ELSEVIER

European Journal of Pharmacology 383 (1999) 169–176

ejp

www.elsevier.nl/locate/ejphar

Effects of K^+ channel inhibitors on relaxation induced by flufenamic and tolfenamic acids in guinea-pig trachea

Liang Li [a,*], Kirsi Vaali [a], Heikki Vapaatalo [a], Hannu Kankaanranta [b]

[a] *Institute of Biomedicine, Department of Pharmacology and Toxicology, P.O. Box 8, University of Helsinki, Helsinki FIN-00014, Finland*
[b] *Medical School, University of Tampere, P.O. Box 607, Tampere FIN-33101, Finland*

Received 25 August 1999; accepted 27 August 1999

Abstract

The effects of different K^+ channel inhibitors on flufenamic- and tolfenamic-acids-induced relaxation were studied in prostaglandin $F_{2\alpha}$ (1 μM) precontracted guinea-pig trachea. Flufenamic and tolfenamic acids (each 0.1–33 μM) and lemakalim (0.01–33 μM), but not indomethacin (0.1–33 μM), caused relaxation. Iberiotoxin (33 and 100 nM) inhibited flufenamic- and tolfenamic-acids-, but not lemakalim-, induced relaxation. Iberiotoxin (100 nM) inhibited nifedipine (10 nM–10 μM)-induced relaxation. Nifedipine (0.1 μM) inhibited the blockade of fenamate-induced relaxation by iberiotoxin. Apamin (0.1 and 1 μM) did not affect flufenamic- and tolfenamic-acids- and lemakalim-induced relaxation. Glibenclamide (10 and 33 μM) inhibited lemakalim-, but not flufenamic- and tolfenamic-acids-, induced relaxation. 4-Aminopyridine (0.5 and 2 mM) inhibited flufenamic- and tolfenamic- acids- and lemakalim-induced relaxation. Flufenamic- and tolfenamic-acids-induced relaxation is likely to be activation of Ca^{2+}-activated K^+ channels and differs from that of lemakalim.

Keywords: Trachea, Guinea-pig; Smooth muscle; Relaxation; Fenamate; K^+ channel

1. Introduction

K^+ channels are an important determinant of smooth muscle excitability and force generation. A diverse range of K^+ channels has been described in the plasmalemma of airway smooth muscle cells. These include the large Ca^{2+}-dependent K^+ channels (K_{Ca}), the ATP-sensitive K^+ channels (K_{ATP}) and the 4-aminopyridine-sensitive, delayed-rectifier K^+ channels (K_{dr}) (Kotlikoff, 1993; Knox and Tattersfield, 1994). The large Ca^{2+}-dependent K^+ channels are present in airway smooth muscle from guinea-pig and several other species and seem to play an important role in maintaining tracheal tone (Marthan et al., 1989; Hisada et al., 1990; Kotlikoff, 1990). Thus, drugs that activate this type of channel should be valuable tools to modify cellular excitability, as well as to reveal mechanisms of the large Ca^{2+}-dependent K^+ channels function.

As a subgroup of non-steroidal anti-inflammatory drugs (NSAIDs), fenamates (Fig. 1), *N*-aryl derivatives of anthranilic acid, are potent inhibitors of prostanoid biosynthesis (Conroy et al., 1991; Moilanen and Kankaanranta, 1994). In addition to inhibition of prostanoid synthesis, fenamates have also been shown to inhibit prostaglandin-induced contractions (Famaey et al., 1977; Vapaatalo et al., 1977; Sanger and Bennett, 1979; Bennett et al., 1980). More recently, they have been reported to inhibit Ca^{2+}-activated Cl^- currents (Janssen and Sims, 1992, 1993; Greenwood and Large, 1995), non-selective cation channels (Gögelein et al., 1990) and receptor-mediated Ca^{2+} influx (Kankaanranta and Moilanen, 1995; Kankaanranta et al., 1996). A voltage-dependent K^+ current from jejunal smooth muscle and corneal epithelium has been shown to be increased by fenamates (Rae and Farrugia, 1992; Farrugia et al., 1993a,b). Fenamates also activate the large Ca^{2+}-dependent K^+ channels of coronary artery membranes reconstituted into lipid bilayer (Ottolia and Toro, 1994) and in rabbit portal vein smooth cells (Greenwood and Large, 1995). In the previous work, we have shown that flufenamic and tolfenamic acids inhibit prostaglandin $F_{2\alpha}$ and other agonists-induced contractions of guinea-pig

* Corresponding author. Department of Physiology and Biophysics, Indiana University, School of Medicine, 635 Barnhill Drive MS 370, Indianapolis, IN 46202-5120, USA. Tel.: +1-317-278-3468; fax: +1-317-274-3318.

E-mail address: liangli@ruby.iupui.edu (L. Li)

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00634-2

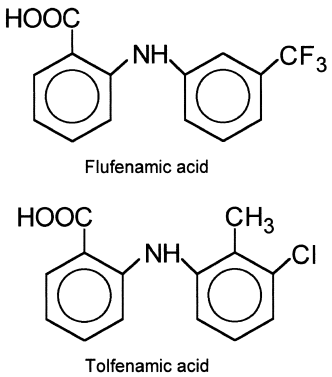

Fig. 1. Chemical structures of flufenamic acid and tolfenamic acid.

airway smooth muscle (Li et al., 1998). Consequently, the aim of the present study was to clarify whether fenamates can cause relaxation of guinea-pig airway smooth muscle by activating the large Ca^{2+}-dependent K^+ channels. For comparison, we used: (1) indomethacin, a chemically different cyclooxygenase inhibitor; (2) lemakalim (BRL 38227), the active enantiomer of cromakalim, a well-known ATP-sensitive K^+ channels activator; and (3) nifedipine, an L-type voltage-dependent Ca^{2+} channel antagonist.

