ELSEVIER

European Journal of Pharmacology 331 (1997) 221–225

ejp

Short communication

Econazole, miconazole and SK&F 96365 inhibit depolarization-induced and receptor-operated contraction of guinea-pig isolated trachea in vitro

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Received 2 April 1997; revised 2 June 1997; accepted 6 June 1997

Abstract

Econazole, miconazole, SK&F 96365 and nifedipine inhibited Ca^{2+}- and depolarization-induced and receptor-operated contraction of guinea-pig isolated trachea. Econazole, miconazole and SK&F 96365 inhibited histamine- and methacholine-induced tracheal contraction more than nifedipine. Nifedipine was more potent in inhibiting KCl-induced contraction. Nifedipine, salbutamol and theophylline, but not econazole, miconazole or SK&F 96365, relaxed KCl, histamine-, and methacholine-precontracted trachea. It appears that in the guinea-pig tracheal smooth muscle, econazole, miconazole and SK&F 96365 behave differently from nifedipine, theophylline and salbutamol. Econazole, miconazole and SK&F 96365 are thus introduced as novel antagonists of receptor-operated airway smooth muscle contraction. © 1997 Elsevier Science B.V.

Keywords: Ca^{2+}; Contraction; Trachea; Econazole; Miconazole; SK&F 96365; Nifedipine

1. Introduction

Contractile mechanisms in airway smooth muscle can be divided into receptor-operated and depolarization-induced contraction mechanisms. Single channel and whole-cell patch clamp studies have shown the existence of voltage-dependent calcium channels in canine, guinea pig, rat and human airway smooth muscle (for review, see Knox and Tattersfield, 1994). The classical L-type voltage-dependent calcium channel antagonists inhibit depolarization-induced contraction of airway smooth muscle. In addition, they have been shown to partially reverse the contractile responses of several receptor agonists (e.g., histamine and cholinergic agonists) in vitro. In contrast, in vivo, dihydropyridine (nifedipine), phenylalkylamine (verapamil) and benzothiazepine (diltiazem) calcium channel antagonists have shown only small and inconsistent effects on the airway response to cold air, histamine, methacholine and antigen (Barnes, 1985; Knox and Tattersfield, 1994). Recently, a novel receptor-operated calcium entry pathway has been shown to be responsible for calcium entry during the maintenance phase of the contractile response in cultured human airway cells (Murray and Kotlikoff, 1991). Antagonists of receptor-operated calcium entry have been lacking and there are no reports on their effects on airway smooth muscle function. Imidazole antimycotic drugs econazole and miconazole have recently been reported to antagonize receptor-mediated calcium influx in neutrophils (Montero et al., 1991) and voltage-dependent Ca^{2+} channels in GH3 pituitary and chromaffin cells (Villalobos et al., 1992). Thus we devised experiments aimed to test whether econazole and miconazole could inhibit Ca^{2+}-induced, receptor-operated and depolarization-induced contraction of guinea-pig isolated trachea. The effects of econazole and miconazole were compared with those of SK&F 96365, an experimental antagonist of receptor-mediated calcium entry as described in neutrophils, lymphocytes, platelets and endothelial cells (Merritt et al., 1990; Nordström et al., 1992); nifedipine, a classical antagonist of L-type voltage-dependent calcium channels; salbutamol, a β_2-agonist; and theophylline, an unspecific phosphodiesterase inhibitor.

2. Materials and methods

2.1. Guinea-pig isolated trachea

English shorthair tricolored guinea pigs (350–530 g) of either sex were used in these studies. Following pento-

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PII S0014-2999(97)01068-6

barbital sodium (75 mg/kg i.p.) anaesthesia and thoracotomy, the lungs were quickly removed. The tracheae (3 mm long rings) were dissected and mounted in organ baths filled with 34 ml of Krebs solution of the following composition (mmol/l): 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_2/4% CO_2 gas mixture. The tracheae were equilibrated under an optimal resting tension of 1 g for about 60 min with replacement of the bath fluid every 15 min. The tone of the tracheal smooth muscle was measured with a Grass 7 B polygraph using FT03 Grass force displacement transducers (Grass Instrument, Quincy, MA, USA). The experimental procedure was approved by the Animal Experimentation Committee of the University of Helsinki, Finland.

