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β_3-adrenoceptor agonists, BRL 37344 and SR 58611A, do not induce relaxation of human, sheep and guinea-pig airway smooth muscle *in vitro*

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ABSTRACT: The existence of atypical- or β_3-adrenoceptors has now been generally accepted. These receptors have been shown to be abundant in adipose tissue and in a number of gastrointestinal smooth muscle preparations. A recent study reported that β_3-adrenoceptor stimulation mediated relaxation of isolated canine bronchial smooth muscle. The aim of the present study was to extend this observation to other species.

We investigated the *in vitro* responses of guinea-pig, human and sheep bronchial smooth muscle to isoprenaline, salbutamol (a selective β_2-adrenoceptor agonist), and BRL 37344 and SR 58611A (two presumably selective β_3-adrenoceptor agonists). The preparations were precontracted to 60–70% of maximal tension with histamine 10^{-6} M for guinea-pig and human bronchi, or acetylcholine 10^{-6} M for sheep bronchi.

In each species, SR 58611A produced a slight fall in tension of about 10% of the effects of theophylline (3 mM), but this decrease in tension was not significantly different from the spontaneous and weak relaxation observed with saline addition during the same duration of the experiment. These relaxations were not modified by either the nonselective β-adrenoceptor antagonist propranolol or the selective β_2-adrenoceptor antagonist ICI 118,551. In contrast, BRL 37344 induced a significant concentration-dependent fall in tension induced by both spasmogens. Its relaxant effects were inhibited both by propranolol and ICI 118,551 in human and guinea-pig airways, whereas on the isolated sheep bronchus BRL 37344-induced relaxations were only slightly, albeit significantly, reduced with either of the β-adrenoceptor antagonists tested. Salbutamol and isoprenaline induced potent relaxations of guinea-pig, human and sheep airway smooth muscle *in vitro*, which were antagonized both by propranolol and ICI 118,551.

Our findings show that β_3-adrenoceptor stimulation does not induce relaxation in guinea-pig, human and sheep bronchial smooth muscle, and that a β_2-adrenoceptor agonistic component might be implicated in the relaxant effects of BRL 37344.
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The existence of atypical or β_3-adrenoceptors has now been generally accepted. The gene encoding for human β_3-adrenoceptor has been identified and sequenced [1]. These receptors have been shown to be abundant in adipose tissue and in a number of gastrointestinal smooth muscle preparations, for example guinea-pig ileum [2], rat proximal colon [3, 4], rat distal colon [5], rat jejunum [6] and rat gastric fundus [7]. They have also been identified in rat skeletal muscle [8], ferret tracheal epithelium [9], and canine airways [10].

Furthermore, stimulation of β_3-adrenoceptors has been shown to induce relaxation of the rat oesophageal muscularis mucosae [11], lipolysis in omental and subcutaneous human fat cells [12], and inotropic effects in human [13] and canine heart [14].

In the lung, β_3-adrenoceptor stimulation increases epithelial fluid movements in ferret trachea [9], and reduces the release of the tachykinins, substance P or neurokinin A, from C-fibre endings of the isolated guinea-pig main bronchus [15, 16].

A recent paper by TAMAOKI *et al.* [10] reported that β_3-adrenoceptor stimulation mediated relaxation of isolated canine bronchial smooth muscle. The aim of the present study was to extend this observation to other species. We investigated the *in vitro* responses of guinea-pig, human and sheep bronchial smooth muscle to isoprenaline, salbutamol (a selective β_2-adrenoceptor agonist), and BRL 37344 [2, 7, 17, 18] and SR 58611A [3, 4, 18] (two presumably selective β_3-adrenoceptor agonists).

Materials and methods

Tissue preparations

Guinea-pig bronchial rings. Main bronchial rings were obtained from tricoloured guinea-pigs of either sex (250–350 g) anaesthetized with urethane (1.25 g·kg^{-1}, *i.p.*), and were suspended under an initial load of 2.0 g in Krebs solution at 37°C aerated with 95% O_2 - 5% CO_2 (pH 7.40). After 1 h of equilibration, resting force was 1.5–2.0 g. Under these conditions, responses to agonists were reproducible over several hours. Changes in force of contraction were measured isometrically with Pioden strain gauges (UF-1), amplified (EMKA, France) and displayed on a recorder (Linseis L65514, France). The composition of the Krebs solution was (mM): NaCl 118.0; KCl 5.4; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.2; $NaHCO_3$ 25.0; and glucose 11.7.