2. Materials and methods

2.1. Tissue preparation

English short-hair tricolor guinea-pigs (450–650 g) of either sex were anesthetized with pentobarbital sodium (75 mg/kg, i.p.). The trachea and attached lungs were quickly removed. Tracheal rings 3 mm long were dissected and then mounted in organ baths filled with 8 ml of Kreb's solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, $CaCl_2 \cdot H_2O$ 1.6, KCl 4.7, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, glucose 11.1. The solution was maintained at 37°C and bubbled with 96% O_2 and 4% CO_2 gas mixture. The tissues were equilibrated under an optimal resting tension of 1 g for at least 60 min with replacement of the bath fluid every 15 min. The tone of the tracheal smooth muscle was measured with a force displacement transducer (FT03) connected to a Model 7 D polygraph (Grass Instrument, Quincy, MA, USA). The experimental procedure was approved by the Animal Experimentation Committee of the University of Helsinki, Finland.

2.2. Experimental procedures

Following the equilibration period, the tracheal preparations were precontracted with prostaglandin $F_{2\alpha}$ (1 μM). After the contraction has reached plateau, the vehicle or one of the K^+ channel blockers was added to the organ bath. After 20 min incubation, concentration–response curves to flufenamic acid, tolfenamic acid, indomethacin (each drug 0.1–33 μM) and lemakalim (0.01–33 μM) were obtained in a cumulative manner. To test the effects of inhibition of the large Ca^{2+}-dependent K^+ channels, relaxation responses to flufenamic acid, tolfenamic acid and lemakalim were obtained in the absence and in the presence of iberiotoxin (33 and 100 nM). The effects of inhibition of the small conductance K^+ channels (SK_{Ca}) on the flufenamic-acid-, tolfenamic-acid- and lemakalim-induced relaxation were investigated in the absence and in the presence of apamin (0.1 and 1 μM). To test the effects of the ATP-sensitive K^+ channels blockers, relaxation responses to flufenamic acid, tolfenamic acid and lemakalim were obtained in the absence or in the presence of glibenclamide (3.3 and 33 μM). The effect of inhibition of the 4-aminopyridine-sensitive, delayed-rectifier K^+ channels was investigated in the absence and in the presence of 4-aminopyridine (0.5 and 2 mM). The control experiments were carried out in the presence of vehicle.

In another set of experiments, the tracheal preparations were precontracted with prostaglandin $F_{2\alpha}$ (1 μM) after the equilibration period. After the contraction has reached plateau, the vehicle or iberiotoxin (100 nM) was added to the organ bath. After 20-min incubation, concentration–response curves to nifedipine (10 nM–10 μM) were obtained in a cumulative manner. To test the effects of iberiotoxin on the combination of nifedipine (0.1 μM) with flufenamic acid or tolfenamic acid, relaxation responses to flufenamic acid and tolfenamic acid were obtained in the absence and in the presence of iberiotoxin (100 nM). The relaxing responses to flufenamic acid and other agonists were expressed as percentage of relaxation of submaximal contraction induced by prostaglandin $F_{2\alpha}$ (1 μM).

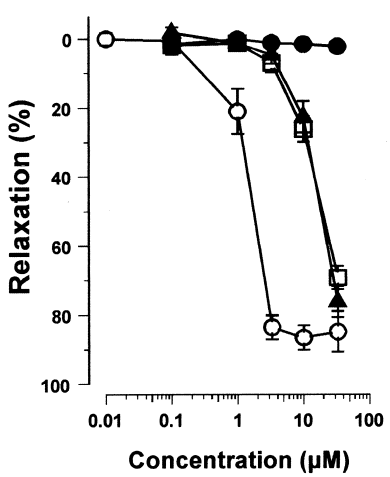

Fig. 2. The relaxant effects of flufenamic acid (□), tolfenamic acid (▲), lemakalim (○) and indomethacin (●) on prostaglandin $F_{2\alpha}$ (1 μM) precontracted guinea-pig isolated trachea. Each point represents mean ± S.E.M. ($n = 5–6$).

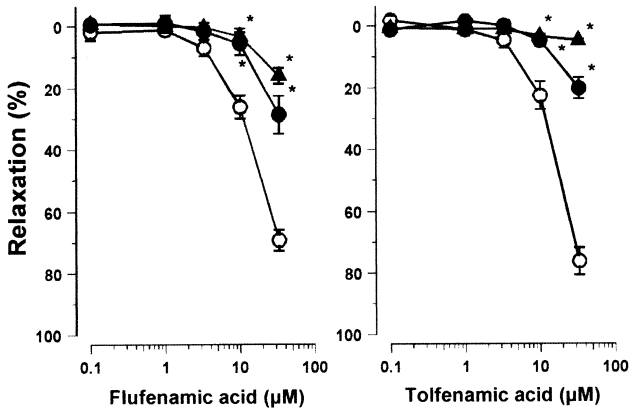

Fig. 3. Effect of iberiotoxin on the relaxation induced by flufenamic acid or tolfenamic acid in prostaglandin $F_{2\alpha}$ (1 μM) precontracted guinea-pig isolated trachea. The concentration–response curves were obtained in the presence of vehicle (○) and 33 nM (●) or 100 nM (▲) iberiotoxin. Each point represents mean ± S.E.M. ($n = 5–6$). Significantly different from control (○): $^{*}P < 0.05$.

2.3. Statistical analysis

The results are expressed as mean ± S.E.M. of the indicated number of animals. Individual EC_{50} values (the concentration of bronchodilator required to cause 50% of the maximal relaxation) for control and test concentration–response curves were determined using linear regression analysis applied to the linear portion of each concentration–response curve. Statistical analysis of the results was performed by analysis of variance (ANOVA) followed by Newman–Keul's test using the Statistica program (StatSoft, Tulsa, OK, USA). Differences were considered significant when $P < 0.05$.