2.2. Effect of EGTA

The tissues were set up in Krebs solutions and equilibrated for 60 min. Thereafter EGTA (3.2 mM), 2 times the concentration of extracellular Ca^{2+} in the organ bath, was added to chelate all extracellular Ca^{2+} and determine its total contribution to KCl-, methacholine- and histamine-induced contraction. After 15 min, the concentration–response curves to KCl (15–30 mM), methacholine (0.03–3.3 μM) or histamine (0.1–10 μM) were measured. In another set of experiments, tracheae were precontracted by KCl (30 mM), methacholine (1 μM) or histamine (1 μM). After the contraction evoked reached plateau, EGTA (3.2 mM) was added to see whether chelation of extracellular Ca^{2+} relaxes precontracted trachea.

2.3. Antagonism of Ca^{2+}

Ca^{2+} concentration–response curves were established according to Foster et al. (1984). In experiments where antagonism of Ca^{2+} was measured, the tracheae were initially mounted in Krebs solutions containing normal amounts of Ca^{2+} and K^+. The tracheae were then washed for 3 times and depolarized with K^+-rich, Ca^{2+}-free solution. This caused marked contraction which was dissipated by regular changes of K^+-rich, Ca^{2+}-free solution every 15 min. This modified Krebs solution was obtained by equimolar substitution of NaCl (84.5 mM) for KCl (40 mM), without Ca^{2+}. When the contraction reached baseline, tissues were treated for 15 min with vehicle (dimethyl sulfoxide; DMSO), econazole (30–100 μM), miconazole (30–100 μM), SK&F 96365 (3–30 μM) or nifedipine (0.1–0.3 μM), and then a concentration–response curve for Ca^{2+}-induced contractions (0.03–3 mM) was measured.

2.4. Cumulative concentration–response curves to KCl, methacholine and histamine

The tissues were set up in Krebs solutions and equilibrated for 60 min. Thereafter vehicle (DMSO), econazole (30 μM), miconazole (30 μM), SK&F 96365 (30 μM) or nifedipine (0.3 μM) was added to the organ bath. After 15 min, concentration–response curves to KCl (15–30 mM), methacholine (0.03–3.3 μM) or histamine (0.1–10 μM) were measured.

2.5. Relaxation of KCl-, methacholine- and histamine-precontracted tissues

After 60 min equilibration, tissues were precontracted by KCl (30 mM), methacholine (1 μM) or histamine (1 μM). After the contraction reached plateau, cumulative concentration–effect curves for econazole, miconazole, SK&F 96365, nifedipine, theophylline (each drug, 1 nM–33 μM for KCl precontraction, 0.1–33 μM for methacholine or histamine precontraction) and salbutamol (0.1–33 nM) were then constructed. Time-matched control tissues received the appropriate amount of vehicle (DMSO).

2.6. Statistical analysis

The results are expressed as mean ± S.E.M. Statistical analysis of the results was performed by Student's *t*-test or analysis of variance (ANOVA) for repeated measures followed by Duncan's multiple range test (Statisca, Statsoft, Tulsa, OK, USA). Differences were considered significant when $P < 0.05$.

2.7. Drugs

Acetyl-β-methylcholine chloride, econazole, EGTA and miconazole were purchased from Sigma (St. Louis, MO, USA). Other reagent sources were as follows: histamine (BDH, Poole, UK), nifedipine (Orion Pharmaceutical, Espoo, Finland), pentobarbital sodium, (Grinsted Products, Grinsted), salbutamol and theophylline (Leiras, Turku, Finland) and SK&F 96365 (Alexis, Laufelfingen, Germany). Unless otherwise stated, all drugs were daily prepared in ultrapure water (MilliQ, Millipore, Bedford, MA, USA) and protected from the light. Econazole, miconazole, nifedipine and SK&F 96365 were dissolved in dimethyl sulfoxide (DMSO) in a stock solution of 10 mM.

3. Results

3.1. Effects of EGTA

EGTA (3.2 mM) abolished tracheal contraction induced by KCl, histamine and methacholine and completely relaxed KCl-, histamine- and methacholine-precontracted tracheae.

