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Effects of Potassium-channel Opener HOE 234 in Guinea-pig Airways

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Abstract

The mechanism of the bronchospasmolytic effect of HOE 234 ((3*S*,4*R*)-3-hydroxy-2,2-dimethyl-4-(2-oxo-1-pyrrolidinyl)-6-phenylsulphonylchromane hemihydrate), a novel opener of ATP-sensitive potassium channels, has been studied by in-vitro testing in ring preparations of trachea and different parts of the principle bronchus of guinea-pigs, using methacholine, histamine and KCl as preconstrictors. The contribution of prostanoids was estimated in the presence and absence of indomethacin. Regional differences in the bronchodilatory effect of HOE 234 were compared with that of salbutamol.

HOE 234 had a concentration-dependent relaxing effect on the smooth muscle preparations. In the absence of indomethacin no regional differences were seen for the effect of HOE 234 against the metacholine or KCl preconstriction, whereas with histamine a particularly strong relaxation was detectable in trachea. In the presence of indomethacin, distal bronchus after methacholine and trachea after KCl preconstriction were relaxed significantly more strongly than the other parts. The relaxation of the histamine-constricted trachea rings was not increased in comparison with that without indomethacin pretreatment. Thus the bronchospasmolytic effect of HOE 234 varied depending on the pretreatment, method of preconstriction and part of the airways examined. It was strongest in trachea rings with histamine preconstriction but its potency was clearly less than that of salbutamol. The latter had regionally different effects, being stronger in trachea than in the bronchi for all methods of preconstriction.

The results suggest that the formation of bronchoconstricting prostanoids can attenuate parts of the relaxing effect of HOE 234. The possible advantages of HOE 234 as an anti-asthma drug might be related to an effect on bronchial hyper-reactivity and mucus secretion, because as a direct bronchodilator it is inferior to salbutamol.

Despite intensive research on the pathophysiology of asthma and the development of new anti-asthma compounds, this disease is still a therapeutic problem because its incidence is increasing world-wide and the late phase reactions are still difficult to abolish (Barnes 1992). As asthma is nowadays widely regarded as an inflammatory process, interest in the mechanisms of bronchodilation had decreased until the renewal of the controversy about the bronchodilatory first-line drugs of acute asthma, especially the β_2-agonist fenoterol, in connection with deterioration of disease control and increased asthma mortality (Lipworth & McDevitt 1992). A group of compounds originally thought to be non-selective smooth muscle relaxants, but later recognized as K^+ channel openers might, in addition to their capacity to relax vessels, also have bronchodilating effects in asthma. They seemed, therefore, to be an exciting approach to the development of new and more effective bronchodilators (Cook & Chapman 1993).

In the smooth muscle of the respiratory tract, opening of ATP-sensitive K^+ channels results in hyper-polarization and possibly reduction of intracellular Ca^{2+} release or storage, which results in relaxation (Raeburn & Karlsson 1991). The novel benzopyrane potassium channel opener HOE 234 ((3*S*,4*R*)-3-hydroxy-2,2-dimethyl-4-(2-oxo-1-pyrrolidinyl)-6-phenylsulphonylchromane hemihydrate) was reported to be more potent and longer acting than lemakalim both in guinea-pig and in human airways in-vitro and in guinea-pigs in-vivo (Englert et al 1992; Miura et al 1993). Our aim was to study the bronchorelaxing effect of HOE 234 in greater detail, in different regions (trachea (T), proximal (B_{prox}) and distal (B_{dist}) principle bronchi) of the guinea-pig airways in-vitro. The contribution of prostanoids was studied using the cyclooxygenase inhibitor indomethacin. The potency of HOE 234 was, moreover, compared with that of the reference compound salbutamol.

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Materials and Methods

Animal housing

Guinea-pigs (350–550 g) of either sex from the short-haired tricoloured breed of the Public Institute of Health, Helsinki (Finland) were housed for at least 7 days before experiments in standardized Macrol Type IV cages (two animals per cage) with bedding from Tapvei (Kaavi, Finland). The cages were kept in a room at an ambient temperature of 21 ± 1°C, with 40% humidity, controlled light cycles (illumination from 0700 to 1900 h) and ventilation (change of the whole air volume of the room 10 times h^{-1}). The animals were provided with a continuous supply of food (K5, Lactamin, Stockholm, Sweden) and water was freely available. All experimental procedures were approved by the Animal Experiment Committee of the University of Helsinki.