2.4. Drugs

Drugs were obtained as follows: 4-aminopyridine, apamin, flufenamic acid, iberiotoxin and indomethacin from Sigma (St. Louis, MO, USA), glibenclamide and nifedipine from Leiras (Turku, Finland), lemakalim (BRL 38227) from SmithKline Beecham Pharmaceuticals (Surrey, UK), pentobarbital sodium from Grinsted Products (Grinsted, Denmark), prostaglandin $F_{2\alpha}$ from Schering (Berlin, Germany) and tolfenamic acid from GEA (Copenhagen, Denmark). Unless otherwise stated, all drugs were prepared daily in ultrapure water (MilliQ, Millipore, Bedford, MA, USA) just before the experiments and protected from the light. Stock solutions of apamin (1 mM), iberiotoxin (20 μM) and prostaglandin $F_{2\alpha}$ (10 mM) were stored at −20°C until use. Lemakalim was dissolved in 70% of ethanol at 10 mM. Flufenamic acid, tolfenamic acid, glibenclamide and nifedipine were prepared in dimethyl sulfoxide at 10 mM.

3. Results

3.1. Effects of fenamates and lemakalim against tone induced by prostaglandin $F_{2\alpha}$

Flufenamic and tolfenamic acids ($-\log EC_{50}$, 4.72 ± 0.19 and 4.74 ± 0.26, respectively) and lemakalim ($-\log EC_{50}$, 5.76 ± 0.23) caused concentration-dependent relaxation of prostaglandin $F_{2\alpha}$ (1 μM) precontracted trachea whereas indomethacin had no relaxant effect. The maximal relaxation induced by flufenamic and tolfenamic acids, and lemakalim was 69 ± 3%, 76 ± 4%, and 86 ± 3%, respectively (Fig. 2).

3.2. Effects of the large Ca^{2+}-dependent K^{+} channel inhibitor on relaxation induced by fenamates and lemakalim

Addition of 33 and 100 nM iberiotoxin increased the level tone by 8 ± 2% and 17 ± 4%, respectively, in the tissues precontracted with prostaglandin $F_{2\alpha}$. Pretreatment with iberiotoxin significantly inhibited the relaxation induced by flufenamic and tolfenamic acids (Fig. 3) but did not modify lemakalim-induced relaxation (Table 1).

Table 1
Effects of different K^{+} channel inhibitors on flufenamic- and tolfenamic-acids- and lemakalin-induced relaxation in prostaglandin $F_{2\alpha}$ (1 μM) precontracted guinea-pig isolated trachea. ND (not determined). Data are mean ± S.E.M. ($n = 5–6$) of $-\log EC_{50}$ and percentage maximal relaxation

	Flufenamic acid		Tolfenamic acid		Lemakalim	
	$-\log EC_{50}$	%Maximal relaxation	$-\log EC_{50}$	%Maximal relaxation	$-\log EC_{50}$	%Maximal relaxation
Control	4.72 ± 0.19	69 ± 3	4.74 ± 0.30	76 ± 4	5.76 ± 0.23	86 ± 3
Apamin (0.1 μM)	4.74 ± 0.12	71 ± 6	4.74 ± 0.28	78 ± 6	5.72 ± 0.30	85 ± 6
Apamin (0.1 μM)	4.72 ± 0.32	68 ± 5	4.72 ± 0.32	71 ± 7	5.74 ± 0.23	82 ± 5
Glibenclamide (3.3 μM)	4.75 ± 0.30	68 ± 3	4.74 ± 0.18	75 ± 6	4.52 ± 0.32[a]	52 ± 7[a]
Glibenclamide (33 μM)	4.69 ± 0.17	72 ± 4	4.73 ± 0.25	73 ± 4	ND	9 ± 4[a]
Iberiotoxin (10 nM)	ND	28 ± 6[a]	ND	20 ± 4[a]	5.79 ± 0.23	91 ± 6
Iberiotoxin (100 nM)	ND	16 ± 3[a]	ND	5 ± 1[a]	5.79 ± 0.16	89 ± 5

[a] Indicates statistically significant difference from control ($P < 0.05$).

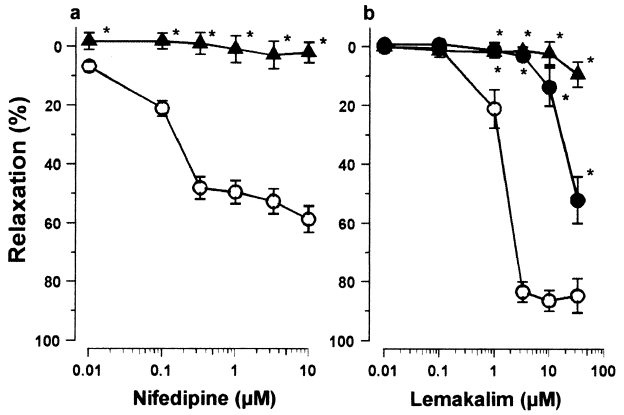

Fig. 4. (a) Effect of iberiotoxin on the relaxation induced by nifedipine in prostaglandin $F_{2\alpha}$ (1 μM) precontracted guinea-pig isolated trachea. The concentration–response curves were obtained in the presence of vehicle (○) and 100 nM (▲) iberiotoxin. (b) Effect of glibenclamide on the relaxation induced by lemakalim in prostaglandin $F_{2\alpha}$ (1 μM) precontracted guinea-pig isolated trachea. The concentration–response curves were obtained in the presence of vehicle (○) and 3.3 μM (●) or 33 μM (▲) glibencalmide. Each point represents mean ± S.E.M. ($n = 5–6$). Significantly different from control (○): $^*P < 0.05$.

3.3. Effects of nifedipine on blockade of fenamate-induced relaxation by the large Ca^{2+}-dependent K^+ channel inhibitor

Nifedipine ($-\log EC_{50}$, 5.88 ± 0.24) relaxed guinea-pig trachea in a concentration-dependent manner. The maximal relaxation induced by nifedipine was 58 ± 4%. Iberiotoxin (100 nM) inhibited nifedipine-induced relaxation (Fig. 4a). Nifedipine (0.1 μM) inhibited the blockade of fenamate-induced relaxation by iberiotoxin (Fig. 5).

