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European Journal of Pharmacology 422 (2001) 203–207

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Short communication

β_3-Adrenoceptors control Cl^- conductance in rabbit nasal epithelium

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Received 9 May 2001; accepted 15 May 2001

Abstract

We have investigated the effects of β_3-adrenoceptor stimulation in vivo on nasal epithelium. We have recorded the transepithelial potential difference in New Zealand white rabbit nostrils. Superfusion of the nasal epithelial surface with a Cl^--free medium supplemented with amiloride, hyperpolarized the nasal potential difference. Isoprenaline produced a hyperpolarization of the nasal potential difference that was not prevented by nadolol, a potent β_1-/β_2-adrenoceptor antagonist, but was abolished by bupranolol, a nonselective β_{1-3}-adrenoceptor antagonist. SR 58611 ((RS)-*N*-[(2S)-7-ethoxycarbonylmethoxy-1,2,3,4-tetrahydronapht-2-yl]-(2R)-2-(3-chlorophenyl)-2 hydroethanamine hydrochloride) and CGP 12177 (4-[3-t-butylamino-2-hydroxypropoxy]benzimidazol-2-1), a preferential and a partial β_3-adrenoceptor agonists, respectively, also produced hyperpolarization of the nasal potential difference. SR 59230 (3-(2-ethylphenoxy)-1-[(1S)1,2,3,4-tetrahydronaphth-1-ylaminol]-(2S)-2-propanol oxalate), a selective β_3-adrenoceptor antagonist, abolished the effects of CGP 12177. We conclude that β_3-adrenoceptor stimulation resulted in modifications in the nasal potential difference. These findings strengthen the view that β_3-adrenoceptors are implicated in controlling water and salt transport in the normal respiratory epithelium.

Keywords: Nasal epithelium, rabbit; Transepithelial potential difference; β_3-Adrenoceptor; Cl^- conductance

1. Introduction

Catecholamines may regulate airway function through the activation of three different β-adrenoceptor subtypes. Stimulation of β_1- and/or β_2-adrenoceptors produces smooth muscle relaxation and stimulates ciliary beat frequency of epithelial cells. The presence of a third β-adrenoceptor subtype, β_3, has previously been suggested (Martin and Advenier, 1995). Similar to other β-adrenoceptor subtypes, β_3-adrenoceptors regulate both epithelium and smooth muscle functions. Stimulation of β_3-adrenoceptors produces a relaxation in isolated canine bronchi (Tamaoki et al., 1993b) but produces only slight effects in isolated human, guinea pig and sheep bronchi (Martin et al., 1994). In guinea pig bronchi, β_3-adrenoceptor stimulation also inhibits nonadrenergic noncholinergic contractions induced by electrical field stimulation (Itabashi et al., 1992). Furthermore, it increases active transport of albumin across the ferret tracheal epithelium (Webber and Stock, 1992) and the ciliary beat frequency in canine bronchial epithelium (Tamaoki et al., 1993a).

Two major active ion transports participate in epithelial function: Na^+ absorption and Cl^- secretion. In a heterologous expression system investigated in vitro, we have recently demonstrated that β_3-adrenoceptor stimulation regulates the activity of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- conductance (Leblais et al., 1999a). In the present study performed in vivo, we have investigated the effects of β_3-adrenoceptor stimulation on the transepithelial potential difference of the rabbit nasal epithelium. We found that β_3-adrenoceptor stimulation in vivo resulted in modifications in the nasal potential difference, which are fully compatible with the activation of apical Cl^- conductance.

2. Materials and methods

2.1. Animals

Twenty three New Zealand White rabbits weighing 3.5–5 kg were investigated. They were anaesthetised with intramuscular injection of mixed xylazine, 10 mg/kg plus

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01060-3

ketamine 35 mg/kg. All experiments were conducted in accordance with our institutional guidelines for animal use in research.

