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Effect of Molsidomine and Linsidomine on the Human Isolated Bronchus and the Guinea-pig Isolated Trachea

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Abstract—The effects of molsidomine and its metabolite linsidomine were studied on the guinea-pig isolated trachea and on the human isolated bronchus. These effects were compared with those of nitrate derivatives (sodium nitroprusside, isosorbide dinitrate), theophylline, zardaverine and isoprenaline. Linsidomine exerted a relaxant effect similar to that of sodium nitroprusside on the two types of preparations precontracted with acetylcholine, histamine or potassium chloride. Molsidomine was about one-hundredth as potent as linsidomine, and less efficacious. The effects of the two substances were not modified by removal of the human bronchial epithelium. The concentration-response curves of linsidomine and sodium nitroprusside were significantly shifted to the right by methylene blue (3×10^{-5} M) but the effects of isoprenaline were unmodified. The effects of linsidomine and sodium nitroprusside were potentiated specifically by zaprinast (10^{-6}–10^{-5} M), an inhibitor of type Ia or V phosphodiesterases, whereas the effects of isoprenaline were potentiated by zardaverine (10^{-9}–10^{-8} M), an inhibitor of class III and IV phosphodiesterases. The effects of all three substances (linsidomine, isoprenaline and sodium nitroprusside) were potentiated equally by theophylline (10^{-5}–10^{-4} M), a nonspecific inhibitor of phosphodiesterases. It is concluded that linsidomine is a potent relaxant of the smooth muscle of the guinea-pig isolated trachea and human isolated bronchus. In terms of potency and efficacy, its effect is much superior to that of the parent compound molsidomine. It is suggested that linsidomine acts, like nitrate derivatives, through the guanylate cyclase-cGMP system.

Molsidomine (*N*-ethoxycarbonyl-3-morpholino-sydnonimine) is a vasodilator agent used in the clinical treatment of ischaemic coronary disease (Guerchicoff et al 1978; Karsch et al 1978; Majid et al 1980; Detry et al 1981). Its vasodilator effects resemble those of nitrovasodilators and are thought to be mediated by release of nitric oxide (NO) and by stimulation of cyclic (c) GMP (Miller & Vanhoutte 1990).

Molsidomine is extensively metabolized in the liver to linsidomine (3-morpholino-sydnonimine, SIN-1) and then, nonenzymatically, to SIN-1A (*N*-nitroso-*N*-morpholino-aminoacetonitrile) and SIN-1C (*N*-cyanomethylamino-morpholine) (Noack & Feelisch 1990). Linsidomine is an active metabolite of the prodrug molsidomine releasing NO spontaneously (Miller & Vanhoutte 1990; Noack & Feelisch 1990). In this study, we endeavoured to determine whether the relaxant effects of molsidomine and its active metabolite linsidomine on blood vessels also applied to the airway smooth muscle.

Materials and Methods

Guinea-pig isolated trachea

Male guinea-pigs, 250–350 g, were killed by a blow on the head and exsanguinated. The trachea was removed and placed in Krebs solution (composition mM; NaCl 118, KCl 5·4, $CaCl_2$ 2·5, KH_2PO_4 0·6, $NaHCO_3$ 25 and glucose 11·7, pH 7·4). Following removal of adhering fat and connective tissue, the trachea was cut into 6–8 rings.

In some experiments, the epithelium of one of the rings was removed by gently rubbing the luminal surface (over both the smooth muscle and cartilage areas) with a cotton-tipped applicator (Devillier et al 1988); the other strips served as paired controls.

The strips were then suspended in 10 mL organ baths containing Krebs solution at 37°C, gassed with 95% O_2–5% CO_2 and equilibrated under an initial tension of 1·80 g. After equilibration for 1·25 h, the resting tension was between 0·6 and 1·4 g. Under these conditions, responses to agonists were reproducible. Tension was measured isometrically with Celaster strain gauges and Celaster amplifiers (Dei Lierre Electronique, Mitry-Mory, France) and displayed on Linseis recorders (Linseis, Selb, Germany).

