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Different Mechanisms of Action of Beta2-adrenergic Receptor Agonists: A Comparison of Reproterol, Fenoterol and Salbutamol on Monocyte Cyclic-AMP and Leukotriene B4 Production in vitro

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Abstract

Background: Beta2-adrenergic receptor agonists have several effects on airway function, most of which are mediated in a variety of cell types resulting in increased c-AMP-production and inhibition of inflammatory mediator production. However, their stimulating effects on cAMP-production became known to be inversed by increasing phosphodiesterase (PDE) activity and degradation of cAMP. Therefore, in this study we have evaluated the efficacy of reproterol, a dual acting beta2-adrenoceptor agonist and PDE-inhibitor, as compared to salbutamol and fenoterol with respect to production of cAMP and LTB_4 in cultured monocytes.

Methods: Isolated human monocytes (10^5/ml) were incubated (n = 9) in suspension with beta2-adrenoceptor agonists (10^{-10}-10^{-4}M) for 30 minutes with and without IBMX. Then, cAMP production was determined following treatment with Triton-X100. Production of LTB_4 was measured following incubation of beta2-adrenoceptor agonists for 4 hrs in the presence of LPS (10 mg/ml). cAMP and LTB_4 were measured in culture supernatants by enzyme immunoassay.

Results: At 10^{-5} M, production of cAMP was significantly stimulated by reproterol > fenoterol > salbutamol in a dose-dependent manner to an extent of *128%, *65%, 13% (*$p<0.04$) respectively. In contrast, LTB_4-production was inhibited significantly to a similar degree by salbutamol and reproterol in a dose-dependent manner by 59% and 49% (10^{-5}M, $p<0.03$), respectively, with decreasing inhibition (15%) after fenoterol. Following co-incubation with IBMX, cAMP production only increased significantly ($p<0.002$) after fenoterol (+110%) compared to salbutamol (+29%) and reproterol (+50%) (ANOVA, $p<0.001$).

Conclusion: These data suggest effects of the theophylline constituent of reproterol to inhibit adenylyl cyclase induced phosphodiesterase activity. The advantageous synergistic effects of reproterol on cAMP-production need to be further explored in trials.

Key words: asthma, reproterol, salbutamol, fenoterol, cAMP, leukotriene B4.

Introduction

Asthma treatment is based on anti-inflammatory and bronchodilator therapy [19]. Beta2-adrenergic receptor agonists act as potent smooth muscle relaxants and are important drugs for the relief of acute asthmatic bronchoconstriction. In addition, they are able to inhibit the release of inflammatory mediators in leukocytes and airway cells, such as histamine and leukotrienes from mast cells [4] and therefore may play a role in suppressing the acute asthmatic inflammatory reaction [1]. Beta 2-adrenergic receptor agonists act by stimulating the intracellular activity of adenylyl cyclase resulting in increased production of cyclic adenosine monophosphate (cAMP) [20]. Cyclic-AMP plays an important regulatory role in many cell types involved in the pathophysiology of asthma. It suppresses the activity of immune and inflammatory cells and leads to acute relaxation of airway smooth muscles [25].

The bronchodilator reproterol is a monomolecular compound synthesised by combination of the non-selective beta-agonist orciprenaline (known in the US as metaproterenol) and the xanthine derivative theophylline [8]. We could recently show that reproterol stimulates cAMP and inhibits leukotriene B4 (LTB_4) production in cultured monocytes in vitro [10]. The effects exerted by reproterol were greater than those of each of its constituents alone, probably due to a synergistic action of the orciprenaline and the theophylline component of the molecule on adenylyl cyclase and phosphodiesterase enzymes. These results are of interest as a combination of reproterol and sodium cromoglycerate is used for asthma therapy in Germany. In a clinical trial, the protective effects of the fixed drug combination of inhaled reproterol and sodium cromoglycate in exercise-induced asthma compared to both substances alone were superior suggesting the use of a combination of reproterol and sodium cromoglycerate in exercise-induced asthma on children and adults [2]. This peculiar importance of reproterol compared to salbutamol and fenoterol may be of further interest.

We have now examined two selective beta2-adrenoceptor agonists, salbutamol and fenoterol, in order to further determine the potential role of the theophylline component of the reproterol molecule. We aimed at investigating whether reproterol is more potent than salbutamol or fenoterol with respect to increasing cAMP levels and related suppression of LTB_4-production in cultured human monocytes.