Tissue preparation

The animals were anaesthetized with pentobarbital sodium (75 mg kg^{-1}) and decapitated. Thereafter trachea and main bronchi were removed immediately, placed in a modified Krebs-Ringer solution and cleaned quickly from connective tissue. Trachea rings were cut including two cartilage rings. From the

principle bronchus of either side one proximal and one distal part were taken, each 3 mm wide. The samples were mounted in water-jacketed organ baths (37°C) containing Krebs-Ringer solution of composition (mM): NaCl 119, $NaHCO_3$ 25, glucose 11·1, $CaCl_2 \cdot H_2O$ 1·6, KCl 4·7, KH_2PO_4 1·2, $MgSO_4 \cdot 7H_20$ 1·2. The solution was oxygenated with a mixture of 96% O_2 and 4% CO_2 and the pH was adjusted to 7·4 at 37°C. The resting tension was set at 1 g, and the tissues were equilibrated for 30 min with an applied tension of 1·5 g. Changes in tension were recorded by means of a Grass 7B polygraph via Grass force displacement transducers connected to a Model 7 D polygraph (Grass Medical Instruments Co., Quincy, MA, USA).

Bronchospasmolytic effect of HOE 234

Eighteen treatment groups were designed (according to the three regions tested and constricting agents used). Half were pre-incubated with indomethacin (1 μM) for 15 min before preconstricting the samples with either methacholine (1 μM), histamine (10 μM) or KCl (30 mM). When the preconstriction had stabilized, concentration–response curves were obtained by adding HOE 234 in a cumulative manner using a range of dosage from 0·01–3·3 μM. The effects of the corresponding vehicles (dimethylsulphoxide for HOE 234, ethanol for indomethacin) on the preconstrictions in each region were found to be negligible.

Bronchospasmolytic effects of salbutamol

The relaxing property of salbutamol was studied in the trachea and proximal and distal principle bronchi using the preconstricting agents mentioned above. Concentration-response curves were obtained by adding salbutamol cumulatively in a range of dosage from 0·1 nM to 1 μM.

Drugs

HOE 234 was kindly provided by Dr Englert (Hoechst AG, Frankfurt, Germany) and salbutamol by Leiras (Turku, Finland). Pentobarbital sodium was purchased from Mebuma (Nord Vacc, Stockholm, Sweden), histamine acid phosphate from BDH Biochemicals (Poole, UK) and indomethacin and methacholine from Sigma Chemicals (St Louis, MO, USA). A 10 mM stock solution of HOE 234 was prepared in dimethylsulphoxide; indomethacin was dissolved in ethanol (96%). All other drugs and the Krebs-Ringer solution were dissolved in ultrapure water (Milli-Q; Millipore Corp., Bedford, MA, USA). Drug concentrations are expressed as final bath concentrations.

Statistical analysis

The effects of the compounds studied were calculated as percent of the maximum preconstriction. Means ± s.e. are shown. Differences between treatment groups at various concentrations of the relaxing agents were analysed by two-way analysis of variance for repeated measures (MANOVA, Statistica for Windows 4·5, Stat. Soft, Inc. 1993). P values < 0·05 were considered to be significant. For salbutamol the concentration producing 50% relaxation (EC50) was calculated by linear interpolation from individual concentration–response curves. For HOE 234 EC50 values were calculated only for the trachea in histamine (as the effect in the other treatment groups did not reach 50%).

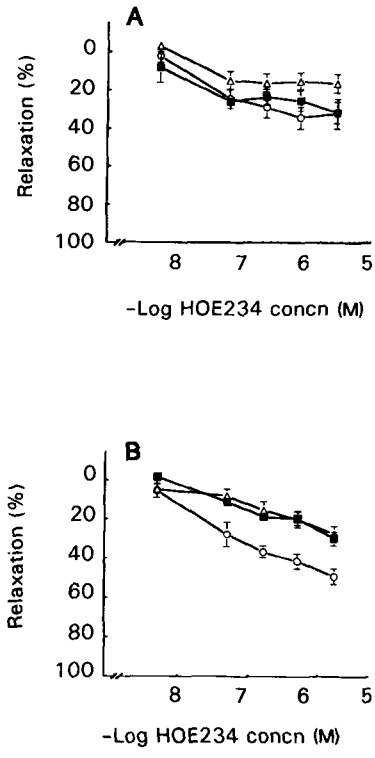

FIG. 1. A. Relaxation induced by HOE 234 in guinea-pig tracheal (△), proximal (■) and distal (○) bronchial rings, preconstricted with metacholine (1 μM). B. Relaxation induced by HOE 234 in guinea-pig tracheal, proximal and distal bronchial rings pretreated with indomethacin (1 μM), preconstricted with methacholine (1 μM). Significantly stronger relaxation in B_{dist} ($P < 0·001$).