Human bronchus

Human bronchial tissues were obtained from patients undergoing surgery for lung cancer. Just after resection, segments of human bronchi with an inner diameter of 4–6 mm were taken as far away as possible from the malignancy. They were dissected free of parenchyma and transported to the laboratory in Krebs solution previously aerated with a mixture of 95% O_2–5% CO_2. The tissue was stored overnight at 4°C, and the experiment was carried out the following day. After removal of adhering fat and connective tissue, two to eight rings of the same bronchus were prepared. Each bronchial ring was suspended in Krebs solution under initial tension of 2·5 g and treated in every respect as the guinea-pig isolated tracheal strips.

Protocols

In all experiments, preparations were first tested for maximal tension with acetylcholine (3 mM), then allowed to rest for at least 1·5 h, during which time, washing was performed every 15 min.

In a first series of experiments, the tracheal rings or the human bronchi were contracted to 70–90% of maximal

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Table 1. Relaxant effects on the human isolated bronchus.

	Acetylcholine (10^{-4} M)			Histamine (10^{-5} M)			KCl (10^{-2} M)		
	n	−log EC50	E_{max}	n	−log EC50	E_{max}	n	−log EC50	E_{max}
Linsidomine	7	4·57±0·09	80±4·1	5	5·06±0·03	96±1·0	6	4·65±0·16	91±2,7
Molsidomine	7	3·38±0·07***	52±8·0*†	5	3·15±0·09***	55±3·3***†	5	3·05±0·05***	54±4·3**†
Sodium nitroprusside	5	5·23±0·38	81±2·5	5	5·01±0·09	91±2·0	6	5·16±0·18	84±2·0
Isosorbide dinitrate	5	3·62±0·17***	56±7·4*†	5	4·56±0·14*	86±4·1†	5	3·43±0·07***	70±3·2**†
Isoprenaline	6	6·47±0·17***	96±1·5*	5	7·74±0·04***	99±1·2	6	5·72±0·19**	84±8·9
Theophylline	6	4·37±0·14	100**	4	4·71±0·21	100*	6	3·93±16**	100*
Zardaverine	6	5·95±0·59*	96±2·7*	5	8·12±0·16***	99±0·5*	6	5·45±0·25*	98±1·0*

The preparations were precontracted with acetycholine, histamine or KCl. Their ability to relax the airway smooth muscle is expressed by E_{max} (maximal effect in relation to theophylline 3 mM) (efficacy) and by −log EC50 (concentration producing 50% of the maximal effect of theophylline 3 mM) (potency). n = number of experiments. Significant differences from linsidomine are shown as: *$P<0·05$; **$P<0·01$; ***$P<0·001$. †Effect observed for the maximal concentration tested: molsidomine 10^{-3} M; isosorbide dinitrate 10^{-3} M.

contraction with acetylcholine (10^{-4} M), histamine (10^{-4} or 10^{-5} M) or KCl (10^{-2} M). When a stable contraction was obtained, cumulative concentration-response curves to drugs were obtained by adding increasing concentrations of the drugs at 5–15 min intervals. Once the concentration-response curve was completed, theophylline (3 mM) was added to the bath to determine maximal relaxation.

In a second series of experiments performed on parallel segments of human bronchi, the concentration-response curves to molsidomine and linsidomine were established on the preparations precontracted with histamine (10^{-5} M) and in the presence or absence of epithelium. The results were expressed as a percentage of maximal relaxation induced by theophylline (3 mM).

In the third series of experiments, the tracheal rings or the human bronchi were pretreated for 1 h with methylene blue (3×10^{-5} M) or for 15 min with theophylline (10^{-4} or 10^{-5} M), zaprinast (10^{-7}–10^{-5} M) or zardaverine (10^{-9}–10^{-6} M), and then contracted with histamine 10^{-5} M (human bronchi) or 10^{-4} M (guinea-pig trachea). When a stable contraction was obtained, cumulative concentration-response curves to isoprenaline, sodium nitroprusside or linsidomine were obtained as previously described. Owing to the development of tachyphylaxis, only one series of concentration-response curves was obtained from each tracheal preparation in each series of experiments.

Experiments performed with isoprenaline, molsidomine and linsidomine were carried out in an organ bath protected from the light.

Relaxation was expressed as a percentage of the effect induced by theophylline (3 mM), and −log EC50 values were calculated from the log concentration-effect curves. They are defined as the −log concentration of the drug that causes 50% of the maximal relaxation induced by 3 mM theophylline.

Statistical analysis

Statistical analysis was performed using analysis of variance or Student's *t*-test. All values were expressed as mean ± s.e.m. *P* values below 0·05 were considered to be significant.