Human bronchus. Human bronchial tissue (with an inner diameter of 2–3 mm) obtained from patients undergoing surgery for lung cancer, but taken at a distance from the malignancy, was dissected free of parenchyma and transported to the laboratory in an ice-cold Krebs solution, previously aerated with a mixture of 95% O_2 - 5% CO_2. The tissue was stored overnight at 4°C and the experiment was carried out the next day. Published data have shown that overnight storage of tissue does not alter its reactivity [19, 20]. Rings from a segmental bronchus were suspended in Krebs solution under an initial load of 2.0 g, and under the conditions described for isolated guinea-pig main bronchi.

Sheep bronchus. Sheep bronchial tissue (with an inner diameter of 2–3 mm) was obtained from sheep of either sex (6–8 months old; Charles River) anaesthetized with an overdose of pentobarbital (60 mg·kg^{-1} in the humeral vein), dissected free of parenchyma, and cut into rings which were suspended in Krebs solution under an initial load of 2.0 g, and under the conditions described for isolated guinea-pig main bronchi.

Protocols

In all experiments, after an equilibration period of 60 min, guinea-pig, human and sheep bronchial rings were contracted with acetylcholine 10^{-3} M and relaxed maximally with theophylline 3×10^{-3} M, or 10^{-2} M for sheep bronchi. They were then allowed to equilibrate for a further 60 min period while they were washed with Krebs solution every 15 min.

Preparations were allowed to remain under resting tone and were contracted to 60–70% of maximal tension with histamine 10^{-6} M for guinea-pig and human bronchi, or acetylcholine 10^{-6} M for sheep bronchi. It was observed that histamine did not produce a potent contraction of the isolated sheep bronchus. Cumulative concentration response curves of SR 58611A (10^{-9}–10^{-5} M), of BRL 37344 (10^{-9}–10^{-5} M), isoprenaline (10^{-9}–10^{-5} M) and salbutamol (10^{-9}–10^{-4} M) were obtained with addition of these compounds every 3–20 min until a plateau was reached. The relaxant effects of β-adrenoceptor agonists were expressed as percentages of the maximal relaxation induced by theophylline (3×10^{-3} M, or 10^{-2} M for sheep bronchi) which was added at the end of the experiment.

To determine which type of β-adrenoceptor was involved in these responses, a nonselective β-adrenoceptor antagonist, propranolol, and a selective β_2-adrenoceptor antagonist, ICI 118,551, were used as pretreatment. After an incubation period of 30 min, cumulative concentration-response curves to β-adrenoceptor agonist were performed.

Statistical analysis of results

Data are expressed as mean±SEM. Statistical analysis of the results was performed with variance analysis and Student's t-test for paired or unpaired data, as appropriate. Probability values of $p<0.05$ were considered significant.

Substances

The substances used were: acetylcholine HCl (PCH, Paris, France); BRL 37344 (sodium-4[2[2-hydroxy-2(3-chlorophenyl)ethyl-amino]propyl]phenoxyacetate) (Beecham Pharmaceuticals, UK); ICI 118,551 (erythro (±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol-hydrochloride) (ZENECA Pharma, France); SR 58611A (ethyl{(7S)-7-[(2R)-2-(3-chlorophenyl)-2-hydroxyethylamino]-5,6,7,8-tetrahydronaphthalen-2-yloxy} acetate, hydrochloride) (SANOFI-Midy S.p.A. Research Center, Milan, Italy), histamine, propranolol, salbutamol (Sigma, St Louis, USA), isoprenaline (Winthrop, Paris, France), theophylline sodium anisate (Bruneau, Paris, France). All drugs were dissolved daily in distilled water and then diluted in Krebs solution.

Results

The relaxant effects of SR 58611A, BRL 37344, salbutamol and isoprenaline were examined on human (fig. 1) and guinea-pig bronchi (fig. 2) precontracted with histamine 10^{-6} M, and on sheep bronchi precontracted with acetylcholine 10^{-6} M (fig. 3).