2.2. Potential difference measurement

Nasal transepithelial potential difference (nasal potential difference) was measured using a method previously described by Knowles et al. (1981) and modified by Alton et al. (1987). The reference electrode consisted of a calomel electrode (K401 Radiometer, Radiometer Analytical, Villeurbanne, France) connected to a 20-gauge needle filled with a 2.5% agar and placed subcutaneously in the back. The exploring electrode was another calomel electrode connected to a vascular catheter (BOC Ohmeda, SE-25106 Helsinborg, Sweden) placed in the nasal cavity and perfused with a control solution containing (in mM): Na^+ 140, K^+ 6, Mg^{2+} 1, Ca^{2+} 2, Cl^- 152, and glucose 10, titrated to pH 7.4 at room temperature with HEPES (*N*-(2-hydroxyethyl)-piperazine-n′-(2-ethane-sulfonic-acid). Both electrodes were connected to a high impedance (10^{11} Ω) voltmeter (pH ion amplifier A–M Systems, Phymep, Paris, France). Prior to use, the two electrodes were placed in close contact in order to calibrate the 0 mV level. The exploring catheter was placed along the floor of the nasal cavity without recourse to direct visual and fixed at the stable potential difference.

Parallel perfusion lines were placed and the nasal epithelial surface was superfused sequentially with different solutions. All measurements were made after a steady state was reached. Solution 1 was the control solution. Solution 2 was a Cl^--free solution using gluconate as a substitute for Cl^-. Solution 2 also contained 0.1 mM amiloride, an epithelial Na^+ channel blocker. Solution 3 was solution 2 supplemented with either a β-adrenoceptor agonist or a β-adrenoceptor antagonist. Solution 4 was solution 2 supplemented with a β-adrenoceptor antagonist plus a β-adrenoceptor agonist. Perfusion with the Cl^--free solution caused a junctional potential which was not corrected.

2.3. Drugs

(−)-Isoprenaline and nadolol were obtained from Sigma (St. Louis, MO) and CGP 12177 (4-[3-t-butylamino-2-hydroxypropoxy]benzimidazol-2-1) from RBI (Natick, MA). SR 58611 ((RS)-*N*-[(25)-7-ethoxycarbonylmethoxy-1, 2, 3, 4-tetrahydronapht-2-yl]-(2R)-2-(3-chlorophenyl)-2 hydroethanamine hydrochloride) and SR 59230 (3-(2-ethylphenoxy)-1-[(1S)1, 2, 3, 4-tetrahydronaphth-1-ylaminol]-(2S)-2-propanol oxalate) were a generous gift from Sanofi Recherche (Montpellier, France) and bupranolol from Schwarz Pharma (Mannheim, Germany). Isoprenaline, SR 58611 and CGP 12177 were prepared as stock solutions in distilled water. Nadolol was dissolved in hydrochloric acid before being neutralised to pH 7.4, and SR 59230 and bupranolol were dissolved in dimethylsulfoxide (DMSO; Sigma), such that the final concentration of the solvent was less than 0.1% v/v. At this concentration, the solvent alone had no effect on the nasal potential difference.

2.4. Statistical analysis

Results are expressed as the mean ± SEM of n experiments. The statistical significance of a drug's effect was assessed using unpaired Student's t-test ($P < 0.05$ being considered as significant).

3. Results

3.1. Nasal response to chloride-free solution

Basal potential difference was determined in the control solution (solution 1). The averaged transepithelial potential difference in rabbit nostrils was -21.9 ± 1.0 mV ($n = 28$). The nasal epithelium was then superfused with a Cl^--free extracellular medium with the aim of increasing the electrochemical gradient for Cl^- efflux at the apical membrane. These experiments were also conducted in the presence of 0.1 mM amiloride, in order to block epithelial Na^+ channels at the apical membrane (solution 2). Under these conditions, nasal potential difference underwent a significant hyperpolarization by -10.3 ± 1.5 mV ($n = 28$; $P < 0.05$ versus control solution).