Results

HOE 234

Generally, HOE 234 had a concentration-dependent relaxing effect on the airway smooth muscle. After preconstriction with methacholine (Fig. 1A) no significant differences in relaxation were seen in the trachea and different parts of the bronchi (maximum relaxation: B_{dist} and B_{prox} 32%, T 18%). After indomethacin pretreatment (Fig. 1B), the maximum relaxation of T increased to 30% and that of B_{dist} to 50%, whereas the reactivity of B_{prox} was not altered (32%). Of all preparations studied, only the relaxation of B_{dist} was most marked and significantly enhanced by indomethacin pretreatment ($P < 0·001$).

After histamine preconstriction (Fig. 2) the relaxation in the trachea in response to HOE 234 was strongest both in the absence and presence of indomethacin pretreatment (maxima in T 70% and 76%, respectively with $P < 0·0005$ in both groups). The corresponding EC50 was 0·4 μM in both groups. For B_{prox} and B_{dist}, maximum relaxation ranged between 18 and 27% and were not significantly altered by indomethacin pretreatment. The comparison between the groups with and without indomethacin showed no significant difference.

After preconstriction with KCl (Fig. 3A) all parts of the respiratory tract relaxed dose-dependently to the same extent (T 32%, B_{dist} 29%, B_{prox} 24%). With indomethacin the maximum relaxation of the principle bronchi was not modified, whereas that of T was significantly enhanced (43%, $P < 0·05$, Fig. 3B).

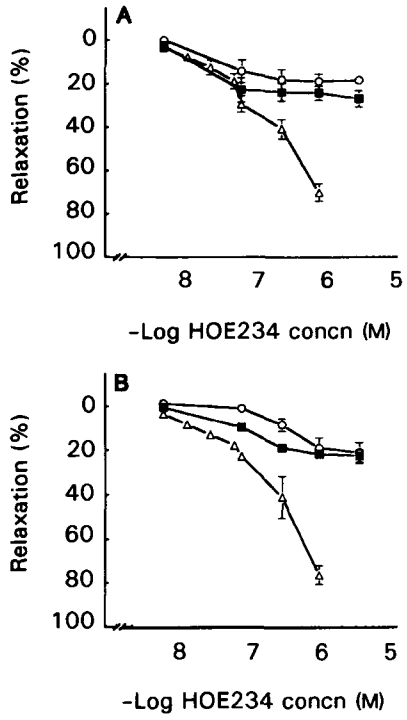

FIG. 2. A. Relaxation induced by HOE 234 in guinea-pig tracheal (△), proximal (■) and distal (○) bronchial rings, preconstricted with histamine (10 μM). Significantly stronger relaxation in trachea ($P < 0.001$). B. Relaxation induced by HOE 234 in guinea-pig tracheal, proximal and distal bronchial rings pretreated with indomethacin (1 μM), preconstricted with histamine (10 μM). Significantly stronger relaxation in trachea ($P < 0.001$).

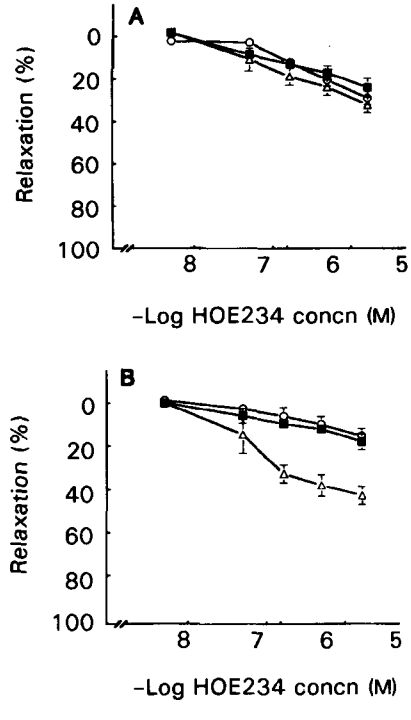

FIG. 3. A. Relaxation induced by HOE 234 in guinea-pig tracheal (△), proximal (■) and distal (○) bronchial rings, preconstricted with KCl (30 mM). B. Relaxation induced by HOE 234 in guinea-pig tracheal, proximal and distal bronchial rings pretreated with indomethacin (1 μM), preconstricted with KCl (30 mM). Significantly stronger relaxation in trachea ($P < 0.05$).

Table 1. EC50 for salbutamol after different preconstrictions in three different parts of guinea-pig large airways.