In each species, SR 58611A produced a slight fall in tension of about 10% of the effects of theophylline, but this decrease in tension was not significantly different from the spontaneous and weak relaxation observed over same time period with saline addition (figs 1–3). These relaxations were not modified both by the nonselective β-adrenoceptor antagonist propranolol and the selective β_2-adrenoceptor antagonist ICI 118,551.

In contrast, BRL 37344 induced a significant concentration-dependent fall in tension induced by both spasmogens. Its relaxant effects were inhibited both by propranolol and ICI 118,551 in human and guinea-pig airways (figs 1 and 2), whereas, on the isolated sheep bronchus BRL 37344 - induced relaxations that were only slightly, albeit significantly, reduced with the β-adrenoceptor antagonists tested (fig. 3).

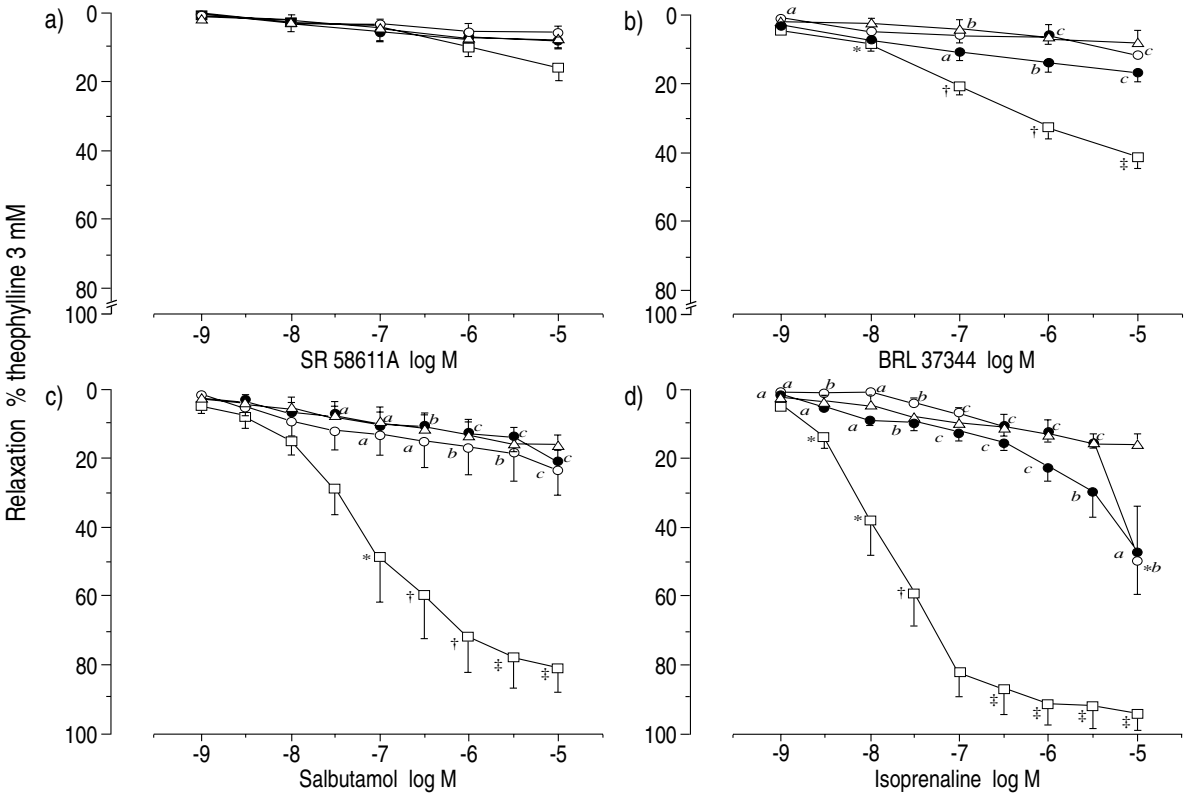

Fig. 1. – Cumulative concentration-response curves of a) SR 58611A; b) BRL 37344; c) salbutamol; d) isoprenaline in the absence (control, □) or presence of propranolol (10^{-6} M, ○) or ICI 118,551 (10^{-6} M, ●), along with response to saline (△) in human bronchi (n=4–6) precontracted with histamine (10^{-6} M). Values are mean±SEM. Significant differences from saline are shown as; *: p<0.05; †: p<0.01 ‡: p<0.001. Significant differences from control are shown as, *a*: p<0.05; *b*: p<0.01; *c*: p<0.001.