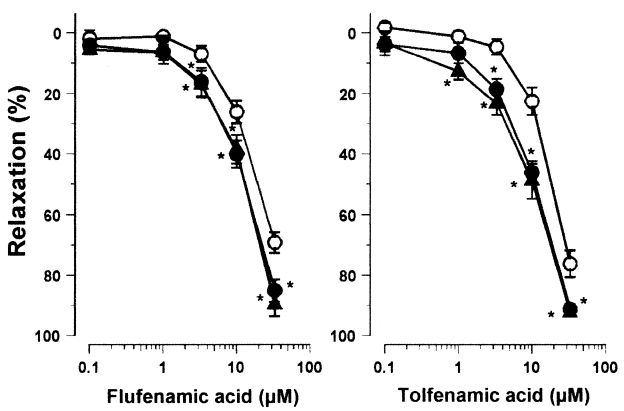

Fig. 5. Effect of iberiotoxin on the relaxation induced by the combination of nifedipine with flufenamic acid or tolfenamic acid in prostaglandin (1 μM) precontracted guinea-pig isolated trachea. The concentration–response curves were obtained for flufenamic acid or tolfenamic acid alone (○) or for 0.1 μM nifedipine plus flufenamic acid or tolfenamic acid (●) or for 0.1 μM nifedipine plus flufenamic acid or tolfenamic acid after pretreatment with 100 nM iberiotoxin (▲). Each point represents mean ± S.E.M. ($n = 5–6$). Significantly different from control (○): $^*P <$ 0.05.

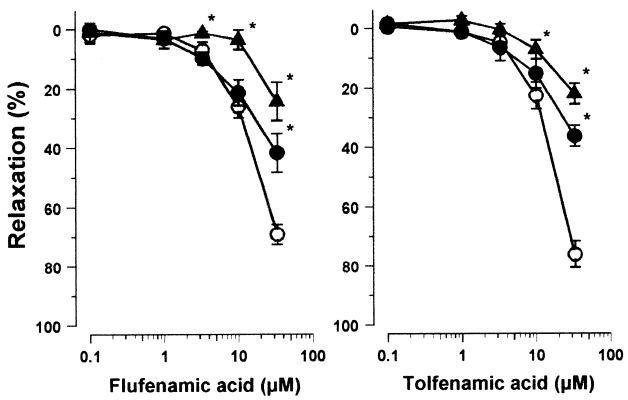

Fig. 6. Effect of 4-aminopyridine on the relaxation induced by flufenamic acid or tolfenamic acid in prostaglandin $F_{2\alpha}$ (1 μM) precontracted guinea-pig isolated trachea. The concentration–response curves were obtained in the presence of vehicle (○) and 0.5 mM (●) or 2 mM (▲) 4-aminopyridine. Each point represents mean ± S.E.M. ($n = 5–6$). Significantly different from control (○): $^*P < 0.05$.

3.4. Effects of the small conductance K^+ channel inhibitor on relaxation induced by fenamates and lemakalim

Addition of apamin (0.1 and 1 μM) had no effect on the level tone in the tissues precontracted with prostaglandin $F_{2\alpha}$. Pretreatment with apamin did not affect the relaxation induced by flufenamic and tolfenamic acids and lemakalim (Table 1).

3.5. Effects of the ATP-sensitive K^+ channel inhibitor on relaxation induced by fenamates and lemakalim

Addition of glibenclamide (3.3 and 33 μM) had no effect on the level tone in the tissues precontracted with

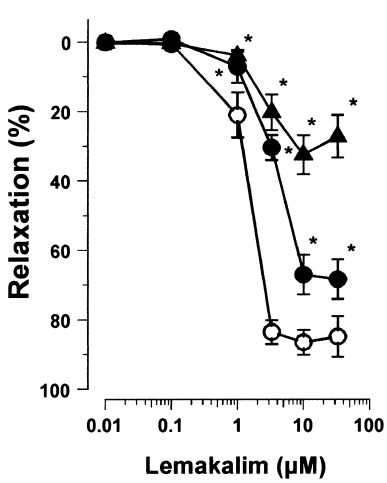

Fig. 7. Effect of 4-aminopyridine on the relaxation induced by lemakalim in prostaglandin $F_{2\alpha}$ (1 μM) precontracted guinea-pig isolated trachea. The concentration–response curves were obtained in the presence of vehicle (○) and 0.5 mM (●) or 2 mM (▲) 4-aminopyridine. Each point represents mean ± S.E.M. ($n = 5–6$). Significantly different from control (○): $^*P < 0.05$.

prostaglandin $F_{2\alpha}$. Pretreatment with glibenclamide inhibited lemakalim-induced relaxation (Fig. 4b) but did not affect the relaxation induced by flufenamic and tolfenamic acids (Table 1).

3.6. Effects of the 4-aminopyridine-sensitive, delayed-rectifier K^+ channel inhibitor on relaxation induced by fenamates and lemakalim

Addition of 0.5 and 2 mM 4-aminopyridine increased the level of tone by $20 \pm 4\%$ and $25 \pm 5\%$, respectively, in the tissues precontracted with prostaglandin $F_{2\alpha}$. Pretreatment with 4-aminopyridine significantly reduced the relaxation induced by flufenamic and tolfenamic acids and lemakalim (Figs. 6 and 7).