Drugs

The drugs used were: theophylline sodium anisate (Laboratoires Delalande, Paris, France), acetylcholine di-HCl (Pharmacie Centrale des Hôpitaux, Paris, France), histamine HCl, KCl (Prolabo, Paris, France), isoprenaline HCl (Winthrop, Paris, France), zardaverine (Byk-Gulden, Konstanz, Germany), linsidomine and molsidomine (Hoechst, Paris la

Table 2. Relaxant effects on the guinea-pig isolated trachea.

	Acetylcholine (10^{-4} M)			Histamine (10^{-4} M)			KCl (10^{-2} M)		
	n	−log EC50	E_{max}	n	−log EC50	E_{max}	n	−log EC50	E_{max}
Linsidomine	11	5·12±0·17	86±3·7	5	5·05±0·14	92±2·6	8	5·60±0·29	92±2·8
Molsidomine	9	2·86±0·15***[a]	46±2·5***†	4	3·39±0·08***	63±7·1*†	4	3·37±0·18**	61±2·9**†
Sodium nitroprusside	6	5·56±0·23	66±7·8*	4	5·03±0·33	85±5·5	8	6·32±0·23	89±2·7
Isosorbide dinitrate	7	3·49±0·16***	65±5·8*†	5	4·64±0·60	89±4·7†	9	4·58±0·39	82±5·6†
Isoprenaline	13	6·58±0·09***	92±2·1	5	7·97±0·13***	98±1·4	8	6·50±0·23*	98±1·1
Theophylline	8	3·68±0·17***	100**	4	4·36±0·05**	100*	8	4·68±0·20*	100*
Zardaverine	5	5·68±0·31	65±4·7*	5	7·35±0·47**	100*	8	6·55±0·23*	98±0·7

The preparations were precontracted with acetycholine, histamine or KCl. Their ability to relax the airway smooth muscle is expressed by E_{max} (maximal effect in relation to theophylline 3 mM) (efficacy) and by −log EC50 (concentration producing 50% of the maximal effect of theophylline 3 mM) (potency). n = number of experiments. Significant differences from linsidomine are shown as: *$P<0·05$; **$P<0·01$; ***$P<0·001$. † Effect observed for the maximal concentration tested: molsidome 10^{-3} M; isosorbide dinitrate 10^{-3} M. [a] Extrapolated value.

Défense, France), isosorbide dinitrate (Merrell Dow, Levallois, France), zaprinast (Rhône Poulenc Rorer, Dagenham, UK). sodium nitroprusside (Sigma, St Louis, USA).

All drugs were dissolved daily in distilled water, except for zardaverine and isosorbide dinitrate which were dissolved in dimethylsulphoxide. Dilutions were made with Krebs solution.

Results

Relaxant effects of molsidomine and linsidomine. Comparison with reference bronchodilators

The characteristics of the relaxant effects of molsidomine, linsidomine and different bronchodilators on the human isolated bronchus and the guinea-pig isolated trachea precontracted with acetylcholine (10^{-4} M), histamine (10^{-4} or 10^{-5} M) or KCl (10^{-2} M) are shown in Tables 1 and 2.

The activity of linsidomine on the guinea-pig tracheal smooth muscle was similar to that of sodium nitroprusside in terms of potency (−log EC50) and efficacy (E_{max}) against histamine and KCl. Against acetylcholine, linsidomine was significantly more efficacious (higher E_{max}) than sodium nitroprusside; it was also more potent and more efficacious than isosorbide dinitrate. Compared with other relaxant agents, linsidomine was less potent than isoprenaline but considerably more potent than theophylline.

In these experiments, molsidomine was about one-hundredth as potent as its metabolite linsidomine, and was also less efficacious.

Similar results were obtained on the isolated human bronchus. In terms of potency, the effect of linsidomine against all bronchoconstrictors was similar to that of sodium nitroprusside, but linsidomine was more active than isosorbide dinitrate. As in experiments conducted on the guinea-pig trachea, the relaxant effect of molsidomine on the human bronchus was considerably less than that of linsidomine.

Neither in guinea-pig trachea nor in human isolated bronchi did zaprinast induce a relaxant response.

Influence of bronchial epithelium removal on the effects of linsidomine and molsidomine

Fig. 1 shows that the concentration-response curves to linsidomine and molsidomine were identical on the human isolated bronchus with or without epithelium (n = 7).