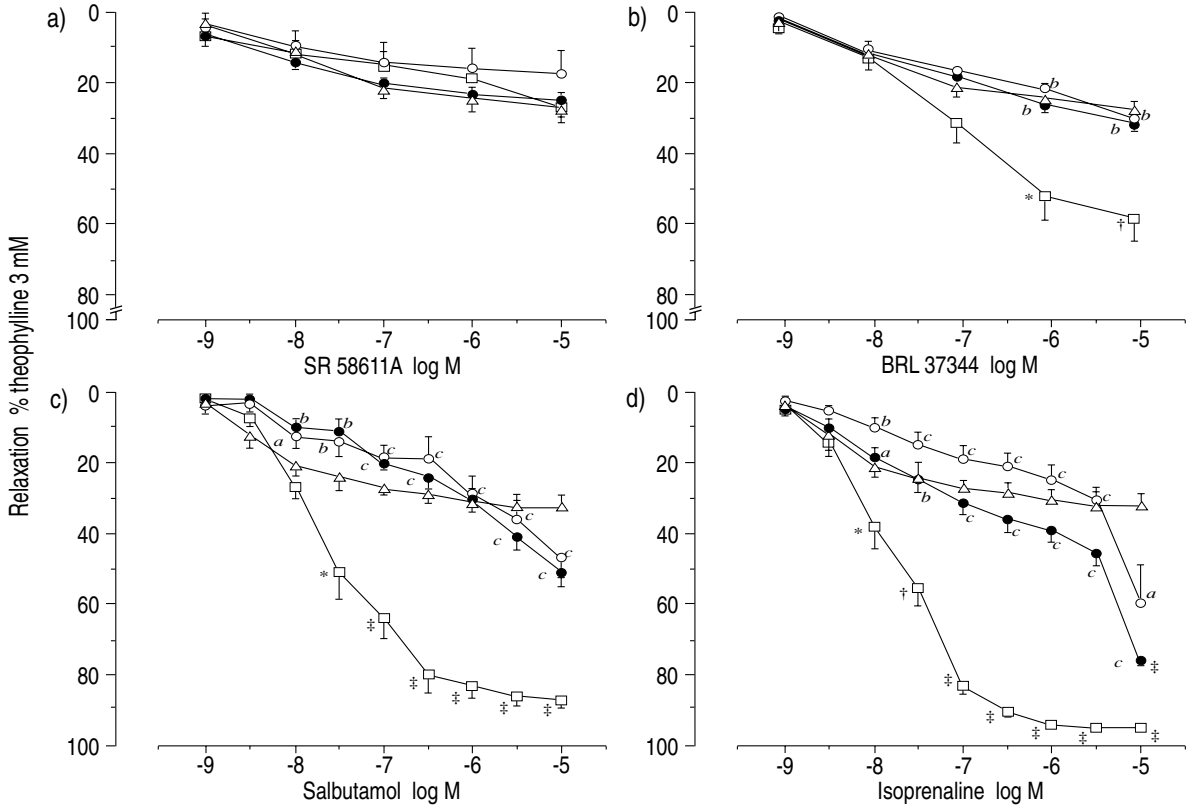

Fig. 2. – Cumulative concentration-response curves of a) SR 58611A; b) BRL 37344; c) salbutamol; d) isoprenaline in the absence (control, □) or presence of propranolol (10^{-6} M, ○) or ICI 118,551 (10^{-6} M, ●), along with response to saline (△) in guinea-pig main bronchi (n=4–6) precontracted with histamine (10^{-6} M). Values are mean±SEM. Significant differences from saline are shown as; *: p<0.05; †: p<0.01 ‡: p<0.001. Significant differences from control are shown as, *a*: p<0.05; *b*: p<0.01; *c*: p<0.001.