4. Discussion

The large Ca^{2+}-dependent K^+ channels have been identified in airway smooth muscles from guinea-pig and several other species (Marthan et al., 1989; Hisada et al., 1990; Kotlikoff, 1990). In the present study, we have shown that flufenamic and tolfenamic acids are relaxants of guinea-pig trachea precontracted with prostaglandin $F_{2\alpha}$. Iberiotoxin, an inhibitor of the large Ca^{2+}-dependent K^+ channels (Galvez et al., 1990), significantly inhibited the relaxant activity of flufenamic and tolfenamic acids. However, inhibitors of the ATP-sensitive K^+ channels and the small conductance K^+ channels failed to affect fenamate-induced relaxation of prostaglandin $F_{2\alpha}$ precontracted guinea-pig trachea. These results indicate that the activation of the large Ca^{2+}-dependent K^+ channels is involved in fenamate-induced relaxation in the guinea-pig airways. On the other hand, lemakalim-induced relaxation was not affected by iberiotoxin, suggesting that the large Ca^{2+}-dependent K^+ channels are not involved in lemakalim-induced airway smooth muscle relaxation. Recently, fenamates have been shown to activate a large conductance K^+-selective channel in rabbit corneal epithelium (Rae and Farrugia, 1992) and in human and canine jejunal circular smooth muscle cells, resulting in membrane hyperpolarization (Farrugia et al., 1993a,b). Fenamates also increase the single-channel open probability of the large Ca^{2+}-dependent K^+ channels from coronary smooth muscle cells (Ottolia and Toro, 1994). Furthermore, fenamates open Ca^{2+}-activated K^+ channels in canine coronary artery smooth muscles (Xu et al., 1994) and in rabbit portal vein smooth muscle cells (Greenwood and Large, 1995). Thus, the present findings agree with the data in the literature that fenamates can act as openers of the large Ca^{2+}-dependent K^+ channels in smooth muscle. Taken together, the present results indicate that in guinea-pig tracheal smooth muscle, the activation of the large Ca^{2+}-dependent K^+ channels appears to be involved in the relaxant activity of flufenamic and tolfenamic acids.

Recent studies have demonstrated that the relaxant ability of isoprenaline and other β_2-selective agents is markedly decreased when the large Ca^{2+}-dependent K^+ channels channel opening is prevented by the selective inhibition of the large Ca^{2+}-dependent K^+ channels using charybdotoxin and iberiotoxin (Jones et al., 1990, 1993; Murray et al., 1991; Miura et al., 1992). Thus, the large Ca^{2+}-dependent K^+ channels seem to play an important part in airway smooth muscle relaxation. These findings also support our results that the activation of the large Ca^{2+}-dependent K^+ channels is involved in the relaxant activity of flufenamic and tolfenamic acids in the guinea-pig trachea.

However, the functional importance of activation of the large Ca^{2+}-dependent K^+ channels in relaxation of the guinea-pig smooth muscle is somewhat controversial (Kotlikoff and Kamm, 1996). Because charybdotoxin and iberiotoxin increase the basal tone in the guinea-pig trachea, and nifedipine reverses this effect (Jones et al., 1993; Issac et al., 1996), this action of the large Ca^{2+}-dependent K^+ channels inhibitors is likely to be due to depolarization of the cell membrane and opening of voltage-dependent Ca^{2+} channels, thus promoting the cellular influx of Ca^{2+} (Small et al., 1993). Although the blockade of the large Ca^{2+}-dependent K^+ channels by charybdotoxin and iberiotoxin inhibits the relaxant action of β_2-adrenoceptor agonists, the ability of nifedipine to prevent this antagonism suggests that it may not be due to specific interaction between the toxins and the β_2-adrenoceptor agonists at the level of the large Ca^{2+}-dependent K^+ channels gating but, instead, from functional antagonism attributable to the toxin promoting the cellular influx of Ca^{2+} (Huang et al., 1993; Cook et al., 1995). Thus, the toxins may simply induce membrane depolarization to inhibit relaxant responses of β_2-adrenoceptor agonists and fenamates. However, this explanation seems unlikely for several reasons. In human airways, charybdotoxin does not increase resting tone (Miura et al., 1992; Fleischmann et al., 1994). Moreover, charybdotoxin does not markedly alter the relaxation caused by a hyperpolarizing agent, lemakalim, in human and guinea-pig airways (Jones et al., 1990; Miura et al., 1992). In the present study, iberiotoxin did not markedly alter lemakalim-induced relaxation. The blockade of lemakalim-induced relaxation by iberiotoxin would have been expected if the effect of the toxin were merely due to a depolarizing action and a functional antagonism at the level of membrane potential. Nifedipine caused a concentration-dependent relaxation of prostaglandin $F_{2\alpha}$ precontracted guinea-pig trachea. Iberiotoxin (100 nM) nearly completely inhibited nifedipine-induced relaxation. Ca^{2+} antagonists exert a highly specific inhibitory effect on contractions induced by agents acting by depolarization such as KCl and tetraethylammonium (Advenier et al., 1984; Foster et al., 1984). Thus, reversal of nifedipine-induced relaxation by iberiotoxin should not have occurred if the action of iberiotoxin were similar to those of the depolarizing agents KCl and tetraethylammonium. Thus,

the action of iberiotoxin is not only due to depolarization of the tissue but also interference with other steps of signaling in nifedipine-induced relaxation. Because iberiotoxin inhibited both nifedipine- and fenamate-induced relaxation, one may conclude that nifedipine and fenamates have the same mechanism in relaxing the precontracted guinea-pig trachea. If this were true, iberiotoxin should have inhibited relaxation even when nifedipine and fenamates were added together. In contrast, the blockade of fenamate-induced relaxation by iberiotoxin was totally inhibited by 0.1 μM nifedipine, thus suggesting that the mechanism of relaxation by nifedipine and fenamates is different.

Apamin, a selective inhibitor of the small conductance Ca^{2+}-activated K^+ channels, failed to antagonize the relaxation induced by flufenamic and tolfenamic acids and lemakalim. The present failure of apamin to antagonize the relaxant responses to lemakalim is consistent with the apamin-resistant action of cromakalim in guinea-pig trachea (Allen et al., 1986). Thus, our results argue against the involvement of apamin-sensitive, small conductance K^+ channels in the tracheal smooth muscle relaxation induced by flufenamic and tolfenamic acids and lemakalim.