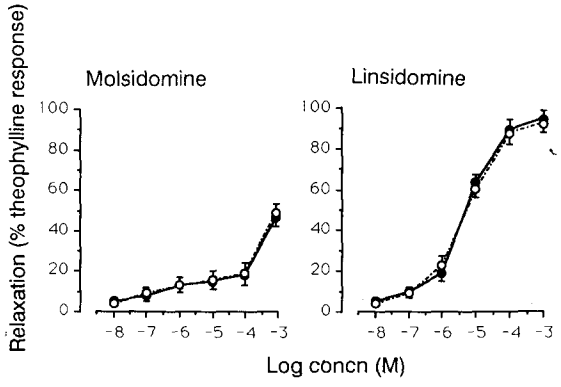

FIG. 1. Influence of epithelium on the effects of molsidomine and linsidomine on human bronchi. ● with epithelium; ○ without epithelium. Values are mean ± s.e.m. n = 7.

Influence of methylene blue on the effects of isoprenaline, sodium nitroprusside and linsidomine on the human isolated bronchus and the guinea-pig isolated trachea

The effects of methylene blue (3×10^{-5} M) on the concentration-response curves to isoprenaline, sodium nitroprusside and linsidomine are shown in Fig. 2. The relaxation of the human bronchus and the guinea-pig trachea induced by sodium nitroprusside and linsidomine was significantly reduced by methylene blue (n = 5, $P < 0.05$ to $P < 0.001$), as indicated by the shift to the right of the concentration-response curves. On the other hand, methylene blue had no significant effect on the isoprenaline concentration-response curves in either type of preparations.

Influence of theophylline, zaprinast and zardaverine on the concentration-response curves to isoprenaline, sodium nitroprusside and linsidomine

Theophylline. At concentrations as low as 10^{-4} M, theophyl-

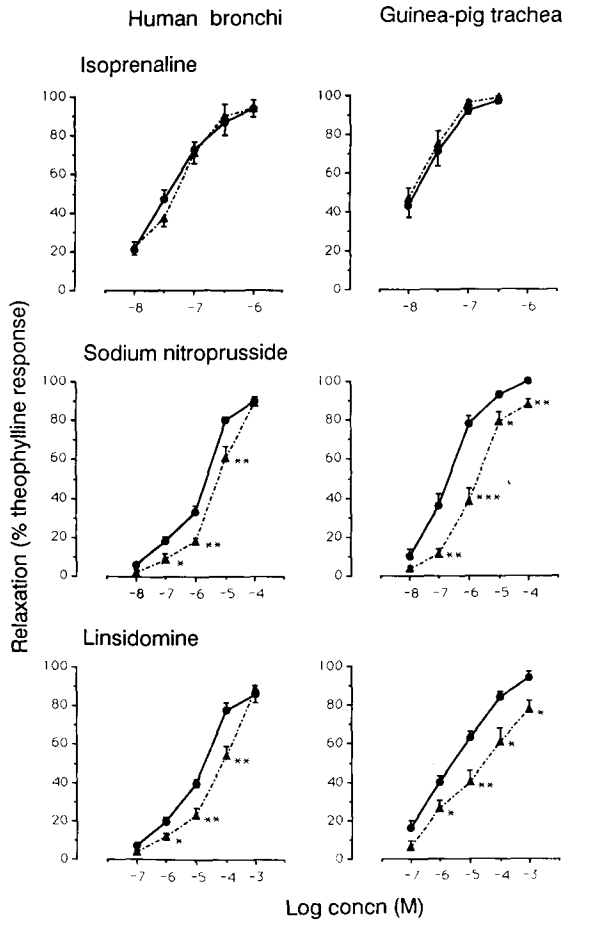

FIG. 2. Influence of methylene blue on the relaxant effect of isoprenaline (n = 5), sodium nitroprusside (n = 5) or linsidomine (n = 5) on the human bronchus and guinea-pig isolated trachea smooth muscle. Concentration-response curves: ● controls, ▲ pretreated with methylene blue 3×10^{-5} M. Values are mean ± s.e.m. Significant differences with the control curves are indicated by $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

