsequent pharmacological experiments.

ACKNOWLEDGMENT

Grateful acknowledgment is made to M. Bretz and F. Stimpfling for valuable technical assistance.

REFERENCES

1. Müller-Schweinitzer E: Cryopreserved human tissue in pharmacological research. *Pharmacol Res* 25:103 (1992).
2. Deschamps C, Trastek VF, Ferguson JL, Martin WJ, Colby TV, Pairolero PC, Payne WS: Cryopreservation of canine trachea: Functional and histological changes. *Ann Thorac Surg* 47:208 (1989).
3. Morris GJ, Buckland R, Rubenstein D, Farrant J: Preservation of human tracheal organ cultures at −196°C. *J Med Microbiol* 6:123 (1973).
4. Wulffraat NM, Veerman AJP, Stamhuis IH: Frequency and coordination of ciliary beat after cryopreservation of respiratory epithelium. *Cryobiology* 22:105 (1985).
5. Gericke R, Harting J, Lues I, Schittenhelm C: 3-Methyl-^{2}H-1-benzopyran potassium channel activators. *J Med Chem* 34:3074 (1991).
6. Müller-Schweinitzer E, Ellis P: Sucrose promotes the functional activity of blood vessels after cryopreservation in DMSO-containing fetal calf serum. *Naunyn-Schmiedeberg's Arch Pharmacol* 345:594 (1992).
7. Ellis P, Müller-Schweinitzer E: Maintenance of functional activity of human pulmonary arteries after cryopreservation. *Br J Pharmacol* 103:1377 (1991).
8. Showalter D, Durham S, Sheppeck R, Berceli S, Greisler H, Brockman K, Makaroun M, Webster M, Steed D, Siewers R, Borovetz H: Cryopreserved venous homografts as vascular conduits in canine carotid arteries. *Surgery* 106:652 (1989).
9. Goldie RG, Fernandes LB, Farmer SG, Hay DWP: Airway epithelium-derived inhibitory factor. *TiPS* 11:67 (1990).
10. Aizawa H, Miyazaki N, Shigematsu N, Tomooka M: A possible role of airway epithelium in modulating hyperresponsiveness. *Br J Pharmacol* 93:139 (1988).
11. Fernandes LB, Preuss JMH, Paterson JW, Goldie RG: Epithelium-derived inhibitory factor in human bronchus. *Eur J Pharmacol* 187:331 (1990).