An antagonist of the ATP-sensitive K^+ channels, glibenclamide did not alter fenamate-induced relaxation of prostaglandin $F_{2\alpha}$ contracted trachea. These results indicate that the activation of the ATP-sensitive K^+ channels is not involved in fenamate-induced relaxation. Lemakalim relaxes guinea-pig, human and mouse airways by activation of the ATP-sensitive K^+ channels (Black et al., 1990; Buckle et al., 1993; Li et al., 1997). In the present study, tracheal relaxation induced by lemakalim was antagonized by glibenclamide. This demonstrates that the relaxant mechanism of fenamates is different from that of lemakalim.

In the present study, 4-aminopyridine inhibited the relaxation induced by fenamates and lemakalim. The 4-aminopyridine-sensitive, delayed-rectifier K^+ channels are voltage-gated, Ca^{2+}-insensitive delayed-rectifier channels, which are relatively insensitive to tetraethylammonium and unaffected by charybdotoxin and glibenclamide but blocked by 4-aminopyridine. The 4-aminopyridine-sensitive, delayed-rectifier K^+ channels have been described in the canine, porcine and ferret smooth muscle cells (Kotlikoff, 1990; Muraki et al., 1990; Boyle et al., 1992; Fleischmann et al., 1993). There are several possible explanations for the inhibitory effect of 4-aminopyridine on fenamate-induced tracheal relaxation. Firstly, it is tempting to explain the inhibition of fenamate-induced relaxation by the blockade of the 4-aminopyridine-sensitive, delayed-rectifier K^+ channels but there is no direct biophysical evidence that the 4-aminopyridine-sensitive, delayed-rectifier K^+ channels exists in guinea-pig airway smooth muscle (Kotlikoff, 1993). Secondly, whole cell experiments have generally consistently reported that Ca^{2+}-activated K^+ currents are tetraethylammonium-sensitive and 4-aminopyridine-resistant in canine airway smooth muscle cells (Kotlikoff, 1990; Muraki et al., 1990). However, the experiments in guinea-pig airway smooth muscles have suggested that Ca^{2+}- and 4-aminopyridine-sensitive currents may exist (Hisada et al., 1990). Thus, the relaxation by fenamates could be mediated through 4-aminopyridine-sensitive channels. Thirdly, 4-aminopyridine may exert actions as a direct or indirect muscarinic agonist in guinea-pig trachea (Urquhart and Broadley, 1991; Issac et al., 1996). Finally, it is possible that the inhibition of fenamate-induced relaxation may be mediated by the combination of above-mentioned effects. In the present study, 4-aminopyridine also inhibited the relaxation induced by lemakalim, which is consistent with the fact that 4-aminopyridine inhibits the relaxant action of cromakalim in guinea-pig trachea (Allen et al., 1986). It has also been shown that at millimolar concentrations, 4-aminopyridine can inhibit the ATP-sensitive K^+ channels (Kakei et al., 1985; Castle and Haylett, 1987; Haworth et al., 1989). However, this does not exclude the possibility that 4-aminopyridine may act as a muscarinic agonist.

Fenamates are potent inhibitors of prostanoid biosynthesis (Lindén et al., 1976; McLean and Gluckman, 1983; Moilanen and Kankaanranta, 1994). However, the inhibition of prostanoid synthesis is not the mechanism responsible for the relaxation induced by flufenamic and tolfenamic acids. In the present study, indomethacin, a chemically different potent prostanoid synthesis inhibitor, did not relax the guinea-pig trachea. The concentration of indomethacin used in the present study is clearly higher than the concentration needed to inhibit cyclooxygenase in the guinea-pig airways (Vane and Botting, 1996). Fenamates have also been shown to inhibit prostaglandin-induced contractions in alimentary muscle, myometrium and respiratory tract (Famaey et al., 1977; Vapaatalo et al., 1977; Sanger and Bennett, 1979; Bennett et al., 1980). Antagonism of prostaglandin action has been proposed to explain some of the clinical features of fenamates (Vapaatalo et al., 1977; Mclean and Gluckman, 1983). We believe that the activation of the large Ca^{2+}-dependent K^+ channels by fenamates may explain part of the earlier described antagonism of prostaglandin action.

Because fenamates also have effects on other ion conductances, the mechanism of relaxation by fenamates still remains open. In receptor-mediated contractions, Ca^{2+} released from the intracellular stores may activate Ca^{2+}-activated CI^- channels. Acetylcholine, substance P and histamine can activate Ca^{2+}-activated CI^- currents in guinea-pig tracheal smooth muscle (Janssen and Sims, 1992, 1993, 1994) and it seems likely that this conductance mechanism is important in producing smooth muscle membrane depolarization and contraction (Large and Wang, 1996). Recently, fenamates have been shown to block Ca^{2+}-activated CI^- currents in micromolar range (Janssen and Sims, 1992, 1993; Greenwood and Large, 1995). Like histamine and carbachol, prostaglandin $F_{2\alpha}$

increases inositol 1,4,5-trisphosphate production in human airway smooth muscle (Marmy et al., 1993). Thus, prostaglandin $F_{2\alpha}$ may also stimulate Ca^{2+}-activated Cl^- currents in guinea-pig trachea. Therefore, inhibition of Ca^{2+}-activated Cl^- channels by fenamates may contribute to their relaxant action in the guinea-pig trachea in addition to their effects on the large Ca^{2+}-dependent K^+ channels.

5. Conclusion

In contrast to indomethacin, flufenamic and tolfenamic acids relax prostaglandin $F_{2\alpha}$ precontracted guinea-pig isolated trachea in a prostanoid-independent manner. The mechanism of the relaxant action of these fenamates is different from that of lemakalim and is mainly due to the activation of the large Ca^{2+}-dependent K^+ channels.

Acknowledgements

We are grateful to Ms. Anneli von Behr for the skillful technical assistance. This study was supported by grants from GEA, Pharmacal Oy, the Academy of Finland, Biomed-2 contract BMH4-CT96-0979 and the Research Fund of Tampere University Hospital.