Methods

Six healthy non-smoking volunteers (age: 25 ± 5 years) with no history of asthma or atopic disease agreed to donate 60 ml of blood for each experiment.

Monocytes were isolated by gradient centrifugation as previously described (Nycodenz Monocytes, 1.068 g/l) resulting in platelet-free samples of $2\text{-}3x10^6$ cells with a purity of >95% as assessed by light microscopy [3]. Cell vitality was >98% as determined by trypan blue exclusion and lactate dehydrogenase (LDH) activity (Boehringer Ingelheim, Germany).

Effects of reproterol (Bronchospamin®, VIATRIS, Frankfurt, Germany), salbutamol and fenoterol (Sigma) were determined following incubation of monocytes (10^5/ml) cultured for 30 minutes in suspension. For the measurement of cAMP cells were made permeable in the presence of Triton-X100 (0.25 mM/ 10 min, Sigma). Monocytes were thereafter pelleted by centrifugation (500 g for 5 minutes at 4°C) and the supernatants were harvested and immediately frozen in liquid nitrogen and stored at -80°C until assayed. Experiments were repeated after non-specific inhibition of phosphodiesterases with IBMX (preincubation of 10^5 cells/ml with 0.25×10^{-3}M IBMX, Sigma, for 5 minutes). For the determination of LTB_4-production, aliquots of monocytes (10^5/ml) were incubated with an optimal concentration of lipopolysaccharide (10µg/ml, LPS) and the test substance on 48-well-plates (Costar) for 4 hours at 37°C in RPMI 1640 containing 10% foetal calf serum.

LTB_4 and cAMP were measured in the culture supernatants by direct enzyme immunoassay as previously described [9] -(materials purchased from Cayman Chem. Corp., Ann Arbor, Michigan, USA; microtiter plates from Nunc, Kamstrup, Denmark).

Statistical Analysis

All results are expressed as means ± standard errors of the means (SEM) for triplicate cultures of 3-4 experiments, if not mentioned otherwise. Mann Whitney U nonparametric tests, paired t-tests and ANOVA were used where appropriate for statistical comparisons of drug effects with the control and different beta2-agonists. P-values were considered significant if <0.05. All analyses were performed using the StatView 5.01 software (SAS Institute Inc., Cary, NC, USA) for Macintosh computers.

Results

cAMP-Production

After reproterol had been added to the test system, production of cAMP (n = 9) by monocytes showed a dose-dependent increase from 24 ± 16% at 10^{-7}M (p>0.05) to 414 ± 14% at 10^{-4}M as compared to the control (p = 0.0012, Fig. 1, Table 1). Fenoterol and salbutamol exerted much smaller effects compared to the control with maximum values of 65 ± 23% at 10^{-5}M fenoterol and of 22 ± 3% at 10^{-4}M salbutamol, respectively. These effects, however, were not statistically significant (Table 1). Stimulation of cAMP-production by reproterol (10^{-5}M, 10^{-4}M) was significantly greater than after salbutamol or fenoterol (n = 9, p = 0.0002 by ANOVA).

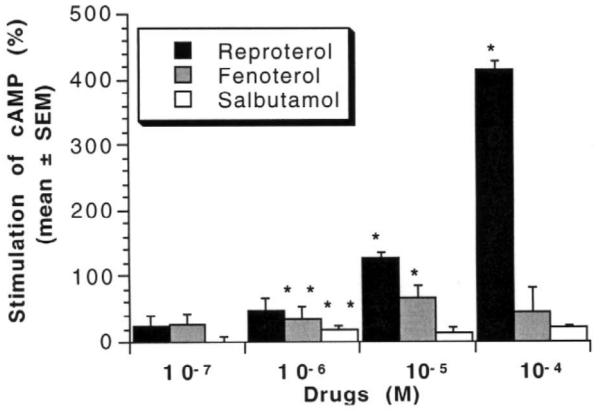

Fig. 1. Increased stimulation of cAMP-production with reproterol compared to fenoterol and salbutamol in vitro. In cultured monocytes, spontaneous cAMP-production (n = 9) was increased significantly (*p<0.04) more by reproterol (10^{-5}M, 10^{-4}M) compared to fenoterol or salbutamol. At 10^{-6} M effects of fenoterol or salbutamol were not significantly different (**p = 0.08, ANOVA) compared to reproterol.