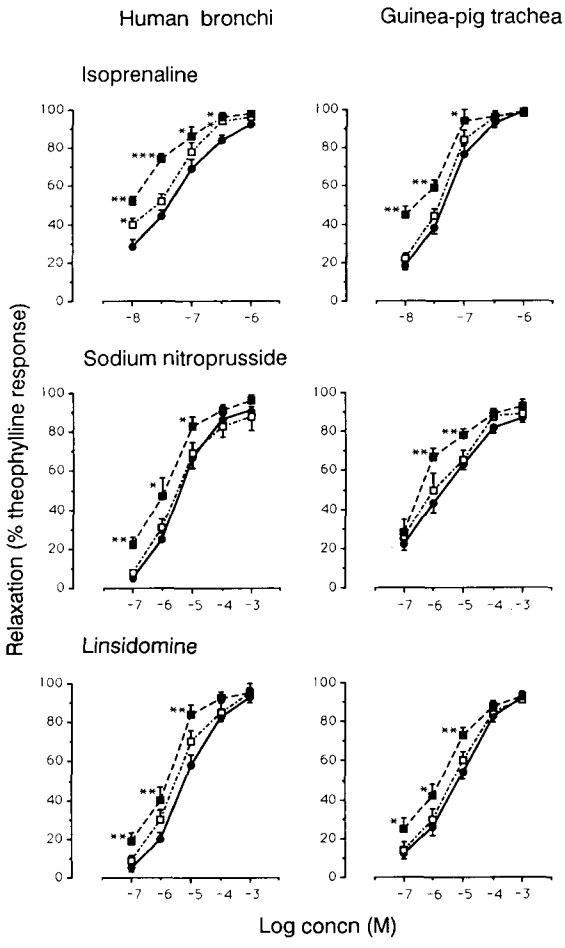

FIG. 3. Influence of theophylline on the relaxant effects of isoprenaline (n = 5), sodium nitroprusside (n = 5) or linsidomine (n = 5) on the human bronchus and guinea-pig isolated trachea smooth muscle. Concentration-response curves: controls (●), pretreated with theophylline 10^{-5} (□) or 10^{-4} M (■). Values are mean ± s.e.m. Significant differences from the control curves are indicated by $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

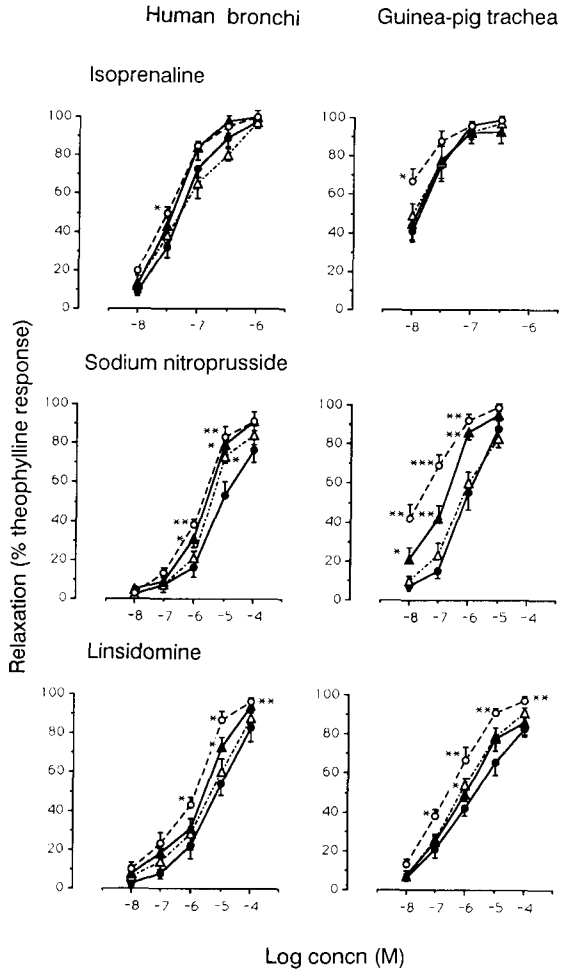

FIG. 4. Influence of zaprinast on the relaxant effects of isoprenaline (n = 5-7), sodium nitroprusside (n = 5) or linsidomine (n = 5-7) on the human bronchus and guinea-pig isolated trachea smooth muscle. Concentration-response curves: controls (●) and pretreated with zaprinast 10^{-6} (△), 10^{-5} (▲) or 10^{-4} M (○). Values are mean ± s.e.m. Significant differences from the control curves are indicated by $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

line potentiated the relaxant effects of sodium nitroprusside, isoprenaline and linsidomine in both types of preparations (Fig. 3).