3.2. Nasal response to isoprenaline

Isoprenaline, a nonselective β-adrenoceptor agonist, was then superfused at a final concentration of 1 μM in the Cl^--free solution containing amiloride. At this concentration, isoprenaline produced a further hyperpolarization of the nasal potential difference by -10.0 ± 2.2 mV ($n = 6$; $P < 0.05$ versus solution 2; Fig. 1(B)). In order to determine the β-adrenoceptor subtypes involved in this hyperpolarization, the effects of isoprenaline were further investigated in the presence of various β-adrenoceptor antagonists. After 20 min of application of 10 μM nadolol, a potent β_1-/β_2-adrenoceptor antagonist (Lee et al., 1975), the isoprenaline-induced hyperpolarization was not significantly affected (-10.6 ± 1.7 mV; $n = 5$; $P < 0.05$ versus solution 2, Fig. 1(B)). This suggested the participation of a third β-adrenoceptor subtype in the isoprenaline effects. The isoprenaline stimulation was thus repeated in the presence of 10 μM SR 59230, a selective β_3-adrenoceptor antagonist (Manara et al., 1996). Under SR 59230 pretreatment, isoprenaline still produced a hyperpolarization by -9.8 ± 1.6 mV ($n = 8$; $P < 0.05$ versus solution 2; Fig. 1(B)) of the nasal potential difference. In contrast, the

isoprenaline-induced hyperpolarization was entirely abolished after 20 min of application of 10 μM bupranolol, a nonselective β_{1-3}-adrenoceptor antagonist (Langin et al., 1991), as illustrated in Fig. 1(C).

3.3. Nasal response to β_3-adrenoceptor agonists

The addition of 0.1 μM M SR 58611, a preferential β_3-adrenoceptor agonist (Nisoli et al., 1994), to the Cl^--free extracellular medium containing amiloride, produced a hyperpolarization of the nasal potential difference by -5.7 ± 1.3 mV ($n = 8$; $P < 0.05$ versus solution 2, Fig. 2(B)). CGP 12177, a partial β_3-adrenoceptor agonist with β_1-/β_2-adrenoceptor antagonistic properties (Blin et al., 1993) at a concentration of 0.1 μM produced a similar hyperpolarization of the nasal potential difference (-7.6 ± 1.7 mV; $n = 5$; $P < 0.05$ versus solution 2; Fig. 2(A) and (B)). The CGP 12177-induced hyperpolarization was entirely abolished after pretreatment of the epithelial surface with 1 μM SR 59230 (-0.7 ± 2.3 mV; $n = 5$; Fig. 2(B)).