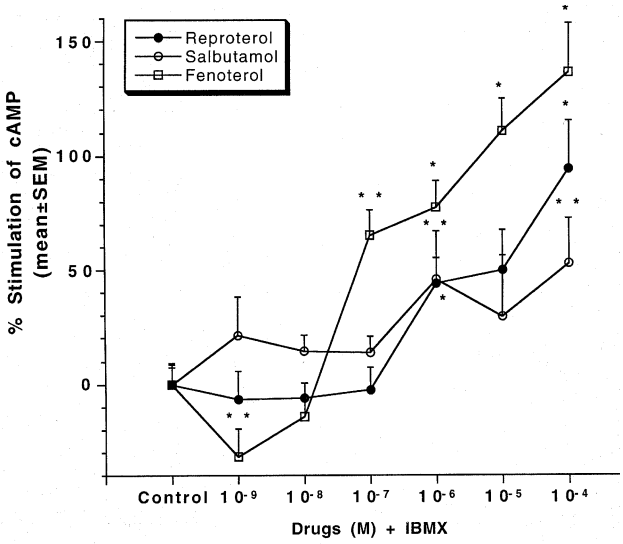

Fig. 2. Stimulation of cAMP production by beta2-adrenergic receptor agonists following PDE-inhibition. Production of cAMP was measured after incubation of normal monocytes (10^5/ml) for 30 min. with IBMX (0.25×10^{-3} M) and beta2-adrenergic receptor agonists. Stimulation of cAMP production following PDE-inhibition was greater for fenoterol than for reproterol and salbutamol but did not significantly increase after salbutamol (*p<0.03, **p<0.09).

Table 1. Increase of spontaneous cAMP production in cultured monocytes after incubation with reproterol (n = 9), salbutamol (n = 7) and fenoterol (n = 9) without IBMX in percent.

	Reproterol			Salbutamol			Fenoterol		
M/30min	**cAMP** (pg/10^5)	***Effect** (%)	**p-value**	**cAMP** (pg/10^5)	***Effect** (%)	**p-value**	**cAMP** (pg/10^5)	***Effect** (%)	**p-value**
Baseline	1.064 ±0.19	-	-	1.433 ±0.03	-	-	1.114 ±0.179	-	-
10^{-9}	1.095 ±0.334	2.9 ±30	0.7963	1.3 ±0.03	-9.3 ±2	0.4386	1.341 ±0.153	20.9 ±11	0.8137
10^{-8}	0.896 ±0.283	-15.8 ±31	1	1.328 ±0.083	-7.3 ±6.2	0.6056	1.182 ±0.061	6.1 ±5	0.711
10^{-7}	1.317 ±0.208	23.8 ±16	0.1826	1.451 ±0.086	1.2 ±6	1	1.4 ±0.229	25.6 ±16	0.191
10^{-6}	1.575 ±0.285	48 ±18	0.1307	1.692 ±0.089	18.7 ±5	0.0865	1.511 ±0.276	35.6 ±18	0.1912
10^{-5}	2.42 ±0.187	127.4 ±8	0.0012	1.618 ±0.119	12.9 ±7	0.2833	1.843 ±0.349	65.4 ±23	0.0833
10^{-4}	5.479 ±0.776	414.9 ±14	0.0012	1.75 ±0.054	22.1 ±3	0.055	1.632 ±0.583	46.5 ±36	0.4629

= % change from control

Table 2. Increase of spontaneous cAMP production in cultured monocytes after incubation with reproterol (n = 9), salbutamol (n = 7) and fenoterol (n = 9) with IBMX ($0.25x10^{-3}$M) as compared to IBMX as control in vitro in percent.