3.2. Effects of imidazoles on Ca^{2+}-induced tracheal contraction

Econazole (30–100 μM), miconazole (30–100 μM), SK&F 96365 (3–30 μM) and nifedipine (0.1–0.3 μM) inhibited tracheal contraction induced by Ca^{2+} (0.03–3

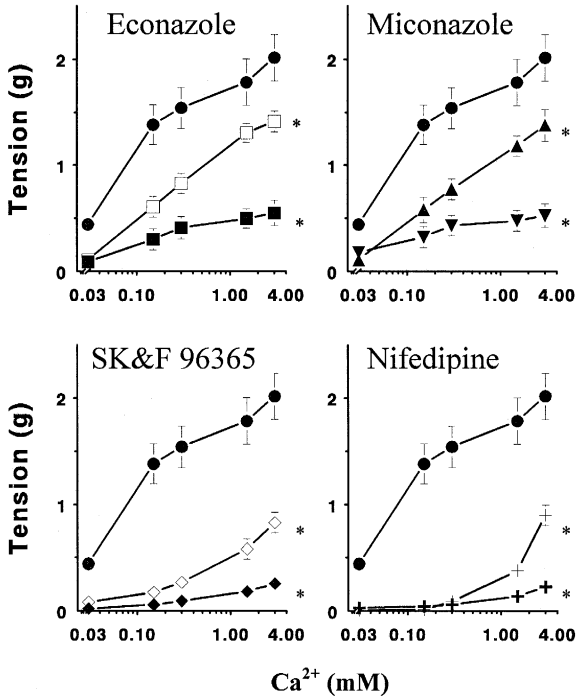

Fig. 1. The effects of econazole (30 μM, □; 100 μM, ■), miconazole (30 μM, ▲; 100 μM, ▼), SK&F 96365 (3 μM, ◇; 30 μM, ◆) or nifedipine (0.1 μM, +; 0.3 μM, +) on Ca^{2+}-induced contraction of guinea-pig isolated trachea. Each point represents mean ± S.E.M. of 6–8 experiments. * Indicates statistically significant difference ($P < 0.01$) between the vehicle (●) and different drug treatments across all concentrations of Ca^{2+} as analyzed with ANOVA for repeated measurements followed by Duncan's multiple range test.

mM) in a concentration-dependent manner. The order of potency was nifedipine > SK&F 96365 > econazole = miconazole. High concentrations of Ca^{2+} (3 mM) partly reversed the inhibitory action of these compounds on Ca^{2+}-induced tracheal contraction (Fig. 1).

3.3. Effects of imidazoles on KCl-, histamine- and methacholine-induced tracheal contraction

In the tracheal preparations, econazole, miconazole and SK&F 96365 had no effect on the resting tone. Econazole (30 μM), miconazole (30 μM), SK&F 96365 (30 μM) and nifedipine (0.3 μM) inhibited significantly KCl-, histamine- and methacholine-induced contraction of guinea-pig tracheal preparations. At these drug concentrations the order of potency for KCl-induced contraction was nifedipine ≫ econazole = miconazole > SK&F 96365 whereas for histamine- and methacholine-induced contractions it was reversed, SK&F 96365 > econazole = miconazole > nifedipine. In the case of KCl-induced tracheal contraction, nifedipine was significantly more potent ($P < 0.05$) than SK&F 96365. Nifedipine was more potent than econazole and miconazole only when the contraction was induced by high concentrations of KCl (25–30 mM). In contrast, the contraction induced by histamine or methacholine was affected less ($P < 0.05$) by nifedipine than econazole, miconazole and SK&F 96365 (Fig. 2).

3.4. Relaxation of KCl-, histamine- and methacholine-precontracted trachea

Econazole, miconazole and SK&F 96365 (up to 33 μM) did not relax guinea-pig isolated tracheal rings precontracted with KCl, histamine or methacholine. In contrast, nifedipine, salbutamol and theophylline relaxed tracheal contraction evoked by KCl, histamine and methacholine in a concentration-dependent manner. In histamine- and methacholine-precontracted tracheal rings, the maximal relaxations produced by nifedipine (33 μM) were 36 ± 4% ($n = 7$) and 19 ± 4% ($n = 6$), respectively. In KCl-precontracted tracheal rings EC_{50} value for nifedipine was 81 ± 2 nM ($n = 7$). In KCl-, histamine- and methacholine-precontracted tracheal rings, the maximal relaxations produced by theophylline (33 μM) were 20 ± 2%, 27 ± 3% and 6 ± 3% (in each case $n = 6$), respectively. In KCl-, histamine- and methacholine-precontracted tracheal rings, EC_{50} values for salbutamol were 7 ± 1, 6 ± 1 and 5 ± 1 nM (in each case $n = 6$), respectively.