Part of the airway	Metacholine (μM)	Histamine (μM)	KCl (μM)
T	0·02	0·005	0·01
B_{prox}	0·008	0·05	0·04
B_{dist}	0·04	0·05	0·02

Salbutamol

After preconstriction with methacholine (Fig. 4A) the maximum relaxations were similar in T 97%, B_{prox} 93% and B_{dist} 89%. The corresponding EC50 values did not differ significantly either (Table 1).

After preconstriction with histamine (Fig. 4B) T relaxed significantly more (T 98%, B_{dist}, 73%, B_{prox} 68%) than the different parts of the bronchus ($P < 0.05$). In accordance with this the EC50 value was smaller for T than it was for B_{prox} or B_{dist} (Table 1).

After preconstriction with KCl (Fig. 4C) T relaxed more strongly than the other parts ($P < 0.005$; T 98%, B_{prox} 84%, B_{dist} 80%). The EC50 values, however, differed only slightly from each other (Table 1).

Discussion

In this study we compared the in-vitro bronchodilatory effect of the new potassium channel opener HOE 234 in trachea and proximal and distal bronchus of the guinea-pig with regard to the contribution of prostanoids and to the efficacy of salbutamol as a compound of standard therapy for bronchoconstriction.

Contribution of prostanoids to the effect of HOE 234

In the absence of indomethacin pretreatment HOE 234 was an equally potent relaxant after all preconstrictions used except for the histamine-pretreated trachea rings, which were relaxed most strongly by HOE 234.

With indomethacin pretreatment, significantly enhanced relaxation, in comparison with that in the absence of indomethacin, was achieved for trachea preconstricted with KCl, and the distal bronchus preconstricted with methacholine. Relaxation of the trachea after histamine preconstriction was not significantly enhanced.

The modulatory role of prostanoids in bronchoconstriction and basal tone of the smooth muscle is well known (Orehek et al 1975). Indomethacin is commonly used to increase the maximum response by eliminating the influence of constricting

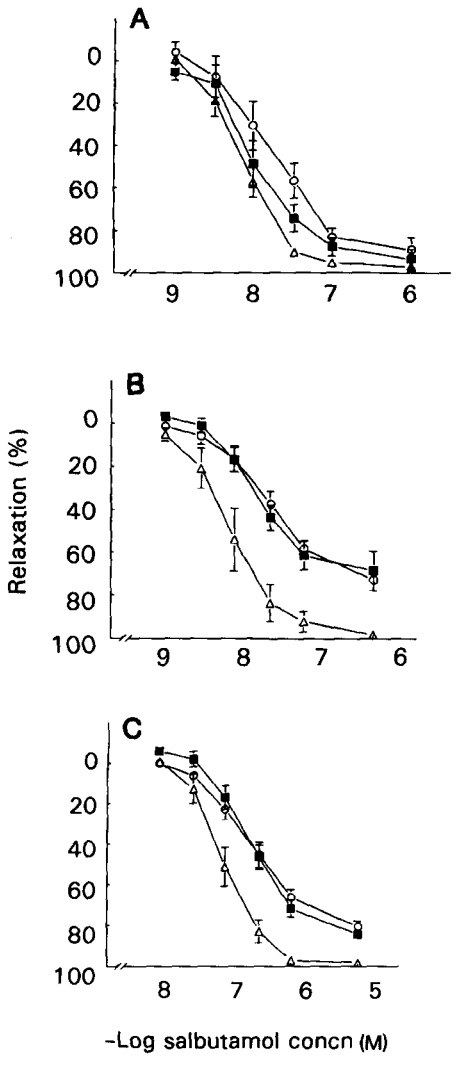

FIG. 4. A. Relaxation induced by salbutamol in guinea-pig tracheal (△), proximal (■) and distal (○) bronchial rings, preconstricted with methacholine (1 μM). B. Relaxation induced by salbutamol in guinea-pig tracheal, proximal and distal bronchial rings preconstricted with histamine (10 μM). Significantly stronger relaxation in trachea ($P < 0.05$). C. Relaxation induced by salbutamol in guinea-pig tracheal, proximal and distal bronchial rings preconstricted with KCl (30 mM). Significantly stronger relaxation in trachea ($P < 0.005$).

and dilating arachidonic acid metabolites on the cyclooxygenase pathway. It has been proposed that to a certain extent indomethacin pretreatment also mimics epithelium denudation (Murlas 1986; Raeburn 1990).