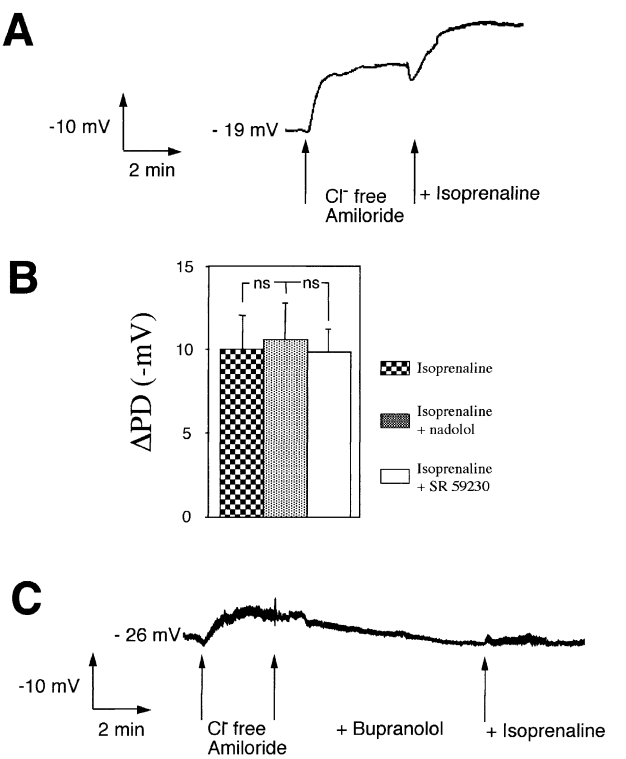

Fig. 1. Effects of isoprenaline on rabbit nasal transepithelial potential difference. (A) Typical nasal potential difference recording. Nasal epithelium surface was sequentially superfused with the control solution, with the Cl^--free solution containing 0.1 mM amiloride and with 1 μM isoprenaline. (B) Comparison of the nasal potential difference response to superperfusion of the nasal epithelium with 1 μM isoprenaline alone ($n = 6$), after pretreatment with 10 μM nadolol, a β_1-/β_2-adrenoceptor antagonist ($n = 5$), or 10 μM SR 59230, a selective β_3-adrenoceptor antagonist ($n = 8$). ns: $P > 0.05$. (C) Typical nasal potential difference recording showing the effects of 1 μM isoprenaline after superfusion of 10 μM bupranolol, a β_{1-3}-adrenoceptor antagonist.

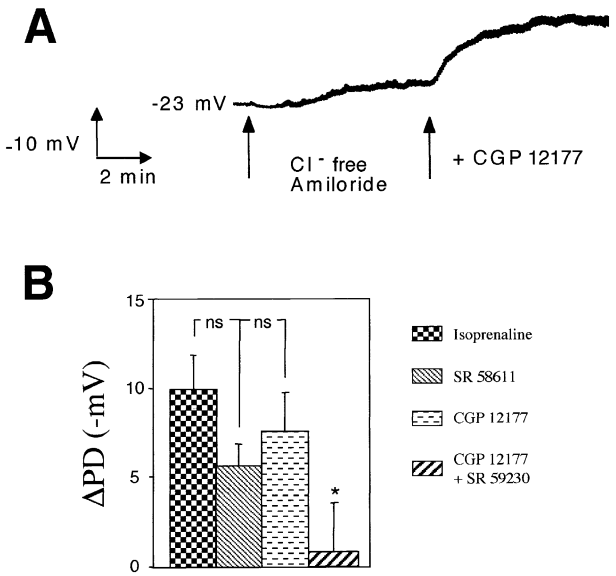

Fig. 2. Effects of β_3-adrenoceptor stimulation on nasal potential difference. (A) Typical nasal potential difference response during superfusion of the nasal epithelium with 0.1 μM CGP 12177, a partial β_3-adrenoceptor agonist with β_1-/β_2-adrenoceptor antagonistic properties. (B) Comparison for the nasal potential difference response to superfusion of the nasal epithelium with 1 μM isoprenaline ($n = 6$), with 0.1 μM SR 58611 ($n = 8$), with 0.1 μM CGP 12177 alone ($n = 5$), or after pretreatment with 1 μM SR 59230, a selective β_3-adrenoceptor antagonist ($n = 5$; $^*P < 0.05$ versus CGP 12177 alone). ns: $P > 0.05$.

4. Discussion

The present work suggests that β_3-adrenoceptor agonists regulate an apical chloride permeability in the rabbit nasal epithelium. This conclusion is based on several lines of evidence: (i) isoprenaline, a nonselective β-adrenoceptor agonist, produced hyperpolarization of the nasal potential difference, that was not prevented by nadolol, a β_1-/β_2-adrenoceptor antagonist, but was lost in the presence of bupranolol, a nonselective β-adrenoceptor antagonist; (ii) similar hyperpolarization was also obtained with two β_3-adrenoceptor agonists, SR 58611 and CGP 12177; (iii) a selective β_3-adrenoceptor antagonist, SR 59230, entirely abolished the effects of CGP 12177.