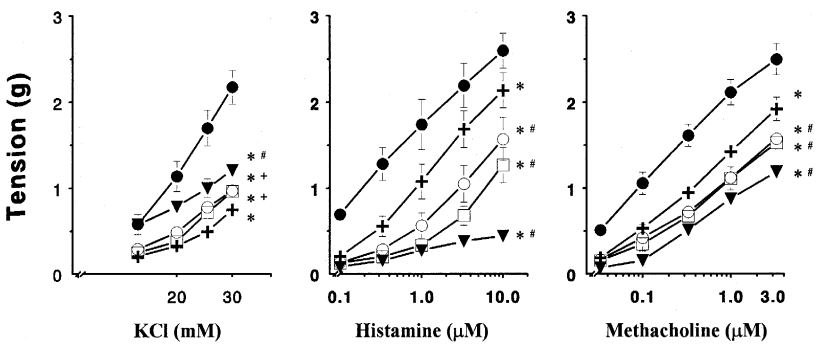

Fig. 2. The effects of econazole (30 μM; ○), miconazole (30 μM; □), SK&F 96365 (30 μM; ▼) or nifedipine (0.3 μM; +) on KCl-, histamine- and methacholine-induced contraction of guinea-pig isolated trachea. Each point represents mean ± S.E.M. of 6–8 experiments. * Indicates a statistically significant difference ($P < 0.01$) between the vehicle (●) and different drug treatments, and # indicates $P < 0.05$ versus nifedipine (+) across all concentrations of the stimuli as analyzed with ANOVA for repeated measures followed by Duncan's multiple range test. + indicates a statistically significant difference ($P < 0.05$) between the effect of econazole or miconazole and nifedipine on 25–30 mM KCl-induced contraction.

4. Discussion

There is a significant body of evidence, from both electrophysiological and ion-influx studies, for the existence of voltage-dependent Ca^{2+} channels in airway smooth muscle (Marthan et al., 1989; Hisada et al., 1990; Worley and Kotlikoff, 1990). In guinea-pig airway smooth muscle, contraction evoked by KCl is due to membrane depolarization with influx of Ca^{2+} through voltage-dependent Ca^{2+} channels (Knox and Tattersfield, 1994). Nifedipine, a classical antagonist of L-type voltage-dependent calcium channels inhibited Ca^{2+}- and KCl-induced contraction of trachea and relaxed KCl-precontracted trachea. Econazole, miconazole and SK&F 96365 inhibited significantly Ca^{2+}- and KCl-induced contraction of isolated trachea, but they were less potent than nifedipine. Inhibition of Ca^{2+} and KCl-induced contraction agrees with the results of Merritt et al. (1990) and Villalobos et al. (1992) who have reported that imidazoles antagonize L-type voltage-dependent Ca^{2+} channels. Our results thus suggest that econazole, miconazole and SK&F 96365 could inhibit Ca^{2+} influx through voltage-dependent Ca^{2+} channels in airway smooth muscle. However, the imidazoles act differently from nifedipine in that they do not relax KCl-precontracted tissue.

Receptor-agonists induce contraction of airway smooth muscle by activation of a guanine nucleotide-binding protein coupled to phospholipase C which leads to hydrolysis of phosphatidylinositol 4,5-bisphosphate, yielding inositol 1,4,5-trisphosphate and diacylglycerol. Inositol 1,4,5-trisphosphate mobilizes Ca^{2+} from intracellular stores and diacylglycerol activates protein kinase C (Chilvers et al., 1994). Activation of airway smooth muscle cells by receptor agonists have been shown to induce a rise in cytoplasmic free calcium concentration. The increase in $[Ca^{2+}]_i$ consists of two phases, a transient release from intracellular stores and a sustained influx across the plasma membrane (Murray and Kotlikoff, 1991; Knox and Tattersfield, 1994). Recently, a novel dihydropyridine-insensitive receptor-operated calcium channel has been shown to be responsible for receptor-agonist-induced calcium entry (Murray and Kotlikoff, 1991; Murray et al., 1993), although methacholine has been reported to also activate a voltage-dependent Ca^{2+} channel (Tomasic et al., 1992).