	Reproterol			Salbutamol			Fenoterol		
M/30min	**cAMP (pmol)**	***Effect (%)**	**p-value**	**cAMP (pmol)**	***Effect (%)**	**p-value**	**cAMP (pmol)**	***Effect (%)**	**p-value**
Baseline	0.446 ±0.048	-	- -	0.472 ±0.072	-	- -	0.364 ±0.049	-	-
IBMX	0.756 ±0.057	69.5±7 (100)	N.D.	0.962 ±0.084	103.8±9 (100)	N.D.	0.635 ±0.055	74.5±9 (100)	N.D.
10^{-9}	0.707 ±0.087	6.4 -±12	0.5184	1.167 0.196	21.3 ±17	0.3017	0.434 ±0.053	-31.6 ±12	0.0662
10^{-8}	0.708 ±0.048	-6.3 ±7	0.7611	1.104 ±0.074	14.7 ±7	0.1238	0.546 ±0.05	-14 ±9	0.4642
10^{-7}	0.738 ±0.073	-2.4 ±10	0.8196	1.097 ±0.084	14 ±7	0.1889	1.05 ±0.118	65.3 ±11	0.087
10^{-6}	1.092 ±0.118	44.4 ±11	0.0027	1.405 ±0.298	46 ±21	0.0929	1.127 ±0.138	77.5 ±12	0.0063
10^{-5}	1.133 ±0.21	49.9 ±18	1.262	1.245 ±0.34	29.4 ±27	0.817	1.338 ±0.193	110.7 ±14	0.0016
10^{-4}	1.472 ±0.317	94.7 ±21	0.0265	1.363 ±0.274	53 ±20	0.0929	1.501 ±0.326	136.4 ±22	0.0084

* = % change from control; N.D.= Not Determined

cAMP-Production after Phosphodiesterase Inhibition with IBMX

In order to evaluate the relative impact of phosphodiesterases (PDE) stimulation by increasing production of the cAMP substrate, experiments were repeated after adding the PDE-inhibitor IBMX (iso-butyl-methyl-xanthine) to the test system (Fig. 2, Table 2). Preincubation with IBMX would prevent the degradation of cAMP to 5'AMP, thereby leading to increased cAMP-production. After addition of beta2-adrenergic receptor agonists, a dose-dependent increase of cAMP-production (n = 9) compared to IBMX without the agonist (control) was observed. Fenoterol showed the highest stimulation of up to 136.4 ± 22% at 10^{-4}M ($p = 0.0084$) and cAMP-induction by fenoterol (10^{-7} and 10^{-5} M) compared to salbutamol and reproterol was significantly stronger (ANOVA, $p<0.001$). In the presence of IBMX cAMP-production was significantly ($p<0.03$) stimulated only at larger concentrations of reproterol (10^{-6}M, 10^{-4}M) with increases of 44.4 ± 11% and 94.7 ± 21%, respectively. Salbutamol had no significant influence on cAMP-production of monocytes after PDE-inhibition, although a mean increase of 53 ± 20% compared to IBMX alone was only observed at high concentrations of 10^{-4}M salbutamol (Table 2).

LTB_4-Production

Reproterol and Salbutamol inhibited LTB_4-production in a dose-dependent manner and to a similar degree ($p>0.05$, ANOVA). A decrease in LTB_4-production (n = 15) at baseline (254.4 ± 38pg/10^5) was observed after reproterol (10^{-8}M, 10^{-6}M) by -29 ± 17% ($p = 0.057$) and -32.5 ± 14% ($p = 0.0095$), respectively (Fig. 3). Salbutamol (10^{-6}M, 10^{-5}M) led to a decline ($p<0.03$) from the LTB_4 baseline (204.2 ± 36pg/ml, n = 9) by -49 ± 17% and by -59 ± 15%, respectively, without any significant difference compared to reproterol or fenoterol at 10^{-6}M ($p>0.05$, ANOVA). Inhibition of LTB_4-production (n = 9) by fenoterol was quantitatively smaller (33 ± 3% at 10^{-7}M, 35 ± 9% at 10^{-6}M, $p = 0.004$) and even subsided (15 ± 12%, $p = 0.3$) at higher concentrations (10^{-5}M).

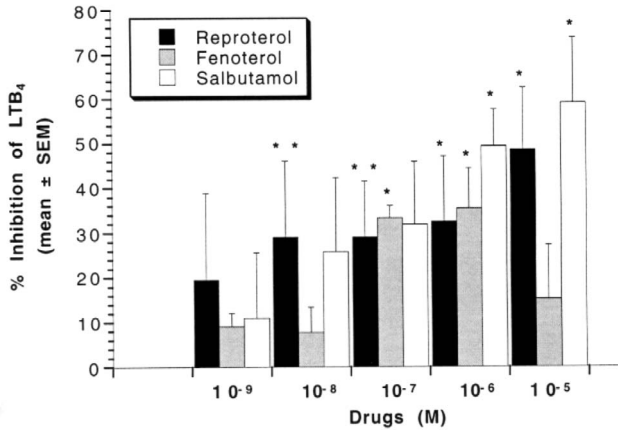

Fig. 3. Inhibition of LTB_4-production by beta2-adrenergic receptor agonists. LPS-stimulated monocyte LTB_4-production was measured following four hours incubation in vitro. Inhibition of LTB_4-production is shown in a dose-dependent manner for all beta2-adrenergic receptor agonists but decreased in the presence of 10^{-5} M fenoterol. At 10^{-6} M, LTB_4-production was significantly inhibited by beta2-adrenergic receptor agonists to a similar extent (*$p<0.025$). Inhibition of LTB_4-production at lower concentrations of reproterol tend towards significance (**$p<0.09$).