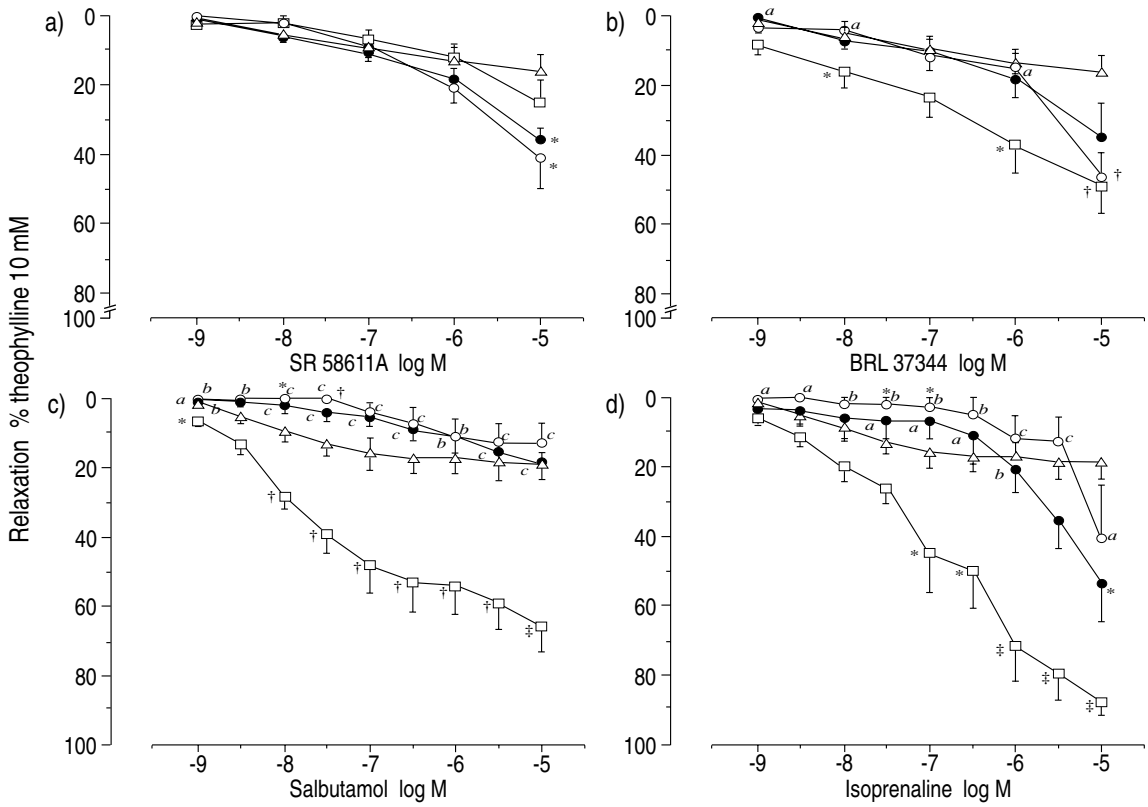

Fig. 3. – Cumulative concentration-response curves of a) SR 58611A; b) BRL 37344; c) salbutamol; d) isoprenaline in the absence (control, □) or presence of propranolol (10^{-6} M, ○) or ICI 118,551 (10^{-6} M, ●), along with response to saline (△) in sheep bronchi (n=4–7) precontracted with acetylcholine (10^{-6} M). Values are mean±SEM. Significant differences from saline are shown as; *: p<0.05; †: p<0.01 ‡: p<0.001. Significant differences from control are shown as, *a*: p<0.05; *b*: p<0.01; *c*: p<0.0 01.