In the presence of SR 59230, isoprenaline stimulation still produces a hyperpolarisation of the nasal potential difference. These effects, which were likely caused by stimulation of β_1-/β_2-adrenoceptors, were very similar in amplitude to those obtained in the absence of SR 59230 (likely caused by the combined stimulation of β_1-/β_2-adrenoceptors and β_3-adrenoceptors). Therefore, isoprenaline produces an activation of Cl^- permeability to a similar extent by activation of either β_1-/β_2-adrenoceptors

or β_3-adrenoceptors. This suggests that either: (i) activation of Cl^- permeability through β-adrenoceptor subtype pathway induces a similar hyperpolarization of nasal potential difference, or (ii) the effects induced by the stimulation of different β-adrenoceptor are not additive on Cl^- conductance, because all subtypes stimulate the same signaling pathway. It may thus be speculated that β_3-adrenoceptors could compensate an impaired β_1-/β_2-adrenoceptor function on the regulation of Cl^- channels.

Activation of a Cl^- permeability by β_3-adrenoceptors is in line with our previous findings in a heterologous expression system, A549 cells. In these cells, co-expression of recombinant CFTR and β_3-adrenoceptor led the β_3-adrenoceptor stimulation (with isoprenaline plus nadolol, with SR 58611 or with CGP 12177) to produce an activation of the CFTR conductance through a pathway independent of cAMP/protein kinase A activation (Leblais et al., 1999a), but dependent of a pertussis toxin-sensitive G protein (Leblais et al., 1999b). An activation of Cl^- secretion by β_3-adrenoceptor stimulation has previously been suggested in cultured canine tracheal epithelial cells. This effect occurred via the accumulation of intracellular cAMP (Tamaoki et al., 1992). In addition, β_3-adrenoceptor stimulation increased ciliary motility through a cAMP-dependent mechanism in cultured rabbit epithelial cells (Takeyama et al., 1993). However, one can envisage that β_3-adrenoceptors is linked to several types of G proteins in airway epithelial cells, and thereafter, stimulates different signaling pathways as is the case in adipocytes (Soeder et al., 1999) and cell lines (Gerhardt et al., 1999).

Characterisation of a β_3-adrenoceptor subtype in airway epithelium, in addition to β_1 and β_2, raises the question of the role of these receptors in this tissue. β_3-adrenoceptor agonists, by increasing Cl^- secretion, could enhance secondary water transport in the airway and mucus secretion. Through these effects, β_3-adrenoceptors could play an important role in the airway mucosal defense system by modifying the mucociliary clearance. Furthermore, since β_3-adrenoceptor stimulation produces a hyperpolarization that was not additive to that produced by β_1-/β_2-adrenoceptor stimulation, it could be speculated that β-adrenoceptors play a redundant function in epithelial cells where β_1-/β_2-adrenoceptor and β_3-adrenoceptors are co-expressed. The specific characteristics of β_3-adrenoceptors could explain their participation in the regulation of epithelial function. β_3-adrenoceptors are activated at higher catecholamine concentrations than β_1- and β_2-adrenoceptors (Strosberg, 1997). In addition, and in opposition with β_1-/β_2-adrenoceptors, β_3-adrenoceptor in vitro and in vivo lack desensitization following activation with agonists. The β_3-adrenoceptor is refractory to short-term agonist-promoted uncoupling of the signaling pathway partly because it does not contain protein kinase A and β-adrenoceptor kinase phosphorylation sites located in the third cytoplasmic loop and the C-terminal region (Strosberg, 1997). This receptor is also resistant to long-term downregulation (Soeder et al., 1999). Thus, β_3-adrenoceptors may be involved when the sympathetic nervous system is overstimulated and/or the other β-adrenoceptor subtypes are downregulated.

Acknowledgements

This work was supported by grants from the Association Française de Lutte contre la Mucoviscidose (AFLM). We are grateful to Agnès Hivonnait for animal care.

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