Discussion

In the present study, the effect of three different short-acting beta2-adrenergic receptor-agonists in cultured human monocytes with regard to activation of adenylyl cyclase-induced stimulation of spontaneous cAMP-production and its response to LPS-stimulated inflammatory mediator production as determined by inhibition of LTB_4 was tested. We found evidence that reproterol compared to salbutamol and fenoterol, demonstrated different effects on monocyte mediator production. Fenoterol stimulated cAMP-production at larger concentrations with subsiding LTB_4-inhibition, whereas salbutamol was more effective in inhibiting LTB_4-production without any demonstrable effect on cAMP. In contrast, the elevation of cyclic AMP levels correlated with the inhibition of LTB_4 production for reproterol.

The mode of action of beta2-adrenoceptor agonists is widely reported to stimulation of cAMP-production on the basis of in vitro-studies [1]. In vivo-data, obtained in healthy volunteers and in patients with asthma, also demonstrated elevated cAMP-plasma levels after inhalation of beta2-adrenoceptor agonists isoprenaline, fenoterol and salbutamol [6,7,13]. Increased cAMP-production in tracheal smooth muscle cells after salbutamol or salmeterol was associated with reversal of methacholine-induced muscle tone [6]. This protective effect is supposed to be in part mediated by anti-inflammatory responses of increased intracellular cAMP levels. Induction of cAMP by fenoterol suppressed in cultured eosinophiles eosinophil peroxidase release [17] and superoxide anion generation to a larger extent compared to salbutamol. These effects, however, were abolished by prolonged incubation time (fenoterol 10^{-6}M >120min) suggesting almost complete desensitization of the beta-receptors [23]. In inflammatory cells cAMP levels are controlled by PDE4 isozymes that were reported to be up-regulated by exposure to endotoxin, dibutyryl-cAMP [26], forskolin and 8-bromo-cAMP [16], isoproterenol [14] and other cAMP-elevating agents, such as salbutamol [24]. Inflammatory mediators, such as histamine, were also reported to produce up-regulation of PDE-activity [5] and to cause hyperresponsiveness to PDE inhibitors [10]. Collectively, PDE-activity is known to be increased markedly after prolonged exposure to agents which increase cAMP content and therefore activation of PDE4 was suggested as one of the mechanisms determining the intensity of the cAMP signal [21].

In the present series of experiments, monocytes were used as a model since they possess beta-receptors on their surfaces [12]. Experiments without PDE-inhibition by IBMX demonstrated a significant stronger stimulation of cAMP-production following 30min in-

cubation with reproterol as compared to fenoterol and salbutamol. As a possible explanation for this observation we reported recently, that reproterol, a dual acting beta2-agonist and phosphodiesterase inhibitor, may act synergistically on cAMP-production through stimulation of adenylyl cyclase activity in the presence of PDE-inhibition by its theophylline component resulting in increased levels of intracellular c-AMP [10]. This mechanism of action of reproterol may be important, since stimulation of at least two of the four PDE4- isozymes (58%) was reported for salbutamol to explain the non-significant effects of salbutamol on cAMP-production in our experiments [15]. However, in experiments with IBMX, cAMP-production was the highest for fenoterol and the lowest for salbutamol with no further increase of cAMP after administration of reproterol. In these experiments we thought to explore the effects of the cAMP-elevating agents on PDE-activation by comparing their effects on cAMP production prior and after nonspecific inhibition of PDE-activities by IBMX. Using cAMP-production in the presence of IBMX as a control (Fig.2) the characteristic profile was changed by the largest increase of cAMP by fenoterol, a non-significant increase by salbutamol and surprisingly less effects for reproterol. These data suggest only weak effects of salbutamol on cAMP production but stronger effects of fenoterol which could only be demonstrated in the presence of PDE-inhibition by IBMX. To further explain these effects of reproterol and IBMX in our experiments, poor efficacy of IBMX in the presence of a previous PDE-inhibition was recently reported only 7-18%, that was not attributable to concomitant increases in cGMP or cAMP. These IBMX effects were shown to be dependent on its blockade of endogenous adenosine effects [18]. These data further indicate that elevation and increased degradation of intracellular cAMP may be important mechanisms contributing to the good, but also adverse clinical effects known for beta2-adrenergic drugs.