The results obtained in the presence of indomethacin would suggest that, particularly in the distal bronchus preconstricted with methacholine and trachea with KCl preconstriction, constricting prostaglandins attenuating the relaxation induced by HOE 234 were inhibited, whereas with histamine preconstriction this effect could not be seen. This observation indicates that KCl and methacholine (but not histamine) are able to induce constricting prostanoid production in the airways. In accordance with this it has been reported that pretreatment with indomethacin does not change histamine contractions of vena cava inferior samples taken from the guinea-pig (Rinkema et al 1993).

Induction of arachidonic acid release by histamine has, on the other hand, been found for equine trachea strips (Gray et al 1992) but the concentrations used, up to 50 μM were much higher than in this study. The relaxation of histamine-stimulated trachea should, therefore, have been an effect solely of HOE 234. The fact that in histamine preconstriction only trachea was efficiently relaxed with HOE 234 could be because of a different distributional density or the existence of ATP-sensitive K^+ channels with different selectivity to HOE 234 in the trachea and bronchi of guinea-pigs. There is evidence for the heterogeneity of ATP-sensitive potassium channels and also regionally different distribution in the airways of the same species (Bray & Quast 1992; Foster et al 1992; Kamei et al 1994). In contrast, if there were regionally different selectivity or distribution, it should have also been reflected in the results we obtained with the other preconstrictions, especially without indomethacin pretreatment.

Bronchospasmolytic effect of salbutamol

With salbutamol, regional differences were seen in KCl- and histamine-preconstricted rings; the trachea relaxed more strongly than the bronchi. In metacholine those differences were less distinct. From the different pretreatments the relaxation was best with KCl for the bronchi and best with histamine for the trachea.

Salbutamol is well known for its equally potent effect in all parts of the human airways and its potency against all possible constricting agents in the human lung (Barnes 1992). It remains unexplained why in the trachea constrictions induced by KCl and histamine the relaxation was not as strong as in the main bronchus. In the guinea-pig trachea there is evidence of the release of prostaglandin E_2 which diminishes with increasing concentrations of salbutamol owing to the induction of new inhibitory protein synthesis (Lew et al 1992). Our results suggest that in the main bronchus, again depending on the preconstrictor used (as there was no marked regional difference in methacholine preconstriction) a different pattern of prostanoid induction is elicited by salbutamol.

Our findings suggest that the trachea of the guinea-pig best reflect the conditions of the human airway with regard to the bronchodilatory effect of salbutamol. Even if guinea-pig trachea is used for its similarities to human bronchi (Kamei et al 1994), however, the limitations of guinea-pig as an animal model for asthma are well known (Muccitelli et al 1987).

Bronchospasmolytic effect of HOE 234 in comparison with salbutamol

We were interested in comparing the bronchodilating activity of HOE 234 with one of the most effective first line therapeutic agents (salbutamol) for acute bronchoconstriction. Our experiment showed that HOE 234 cannot compete with salbutamol in terms of bronchodilatory effect. As HOE 234 is not selective for lung tissue (Linz et al 1992) the systemic use of this compound as an anti-asthma drug is limited, whereas local application as an aerosol still remains to be tested. There are many reports of the strong bronchodilating effects of potassium channel openers and of the weak effects in animals and human, potassium channel openers being 30 times less potent than salbutamol. Our results for methacholine preconstriction are in accordance

with other reports (e.g., Imagawa et al 1993) showing that ATP-sensitive K^+ channel openers are not very effective on contractions induced by cholinergic agonists in the tracheal smooth muscle of guinea-pigs, indicating that the mechanism of opening of ATP-sensitive K^+ channels might be of less importance after preconstriction with methacholine and, according to our results, after KCl preconstriction also.

Generally the effects of potassium channel openers seem to vary with the experimental conditions used (for reviews, see Black & Barnes 1990; Raeburn & Karlsson 1991). Their effects are, furthermore, reported to be stronger in sensitized animals than in unsensitized (Morley 1994). It must be kept in mind that our results with HOE 234 were obtained with unsensitized animals. There are also reports of beneficial effects of these compounds on mucus secretion (Weston & Edwards 1992; Griffin & Scott 1994).

Conclusion

Our results show that the ATP-sensitive potassium channel opener HOE 234 has clear bronchodilating properties, the degree of which depend on the preconstrictor used and the part of the respiratory tract studied. In some parts of the airways, at least, arachidonic acid metabolites formed by the cyclooxygenase pathway attenuate the relaxing effect of HOE 234, owing to the formation of bronchoconstricting prostanoids (see also Raeburn & Brown 1991).

As the bronchodilating potency of HOE 234 was clearly not as great as that of salbutamol, the other possible advantageous effects of this compound should be studied in-vivo under different pathophysiological conditions such as hyper-reactivity and increased mucus secretion.

Acknowledgements

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