Table 1. – -log EC_{50} and Emax of isoprenaline and salbutamol on human, guinea-pig and sheep bronchi after precontraction with histamine (Hist; 10^{-6} M) (guinea-pig and human bronchi) or acetylcholine (Ach, 10^{-6} M) (sheep bronchi)

	Human	Guinea-pig	Sheep
Isoprenaline			
Basal tone g	1.83±0.30 (5)	1.69±0.17 (5)	1.47±0.35 (7)
Ach or Hist induced tone g	2.36±0.26 (5)	2.95±0.28 (5)	1.98±0.22 (7)
-log EC_{50}	7.63±0.20 (5)	7.72±0.13 (5)	6.47±0.31 (7)
Emax g	0.75±0.23 (5)	0.76±0.07 (5)	0.81±0.18 (7)
Theophylline %	93±5.5 (5)	95±0.7 (5)	88±4.0 (7)
Salbutamol			
Basal tone g	1.59±0.32 (6)	1.82±0.32 (6)	1.05±0.21 (7)
Ach or Hist induced tone g	2.29±0.37 (6)	3.42±0.31 (6)	1.74±0.17 (7)
-log EC_{50}	6.65±0.41 (6)	7.37±0.16 (6)	6.34±0.51 (7)
Emax g	0.69±0.12 (6)	1.14±0.13 (6)	1.02±0.16 (7)
Theophylline %	80±7.1% (6)	87±2.4% (6)	66±7.2% (7)

Emax are expressed as grams (g) or as percentages of the maximal relaxation induced by theophylline (3×10^{-3} M for guinea-pig and human bronchi, or 10^{-2} M for sheep bronchi). Values are presented as mean±SEM. The number of experiments is given in parenthesis. EC_{50}: median effective concentration; Emax: maximal efficacy.

Salbutamol and isoprenaline strongly relaxed guinea-pig, human and sheep airway smooth muscle *in vitro* (figs 1–3). However, in the case of sheep bronchi, a tendency for additional relaxation was observed at the higher concentrations of salbutamol.

The potency of salbutamol on guinea-pig bronchi (median effective concentration (EC_{50})=4.20×10^{-8} M; n=6) was greater than those observed on human (EC_{50}=2.23×10^{-7} M; n=6) and sheep (EC_{50}= 4.57×10^{-7} M; n=7) bronchi (table 1). Efficacy was greater in human (maximal efficacy (Emax % theophylline relaxation)=80±7.1%; n=6) and guinea-pig (Emax=87±2.4%; n=6) than in sheep (Emax=66±7.2%; n=7) (table 1).

Isoprenaline had a higher potency on human (EC_{50}= 2.30×10^{-8} M; n=5) and guinea-pig bronchi (EC_{50}=1.90×10^{-8} M; n=5) than on sheep bronchi (EC_{50}=3.38×10^{-7} M;

n=7) (table 1). Efficacy was similar in all species (human Emax=93±5.5%; n=5; guinea-pig Emax=95±0.7; n=5; sheep Emax=88±4.0; n=7) (table 1).

In each species, relaxations induced by salbutamol or isoprenaline were antagonized both by propranolol and ICI 118,551 (figs 1–3).

Discussion

Our results have clearly shown that in the three species studied, and under the experimental conditions selected, an intense relaxation of the bronchial smooth muscle can be obtained with salbutamol and isoprenaline, and that this effect is principally β_2-adrenergic, since the relaxations induced by these substances are abolished by ICI 118,551 10^{-6} M, a concentration at which this substance can be considered a specific antagonist of β_2-adrenoceptors [10]. However, we observed that on sheep bronchi salbutamol has a partial agonistic effect that is weaker than in human and guinea pig bronchi, as testified by a lower Emax. This difference might indicate a smaller number of β_2-adrenoceptors in sheep or that β_1-adrenoceptors could be involved in the response elicited by salbutamol, but there is no specific study to support this hypothesis. Furthermore, it must be noted that in the case of sheep bronchi acetylcholine was used to induce tone instead of histamine as in human and guinea-pig airways. Differences dependent on the nature of the agonist used, on the response of β_2-adrenoceptor agonist, were previously observed in the study of Van Amsterdam *et al*. [21], suggesting that acetylcholine induces a higher functional antagonism than histamine.