References

Advenier, C., Cerrina, J., Duroux, P., Floch, A., Renier, A., 1984. Effects of five different organic calcium antagonists on guinea-pig isolated trachea. Br. J. Pharmacol. 82, 727–733.

Allen, S.L., Boyle, J.P., Cortijo, J., Foster, R.W., Morgan, G.P., Small, R.C., 1986. Electrical and mechanical effects of BRL 34915 in guinea-pig isolated trachealis. Br. J. Pharmacol. 89, 395–405.

Bennett, A., Pratt, D., Sanger, G.J., 1980. Antagonism by fenamates of prostaglandin action in guinea-pig and human alimentary muscle. Br. J. Pharmacol. 68, 357–362.

Black, J.L., Armour, C.L., Johnson, P.R., 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.

Boyle, J.P., Tomas, M., Kotlikoff, M.I., 1992. Delayed rectifier potassium channels in canine and porcine airway smooth muscle cells. J. Physiol. 447, 329–350.

Buckle, D.R., Arch, J.R., Bowring, N.E., Foster, K.A., Taylor, J.F., Taylor, S.G., Shaw, D.J., 1993. Relaxant effects of the potassium channel activators BRL 38227 and pinacidil on guinea-pig and human airway smooth muscle, and blockade of their effects by glibenclamide and BRL 31660. Pulm. Pharmacol. 6, 77–86.

Castle, N.A., Haylett, D.G., 1987. Effects of channel blockers on potassium efflux from metabolically exhausted frog skeletal muscle. J. Physiol. 383, 31–43.

Conroy, M.C., Randinitis, E.J., Turner, J.L., 1991. Pharmacology, pharmacokinetics, and therapeutic use of meclofenamate sodium. Clin. J. Pain 7 (Suppl. 1), S44–S48.

Cook, S.J., Archer, K., Martin, A., Buchheit, K.H., Fozard, J.R., Muller, T., Miller, A.J., Elliott, K.R., Foster, R.W., Small, R.C., 1995. Further analysis of the mechanisms underlying the tracheal relaxant action of SCA40. Br. J. Pharmacol. 114, 143–151.

Famaey, J.P., Fotaine, J., Reuse, J., 1977. Effect of high concentrations of non-steroidal and steroidal anti-inflammatory drugs on prostaglandin-induced contractions of the guinea-pig isolated ileum. Prostaglandins 13, 107–114.

Farrugia, G., Rae, J.L., Sarr, M.G., Szurszewski, J.H., 1993a. Potassium current in circular smooth muscle of human jejunum activated by fenamates. Am. J. Physiol. 265, G873–G879.

Farrugia, G., Rae, J.L., Szurszewski, J.H., 1993b. Characterization of an outward potassium current in canine jejunal circular smooth muscle and its activation by fenamates. J. Physiol. 468, 297–310.

Fleischmann, B.K., Washabau, R.J., Kotlikoff, M.I., 1993. Control of resting membrane potential by delayed-rectifier potassium currents in ferret airway smooth muscle cells. J. Physiol. 469, 25–638.

Fleischmann, B.K., Hay, D.W.P., Kotlikoff, M.I., 1994. Control of basal tone by delayed rectifier potassium channels in human airways. Am. Rev. Respir. Dis. 149, A1080.

Foster, R.W., Okpalugo, M.I., Small, R.C., 1984. Antagonism of calcium and other actions of verapamil in guinea-pig isolated trachea. Br. J. Pharmacol. 81, 499–507.

Galvez, A., Gimenez-Gallego, G., Reuben, J.P., Roy-Contancin, L., Feigenbaum, P., Kaczorowski, G.J., Garcia, M.L., 1990. Purification and characterization of a unique, potent, peptidyl probe for the high conductance, calcium-activated potassium channel from venom of the scorpion, *Buthus tamulus*. J. Biol. Chem. 265, 11083–11090.

Gögelein, H., Dahlem, D., Englert, H.C., Lang, H.J., 1990. Flufenamic acid, mefenamic acid and niflumic acid inhibit single nonselective cation channels in the rat exocrine pancreas. FEBS Lett. 268, 79–82.

Greenwood, I.A., Large, W.A., 1995. Comparison of the effects of the fenamates on Ca-activated chloride and potassium currents in rabbit portal vein smooth muscle cells. Br. J. Pharmacol. 116, 2939–2948.

Haworth, R.A., Goknur, A.B., Berkoff, H.A., 1989. Inhibition of ATP-sensitive potassium channels of adult rat heart cells by anti-arrhythmic drugs. Circ. Res. 65, 1157–1160.

Hisada, T., Yoshihisa, K., Tsuneaki, S., 1990. Properties of membrane current from guinea-pig trachea. Pflug. Arch. 416, 151–161.

Huang, J.C., Garcia, M.L., Reuben, J.P., Kacsorowski, G.J., 1993. Inhibition of beta-adrenoceptor agonist relaxation of airway smooth muscle by Ca^{2+}-activated K^+ channel blockers. Eur. J. Pharmacol. 235, 37–43.

Issac, L., Mcardle, S., Miller, N.M., Foster, R.W., Small, R.C., 1996. Effects of some K^+ channel inhibitors on the electrical behavior of guinea-pig isolated trachealis and on its responses to spasmogenic drugs. Br. J. Pharmacol. 117, 1653–1662.

Janssen, L.J., Sims, S.M., 1992. Acetylcholine activates non-selective cation and chloride conductances in canine and guinea-pig tracheal myocytes. J. Physiol. 453, 197–218.

Janssen, L.J., Sims, S.M., 1993. Histamine activates Cl^- and K^+ currents in guinea-pig tracheal myocytes: convergence with muscarinic signaling pathway. J. Physiol. 465, 661–677.

Janssen, L.J., Sims, S.M., 1994. Substance P activates Cl^- and K^+ conductances in guinea-pig tracheal smooth muscle cells. Can. J. Physiol. Pharmacol. 72, 705–710.