LPS-induced production of LTB_4 decreased significantly to a similar degree in the present study after administration of 10^{-6}M reproterol (-32%), salbutamol (-49%) and fenoterol (-35%). Particularly at smaller drug concentrations (10^{-7}M) LTB_4 production was suppressed by fenoterol with no significant effect for reproterol and salbutamol but in line with the measurements of spontaneous cAMP there was a decrease in inhibition at higher concentrations. Effects of reproterol at smaller concentrations ($<10^{-6}$ M) on LTB_4 production seemed to be notable but did not reach significance compared to the other agents. Inhibition of LTB_4 was also measured in a comparable manner with forskolin (data not shown). When PGE_2, the representative cyclooxygenase metabolite in monocytes, was measured in culture supernatants, neither reproterol nor salbutamol or fenoterol had any influence on monocytic production of this prostaglandin (data not shown). In an earlier study, fenoterol was able to inhibit the production of antigen-induced leukotriene release in vitro [22] but these results were not confirmed in zymosan-stimulated macrophages [27].

In conclusion, we have shown that short-acting beta2-adrenoceptor agonists differ with respect to their activation on adenylyl cyclase and PDE-isoenzymes, and this may at least partly explain the different effects on cAMP-and LTB_4-production observed in the present study. Reproterol, a dual acting beta2-agonist and PDE-inhibitor, stimulates cAMP to a greater degree than the beta2-adrenoceptor agonists salbutamol and fenoterol and similar to salbutamol inhibits LTB_4-production. This may be due to a combined action of the orciprenaline and theophylline portions of the reproterol molecule on the activity of both adenylyl cyclase and phosphodiesterases resulting in more pronounced metabolic effects.

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REFERENCES

1. Barnes PJ. Pauwels R, O'Byrne PM, editors (1997) Beta2-Agonists in Asthma Treatment. New York: Marcel Dekker, Inc. 3, Effect of beta-agonists on airway effector cells. p. 35-66.
2. Berg A, Albrecht B, Darlath W, Voß HW, Berdel D (2002) Intraindividual, randomised, double-blind comparison between sodium cromoglycate and reproterol to assess the protective effect of the single drugs and their combination in children with exercise-induced asthma. Allergologie 25 (11): 557-564.
3. Boyum A (1983) Isolation of human monocytes with Nycodenz, a new non-ionic iodinated gradient medium. Scand J Immunol 17: 429-436
4. Church MK, Hiroi J (1987) Inhibition of IgE-dependent histamine release from human dispersed lung mast cells by anti-allergic drugs and salbutamol. Br J Pharmacol 90 (2): 421-429.
5. Dasi FJ, ortiz Jl, Cortijo J, Morcillo EJ (2000) Histamine up-regulates phosphodiesterase 4 activity and reduces prostaglandin E2-inhibitory effects in human neutrophils. Inflamm Res 49(11):600-609.
6. Ellis KE, Mistry R, Boyle JP, Challiss RA. Correlation of cyclic AMP accumulation and relaxant actions of salmeterol and salbutamol in bovine tracheal smooth muscle (1995) Br J Pharmacol 116(5):2510-2516.
7. Fairfax AJ, Rehahn M, Jones D, O'Malley B (1984) Comparison between the effects of inhaled isoprenaline and fenoterol on plasma cyclic AMP and heart rate in normal subjects. Br J Clin Pharmacol 17(2):165-170.
8. Habersang S, Leuschner F, von Schlichtegroll A (1977) [Toxicological investigation of reproterol (author's transl)]. Arzneimittelforschung 27 (12): 45-52.
9. Juergens UR, Christiansen SC, Stevenson DD, Zuraw BL. (1992) Arachidonic acid metabolism in monocytes of aspirin-sensitive asthmatic patients before and after oral aspirin challenge. J Allergy Clin Immunol 90: 636-645.
10. Juergens UR, Stöber M, Vetter H (1999) Reproterol - a monomolecular combination of orciprenaline and theophylline: novel aspects of its mode of action in asthma. Respiration 66: 220-224.
11. Kirino Y, Mio M, Fujii Y, Kamel C (2003) Role of Src in hypersensitization to phosphodiesterase inhibitors in beta2-adrenoceptor-desensitized eosinophiles. Methods Find Exp Cli Pharmacol 25(7):517-520.
12. Liggett SB (1989) Identification and characterization of a homogeneous population of beta 2- adrenergic receptors on human alveolar macrophages. Am Rev Respir Dis 139(2):552-555.