Our results also show that BRL 37344 induces a weak relaxation of the smooth muscle in man, sheep or guinea-pig, whereas SR 58611A has no significant effect. Moreover, the weak effect of BRL 37344 is abolished by ICI 118,551, which suggests that this compound exert relaxation through a β_2-agonistic effect. Such a β_2-agonistic effect has previously been reported by Martin *et al*. [15] on the guinea-pig isolated bronchus and against the nonadrenergic noncholinergic contraction induced by electrical field stimulation. Thus, under the experimental conditions used by Martin *et al*. [15], part of the protective effect of BRL 37344 at 10^{-6} M is inhibited by ICI 118,551. Similarly, Newnham *et al*. [22] have demonstrated, in man, that BRL 37344 induces a fall in airways resistance, as measured by body plethysmography, but that effect was completely attenuated by nadolol, which is known to antagonize both β_1- and β_2- but not β_3-adrenoceptors [1] when compared with placebo, suggesting that this effect is mediated by β_2-, but not β_3-adrenoceptors. Conversely, SR 58611A appears, in our functional study on airway smooth muscle, devoid of β_2-adrenoceptor agonist effect, in contrast to the results of Blin *et al*. [23] on Chinese hamster ovary cells transfected with human β_3-adrenoceptor.

Conversely, SR 58611A seems to be more specific to the β_3-adrenoceptors. Its β_3-agonistic effects have been demonstrated in very low concentrations. This compound has been known to produce an inhibition of spontaneous motility of rat isolated proximal colon with an EC_{50} of 3.5×10^{-9} M. Elsewhere, SR 58611A has no chronotropic action (EC_{50} >3×10^{-5} M) on the guinea-pig isolated atrium (a β_1-adrenoceptor-mediated response) [3, 4]. The absence of β_2-agonistic effect of SR 58611A has been reported by Martin *et al*. [15] in their study on electrical field stimulation of the isolated guinea-pig main bronchus.

The results we obtained *in vitro* on preparations of man, guinea-pig and sheep airways were, therefore, different from those obtained by Tamaoki *et al*. [10] who reported β_3-adrenoceptor mediated relaxation of isolated canine bronchial smooth muscle. This discrepancy seems to be species-related, since the experimental conditions in the study by Tamaoki *et al*. [10] were similar to ours. However, it must be noted that these authors used bronchi that were slightly wider than ours (4–6 mm), taken from dogs weighing 17–32 kg; also, their animals were young (2–4 yrs), which brings them nearer to our guinea-pigs or sheeps, and their procedures were close to ours (precontraction with acetylcholine 10^{-5} M). Tamaoki *et al*. [10] also used BRL 37344, and showed that ICI 118,551 did not modify the concentration-response curves of BRL 37344, unless high concentrations were used (pA_2 *vs* BRL 37344 = 5.66 and pA_2 *vs* salbutamol = 7.01), pA_2 being the negative logarithm of the molar concentration of antagonist with which the ratio of equi effective concentrations of agonist in the presence and absence of antagonist is two.

In conclusion, our findings showed that β_3-adrenoceptor stimulation does not induce relaxation in human, guinea-pig and sheep bronchial smooth muscle, and that a β_2-adrenoceptor agonistic component might be involved in the relaxant effects of BRL 37344.

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References

1. Emorine LJ, Marullo S, Briend-Sutren M-M, *et al*. Molecular characterization of the human β_3-adrenergic receptor. *Science* 1989; 245: 1118–1121.
2. Bond RA, Clarke DE. Agonist and antagonist characterization of a putative adrenoceptor with distinct pharmacological properties from the α- and β-subtypes. *Br J Pharmacol* 1988; 95: 723–734.
3. Bianchetti A, Manara L. *In vitro* inhibition of intestinal mobility by phenylethanolaminotetralines: evidence of atypical β-adrenoceptors in rat colon. *Br J Pharmacol* 1990; 100: 831–839.
4. Croci T, Bianchetti A, Poggesi E, Boigerain R, Manara L. Gut-specific beta-adrenergic agonists inhibit rat colon mobility *in vitro* and *in vivo*. *Digest Dis Sci* 1987; 32: 900–932.
5. McLaughlin DP, MacDonald A. Evidence for the existence of "atypical" β-adrenoceptors (β_3-adrenoceptors) mediating relaxation in the rat distal colon *in vitro*. *Br J Pharmacol* 1990; 101: 569–574.