Zaprinast. The relaxant effects of sodium nitroprusside and linsidomine were significantly potentiated by zaprinast in concentrations of 10^{-5} M on the human bronchus and on the guinea-pig trachea (n = 5-6, $P < 0.05$). This effect of zaprinast was dose-dependent. Under the same conditions, zaprinast had little influence on the effect of isoprenaline (Fig. 4).

Zardaverine. Fig. 5 shows that zardaverine at a concentration of 10^{-9} M significantly potentiated the effect of isoprenaline on the human bronchus and the guinea-pig trachea (n = 5, $P < 0.05$). The effect of sodium nitroprusside was potentiated at a concentration of 10^{-8} M in the human bronchus (n = 5, $P < 0.05$) and at a concentration of 10^{-6} M in the guinea-pig trachea (n = 5, $P < 0.05$). The relaxant effect of linsidomine on both types of preparations was potentiated by zardaverine at concentrations of 10^{-8} or 10^{-7} M (n = 5, $P < 0.05$).

Discussion

Our results clearly show that linsidomine exerts a considerable relaxant effect on the bronchial smooth muscle and that this effect is comparable, in both potency and efficacy, from that of sodium nitroprusside on the human bronchus and guinea-pig trachea. Linsidomine proved to be 3- to 40-fold more potent than isosorbide dinitrate. Its effect was little modified by the nature of the contracting agent.

These findings are similar to those of Gruetter et al (1981), who worked on the bovine isolated trachea and observed that isosorbide dinitrate was less potent and less efficacious than sodium nitroprusside. They are also in agreement with the results of Miller & Vanhoutte (1990), who found that in terms of potency and efficacy linsidomine and sodium nitroprusside had about the same vasodilator effects on the dog femoral artery and femoral vein. Those authors also

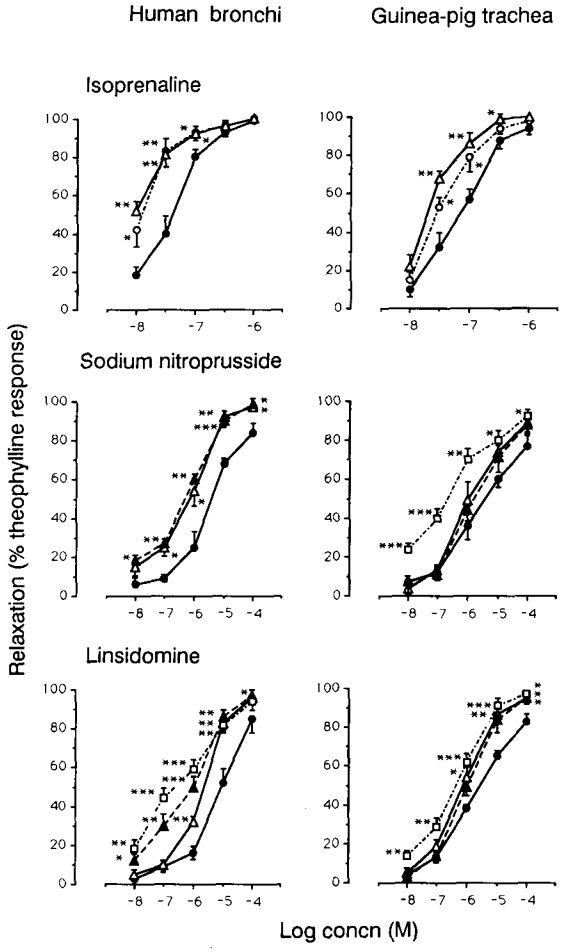

FIG. 5. Influence of zardaverine on the relaxant effects of isoprenaline (n = 5–7); sodium nitroprusside (n = 5) or linsidomine (n = 5–7) on the human bronchus and guinea-pig trachea smooth muscle. Concentration-response curves: controls (●) and pretreated with zardaverine 10^{-9} (○), 10^{-8} (△), 10^{-7} (▲) or 10^{-6} M (□). Values are mean ± s.e.m. Significant differences from control curves are indicated by $*P<0.05$; $**P<0.01$; $***P<0.001$.

observed that the effects of the two substances were independent of the contracting agent on the femoral artery model but not on the femoral vein model. We too observed this lack of influence of the contracting agents.