13. Lipworth BJ, Clark RA, Dhillon DP, Brown RA, McDevitt DG (1988) Beta-adrenoceptor responses to high doses of inhaled salbutamol in patients with bronchial asthma. Br J Clin Pharmacol 26(5):527-533.
14. Madelian V, La Vigne E (1996) Rapid regulation of a cyclic AMP-specific phosphodiesterase (PDE IV) by forskolin and isoproterenol in LRM55 astroglial cells. Biochem Pharmacol 28;51(12):1739-1747.
15. Manning CD, McLaughlin MM, Livi GP, Cieslinski LB, Torphy TJ, Barnette MS (1996) Prolonged beta adrenoceptor stimulation up-regulates cAMP phosphodiesterase activity in human monocytes by increasing mRNA and protein for phosphodiesterases 4A and 4B. J Pharmacol Exp Ther 276:810-818.
16. Maurice DH (1998) Cyclic nucleotide-mediated regulation of vascular smooth muscle cell cyclic nucleotide phosphodiesterase activity. Selective effect of cyclic AMP. Cell Biochem Biophys 29(1-2):35-47.
17. Mio M, Kirino Y, Kamei C (2000) Desensitization of beta2-adrenoceptor and hypersensitization to phosphodiesterase inhibitors elicited by beta2-agonists in guinea pig eosinophils. J Allergy Cli Immunol 106 (5):896-903.
18. Morgan AJ, Murray KJ, Challiss RA (1993) Comparison of the effect of isobutylmethylxanthine and phosphodiesterase-selective inhibitors on cAMP levels in SH-SY5Y neuroblastoma cells. Biochem Pharmacol 45(12):2373-2380.
19. National Heart, L.a.B.I. and WHO (1995) Global strategy for asthma management and prevention NHLBI/WHO Workshop. National Institutes of Health 95-3659: 40.
20. Nyberg L. Pauwels R, O'Byrne PM, editors (1997) Beta2-Agonists in Asthma Treatment. New York: Marcel Dekker, Inc. 5, Pharmacokinetics of beta-2-adrenoceptor-stimulating drugs. p. 87-130.
21. Oki N, Takahashi SI, Hidaka H, Conti M (2000) Short term feedback regulation of cAMP in FRTL-5 thyroid cells. Role of PDE4D3 phosphodiesterase activation. J Biol Chem 275:10831-10837.
22. Pacheco Y, Hosni R, Chabannes B, Gormand F, Moliere P, Grosclaude M, Piperno D, Lagarde M, Perrin-Fayolle M (1992) Leukotriene B4 level in stimulated blood neutrophils and alveolar macrophages from healthy and asthmatic subjects. Effect of beta-2 agonist therapy. Eur J Clin Invest 22(11):732-739.
23. Tachibana A, Kato M, Kimura H, Fujiu T, Suzuki M, Morikawa A (2002) Inhibition by fenoterol of human eosinophil functions including beta2-adrenoceptor-independent actions. Clin Exp Immunol 130 (3):415-423.
24. Torphy TJ, Zhou HL, Cieslinski LB (1992) Stimulation of beta adrenoceptors in a human monocytic cell line (U937) up-regulates cyclic AMP-specific phosphodiesterase activity. J Pharmacol Exp Ther 263(3):1195-1205.
25. Torphy TJ (1998) Phosphodiesterase isozymes: molecular targets for novel antiasthma agents. AmJ Respir Crit Care Med 157 (2): 351-370.
26. Verghese MW, McConnell RT, Lenhard JM, Hamacher L, Jin SL (1995) Regulation of distinct cyclic AMP-specific phosphodiesterase (phosphodieesterase type 4) isozymes in human monocytic cells. Mol Pharmacol 47(6):1164-1171.
27. Zetterlund A, Linden M, Larsson K (1998) Effects of beta2-agonists and budesonide on interleukin-1beta and leukotriene B4 secretion: studies of human monocytes and alveolar macrophages. J Asthma 35(7):565-573.

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