Econazole, miconazole and SK&F 96365 inhibited histamine- and methacholine-induced tracheal contraction more than nifedipine. The concentration of nifedipine (0.3 μM) is considered to be sufficient to block most L-type voltage-dependent calcium channels as it almost totally abolished KCl-induced contraction. This suggests that only part of receptor-agonist-induced contraction is due to influx of Ca^{2+} through voltage-dependent Ca^{2+} channels and the rest is due either to release of Ca^{2+} from the intracellular stores or influx of calcium through voltage-independent channels. Our results suggest the latter as econazole, miconazole and SK&F 96365 are known to antagonize receptor-operated Ca^{2+} influx in other cell types but not to inhibit the release of Ca^{2+} from the intracellular stores at the concentrations used in the present study (Merritt et al., 1990; Nordström et al., 1992; Kankaanranta and Moilanen, 1995). Furthermore, chelation of extracellular Ca^{2+} by EGTA abolished histamine and methacholine induced contraction of guinea-pig trachea. We consider the effect of EGTA to be mainly due to chelation of extracellular calcium and not to depletion of intracellular Ca^{2+} stores because stimulation of airway smooth muscle cells by histamine induces a calcium transient followed by a sustained increase lasting several minutes and EGTA abolishes the sustained phase, but not histamine-induced release of calcium from the intracellular stores (Murray and Kotlikoff, 1991). The results therefore suggest that the imidazole sensitive Ca^{2+} entry pathway is important in airway smooth muscle contraction.

The antifungal agents did not relax tracheal rings precontracted with histamine or methacholine whereas nifedipine at high (33 μM) drug concentrations relaxed the tracheal rings by 36 and 19%, respectively. In contrast, nifedipine (0.3 μM) almost totally relaxed KCl-precontracted tissue, reflecting nearly complete L-type calcium channel block. In the study of Murray and Kotlikoff (1991), the sustained calcium uptake by histamine was not affected by nifedipine (10 μM). The 100-fold difference between the action of nifedipine on receptor-mediated and depolarization-induced contractions and the lack of effect on histamine-induced calcium uptake suggest that the relaxation of receptor-mediated contraction by nifedipine is not due to inhibition of calcium influx. Rather the effect could be explained by the possible effects of calcium antagonists on inositol–phosphate accumulation (Chilvers et al., 1994) or other possibly Ca^{2+} antagonism-unrelated effects (see Nayler and Dillon, 1986).

Our present data show that econazole, miconazole and SK&F 96365 are potent inhibitors of receptor-mediated contraction of guinea-pig isolated trachea and suggest that their mechanism of action in trachea is inhibition of calcium entry that occurs through the recently described voltage-independent pathway (Murray and Kotlikoff, 1991; Murray et al., 1993). The reason why econazole, miconazole and SK&F 96365 inhibited receptor-mediated contraction but did not relax precontracted tissue remains open. Whether they inhibit the production but not the activity of the presently unknown second messenger regulating receptor-mediated calcium influx is not known. However, this would explain the effect on contraction and the absence of any relaxant effect. There is evidence for the involvement of P450-related enzyme(s) in Ca^{2+} entry in non-excitable cells, such as neutrophils (Montero et al., 1991), although recently imidazoles have been proposed to inhibit calcium entry by an unknown mechanism, not by inhibiting a cytochrome P-450 oxidase (Koch et al., 1994). Furthermore, econazole, miconazole and SK&F 96365 may also have additional properties such as inhibition of

sarcoplasmic Ca^{2+} ATPases and inhibition of K^+ channels which may also contribute to their action.

In conclusion, we have shown in the present study for the first time that econazole, miconazole and SK&F 96365 inhibit both depolarization-induced and receptor-operated contraction of guinea-pig isolated trachea and behave differently from nifedipine, theophylline and salbutamol. Econazole, miconazole and SK&F 96365 are thus introduced as novel antagonists of receptor-operated airway smooth muscle contraction.

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