It must be noted that sodium nitroprusside and linsidomine were less potent on the airways of guinea-pig and man than on dog vessels, since when the preparations were contracted by noradrenaline the −log EC50 of linsidomine was 6·8 on the femoral vein and 7·5 on the femoral artery (Miller & Vanhoutte 1990). As regards in-vitro studies of human preparations, Lüscher et al (1989), working on the human internal mammary artery contracted with noradrenaline, reported a −log EC50 of 6·6 ± 0·1 (n = 8) with linsidomine, whereas on the human bronchus the −log EC50 of this compound ranged from 4·57 to 5·06, depending on the contracting agent.

Our experiments also show that, unlike linsidomine, molsidomine exerts a relaxant effect on the airway smooth muscle only in concentrations of 10^{-4}–10^{-3} M; its maximal effect seems to be 45–60% of that of theophylline. These findings are concordant with the fact that linsidomine is the active metabolite of molsidomine, and they suggest that the tracheal and bronchial preparations used in our experiments were devoid of the enzymes required to transform molsidomine into linsidomine. This transformation seems to take place mainly in the liver (Tanayama et al 1974).

Another result of our study was that removing the epithelium did not modify bronchial relaxation. The epithelium plays a major role in regulating the effects of numerous pharmacological agents, and several theories have been put forward to explain the epithelium-related modulation of the airways. The epithelium may act as a diffusion barrier (Small et al 1990; Iriarte et al 1990). Other authors have suggested that the epithelium releases a relaxant factor (Ep-DIF) which could be a prostanoid derived from the arachidonic acid cascade (PGE_2) (Gao & Vanhoutte 1988; Ullman et al 1990), or a non-prostanoid substance (Fernandes & Goldie 1990; Goldie et al 1990).

The influence of epithelium on the effects of bronchial smooth muscle relaxants has not yet been fully established, and it has given rise to conflicting reports. Thus, Farmer et al (1986) and Candenas et al (1990) have shown that the relaxant effect of papaverine or theophylline is not modified by epithelium abrasion, whereas Goldie et al (1986) and Lundblad & Persson (1988) have observed a reduction of theophylline E_{max} after removal of epithelium. Our present study shows that none of the effects attributed to the epithelium plays a role in the relaxant action of linsidomine and molsidomine, and in particular, no metabolic effects which might have revealed the action of molsidomine in our experiments.

Concerning the mechanism of action of linsidomine, our results clearly support the hypothesis that NO spontaneously released from the metabolite of molsidomine triggers the cGMP system (Katsuki et al 1977; Ignarro & Kadowitz 1981; Griffith et al 1985; Försterman et al 1986). The action of linsidomine, as that of sodium nitroprusside, is reduced by methylene blue, a substance that inhibits the activation of guanylate cyclase in its soluble form by NO donating drugs (Katsuki et al 1977; Gruetter et al 1981). Rhoden & Barnes (1990) have already shown that methylene blue inhibits the effect of sodium nitroprusside on the guinea-pig isolated trachea. This is further demonstrated by the use of phosphodiesterase inhibitors: the effects of linsidomine on the human bronchus and the guinea-pig trachea are potentiated by theophylline, a nonspecific inhibitor of cGMP and cAMP phosphodiesterases (Fredholm et al 1979), and by zaprinast, a specific inhibitor of cGMP low specific affinity phosphodiesterase (Frossard et al 1981; Torphy et al 1985; Weishaar et al 1986; Rhoden & Barnes 1990; Chilvers et al 1991), or the Ca^{2+}/calmodulin-independent phosphodiesterase Ia (Hidaka & Endo 1984; Torphy & Cieslinski 1990), also called phosphodiesterase V by Nicholson et al (1991) and Beavo & Reifsnyder (1990).

Conversely, the effects of linsidomine are potentiated by zardaverine only in concentrations up to 10^{-7} and 10^{-8} M, whereas zardaverine potentiates isoprenaline in concentrations of 10^{-9} M. This supports the contention that zardaverine is a specific inhibitor of the cAMP-degrading phosphodiesterases type III and IV (Schulz et al 1989; Galvan & Schudt 1990; Nicholson et al 1991).

This study has shown that molsidomine has little relaxant effect on the bronchial smooth muscle, whereas linsidomine, its active metabolite, is a potent relaxing agent acting, like nitrate derivatives, through the cGMP system.

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