6. Van Der Vliet A, Rademaker B, Bast A. A beta-adrenoceptor with atypical characteristics is involved in the relaxation of the rat small intestine. *J Pharmacol Exp Ther* 1990; 255: 218–226.
7. Coleman RA, Denyer LH, Sheldrick KE. β-adrenoceptors in guinea-pig gastric fundus: are they the same as the "atypical" β-adrenoceptors in the rat adipocytes? *Br J Pharmacol* 1987; 90: 40P.
8. Challiss RAJ, Leighton B, Wilson S, Thurlbly PL, Arch JRS. An investigation of the β-adrenoceptor that mediates metabolic responses to the novel agonist BRL 28410 in rat soleus muscle. *Biochem Pharmacol* 1988; 37: 947–950.
9. Webber SE, Stock MJ. Evidence for an atypical or β_3-adrenoceptor in ferret tracheal epithelium. *Br J Pharmacol* 1992; 105: 857–862.
10. Tamaoki J, Yamauchi F, Chiyotani A, Yamawaki I, Takeuchi S, Konno K. Atypical β-adrenoceptor (β_3-adrenoceptor) mediated relaxation of canine isolated bronchial smooth muscle. *J Appl Physiol* 1993; 74: 297–302.
11. De Boer REP, Brouwer F, Zaagsma J. The β-adrenoceptors mediating relaxation of rat oesophageal muscularis mucosae are predominantly of the β_3-, but also of the β_2-subtype. *Br J Pharmacol* 1993; 110: 442–446.
12. Lönnqvist F, Krief S, Strosberg AD, Nyberg B, Emorine LJ, Arner P. Evidence for a functional β_3-adrenoceptor in man. *Br J Pharmacol* 1993; 110: 929–936.
13. Wheeldon NM, McDevitt DG, Lipworth BJ. Investigation of putative cardiac β_3-adrenoceptors in man. *Br J Pharmacol* 1993; 35: 87P.
14. Berlan M, Galitzky J, Bousquet-Mélou A, Lafontan M, Montastruc JL. β_3-adrenoceptor mediated increase in cutaneous blood flow in the dog. *Fund Clin Pharmacol* 1993; 7: 348.
15. Martin CAE, Naline E, Manara L, Advenier C. Effects of two β_3-adrenoceptor agonists, SR 58611A and BRL 37344, and of salbutamol on cholinergic and NANC neural contraction in guinea-pig main bronchi *in vitro*. *Br J Pharmacol* 1993; 110: 1311–1316.
16. Itabashi S, Aikawa T, Sekizawa K, Sasaki H, Takishima T. Evidence that an atypical β-adrenoceptor mediates the prejunctional inhibition of nonadrenergic noncholinergic contraction in guinea-pig bronchi. *Eur J Pharmacol* 1992; 218: 187–190.
17. Arch JRS, Ainsworth AT, Cawthorne MA, *et al*. Atypical β-adrenoceptor on brown adipocytes as target for anti-obesity drugs. *Nature* 1984; 309: 163–165.
18. Landi M, Croci T, Manara L. Similar atypical β-adrenergic receptors mediate *in vitro* rat adipocyte lipolysis and colonic motility inhibition. *Life Sci* 1993; 53: 297–302.
19. Brink C, Grimaud C, Guillot C, Orehek J. The interaction between indomethacin and contractile agents on human isolated airway muscle. *Br J Pharmacol* 1980; 69: 383–388.
20. Ghelani AM, Holroyde MC, Sheard P. Response of human isolated bronchial and lung parenchymal strips to SRS-A and other mediators of asthmatic bronchospasm. *Br J Pharmacol* 1980; 71: 107–112.
21. Newnham DM, Ingram CG, Mackie A, Lipworth BJ. β-adrenoceptor subtypes mediating the airways response to BRL 35135 in man. *Br J Clin Pharmacol* 1993; 36: 567–571.
22. Van Amsterdam RGM, Meurs H, Brouwer F, Postema JB, Timmermans A, Zaagsma J. Role of phosphoinositide metabolism in functional antagonism of airway smooth muscle contraction by β-adrenoceptor agonists. *Eur J Pharmacol* 1989; 172: 175–183.
23. Blin N, Camoin B, Maigret B, Strosberg AD. Structural and conformational features determining selective signal transduction in the β_3-adrenergic receptor. *Mol Pharmacol* 1994; 44: 1091–1104.