Jones, T.R., Charette, L., Garcia, M.L., Kaczorowski, 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.

Jones, T.R., Charette, L., Garcia, M.L., Kaczorowski, G.J., 1993. Interaction of iberiotoxin with beta-adrenoceptor agonists and sodium nitroprusside on guinea-pig trachea. J. Appl. Physiol. 74, 1879–1884.

Kakei, M., Noma, A., Shibasaki, T., 1985. Properties of adenosine-triphosphate-regulated potassium channels in guinea-pig ventricular cells. J. Physiol. 363, 441–462.

Kankaanranta, H., Moilanen, E., 1995. Flufenamic and tolfenamic acids inhibit calcium influx in human polymorphonuclear leukocytes. Mol. Pharmacol. 47, 1006–1013.

Kankaanranta, H., Luomala, M., Kosonen, O., Moilanen, E., 1996. Inhibition by fenamates of calcium influx and proliferation of human lymphocytes. Br. J. Pharmacol. 119, 487–494.

Knox, A.J., Tattersfield, A.E., 1994. Airway smooth muscle. In: Szekeres, L., Papp, J.G. (Eds.), Handbook of Experimental Pharmacology 111 Springer-Verlag, Berlin, pp. 405–443.

Kotlikoff, M.I., 1990. Potassium currents in canine airway smooth muscle cells. Am. J. Physiol. 259, L384–L395.

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

Kotlikoff, M.I., Kamm, K.E., 1996. Molecular mechanisms of β-adrenergic relaxation of airway smooth muscle. Annu. Rev. Physiol. 58, 115–141.

Large, W.A., Wang, Q., 1996. Characteristics and physiological role of the Ca^{2+}-activated Cl^- conductance in smooth muscle. Am. J. Physiol. 271, C435–C454.

Li, L., Vaali, K., Paakkari, I., Vapaatalo, H., 1997. Bradykinin, lemakalim and sodium nitroprusside relax the mouse trachea in vitro by different mechanisms. Life Sci. 61, PL67–PL73.

Li, L., Vapaatalo, H., Vaali, K., Paakkari, I., Kankaanranta, H., 1998. Fenamates inhibit contraction of guinea-pig isolated bronchus in vitro independent of prostanoid synthesis inhibition. Life Sci. 62, PL289–PL294.

Lindén, I., Parantainen, J., Vapaatalo, H., 1976. Inhibition of prostaglandin biosynthesis by tolfenamic acid in vitro. Scand. J. Rheum. 5, 129–132.

Marmy, N., Mottas, J., Durand, J., 1993. Signal transduction in smooth muscle cells from human airways. Respir. Physiol. 91, 295–306.

Marthan, R., Martin, C., Amedee, T., Mirroneau, J., 1989. Calcium channel currents in isolated smooth muscle cells from human bronchus. J. Appl. Physiol. 66, 1706–1714.

Mclean, J.R., Gluckman, M.I., 1983. On the mechanism of the pharmacological activity of meclofenamate sodium. Drug Res. 33, 627–631.

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.

Moilanen, E., Kankaanranta, H., 1994. Tofenamic acid and leukotriene synthesis inhibition. Pharmacol. Toxicol. 75 (Suppl. 2), 60–63.

Muraki, K., Imaizumi, Y., Kojima, T., Kawai, T., Watanabe, M., 1990. Effects of tetraethylammonium and 4-aminopyridine on outward currents and excitability in canine tracheal smooth muscle cells. Br. J. Pharmacol. 100, 507–515.

Murray, M.A., Berry, J.L., Cook, S.J., Foster, R.W., Green, K.A., Small, R.C., 1991. Guinea-pig isolated trachealis: the effects of charybdotoxin on mechanical activity, membrane potential changes and the activity of plasmalemmal K^+ channels. Br. J. Pharmacol. 98, 865–874.

Ottolia, M., Toro, L., 1994. Potentiation of large conductance K_{Ca} channels by niflumic, flufenamic and mefenamic acid. Biophys. J. 67, 2272–2279.

Rae, J.L., Farrugia, G., 1992. Whole-cell potassium current in rabbit corneal epithelium activated by fenamates. J. Membr. Biol. 129, 81–97.

Sanger, G.J., Bennett, A., 1979. Fenamates may antagonize the actions of prostaglandin endoperoxides in human myometrium. Br. J. Clin. Pharmacol. 8, 482–497.

Small, R.C., Chiu, P., Cook, S.J., Foster, R.W., Isaac, L., 1993. β-adrenoreceptor agonists in bronchial asthma: role of K^+-channel opening in mediating their bronchodilator effects. Clin. Exp. Allergy 23, 802–811.

Urquhart, R.A., Broadley, K.J., 1991. Blockade by 4-AP of the muscarinic receptor responses of guinea-pig atria and trachea. J. Pharm. Pharmacol. 43, 417–420.

Vane, J., Botting, R., 1996. Overview — mechanisms of action of anti-inflammatory drugs. In: Vane, J., Botting, J., Botting, R. (Eds.), Improved Non-Steroid Anti-Inflammatory Drugs: COX-2 Enzyme Inhibitors. Kluwer Academic Publishers and William Harvey Press, London, pp. 1–27.

Vapaatalo, H., Parantainen, J., Linden, I.-B., Hakkarainen, H., 1977. Prostaglandins and vascular headache. Action of tolfenamic acid, a new anti-inflammatory analgesic, on migraine. In: Scooter, F. (Ed.), Headache, New Vistas. Biochemical Press, Florence, pp. 287–300.

Xu, X., Tsai, T.D., Wang, J., Lee, E.W., Lee, K.S., 1994. Modulation of three types of K^+ currents in canine coronary artery smooth muscle cells by NS-004, or 1-(2′-hydroxy-5′-chlorophenyl-5-trifluoromethyl-2(3*H* benzimidazolone. J. Pharmacol. Exp. Ther. 271, 362–369.