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In vitro inhibition of human colonic motility with SR 59119A and SR 59104A: evidence of a β_3-adrenoceptor-mediated effect

Marc Bardou [a,d,*], Bertrand Dousset [b], Catherine Deneux-Tharaux [a], Claude Smadja [c], Emmanuel Naline [a], Jean-Claude Chaput [d], Sylvie Naveau [d], Luciano Manara [e], Tiziano Croci [e], Charles Advenier [a]

[a] *Département de Pharmacologie, Faculté de Médecine Paris-Ouest, 15 rue de l'Ecole de Médecine, F-75270 Paris Cedex 06, France*
[b] *Service de Chirurgie Viscérale, Hôpital Cochin, 27 rue du Fbg Saint-Jacques, F-75679 Paris Cedex 14, France*
[c] *Service de Chirurgie Viscérale, Hôpital Antoine Béclère, 157 rue de la Porte de Trivaux, F-92141 Clamart Cedex, France*
[d] *Service d'Hépato-Gastroentérologie, Hôpital Antoine Béclère, 157 rue de la Porte de Trivaux, F-92141 Clamart Cedex, France*
[e] *Research Centre Sanofi-Midy, Via G.B. Piranesi, 38, 20137 Milan, Italy*

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Abstract

The new β_3-adrenoceptor is present in the gastrointestinal tract of various species. This study aimed to show that this receptor modulates human colonic motility in vitro. We used circular muscle strips from the human colon suspended in single organ baths containing Krebs solution and subjected to an initial 1.5–2 g tension. We measured the effects of different β_3-adrenoceptor agonists, including SR 59104A (*N*-[(6-hydroxy-1,2,3,4-tetrahydronaphthalen-(2*R*)-2-yl)methyl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride), SR 59119A (*N*-[(7-methoxy-1,2,3,4-tetrahydronaphthalen-(2*R*)-2-yl)methyl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride), BRL 37344 (*R*,*R* + *S*,*S*) [4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino] propyl] phenoxy] acetic acid), and of isoprenaline and salbutamol in the absence or in the presence of propranolol alone or in combination with the β_3-adrenoceptor antagonist SR 59230A (3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydro-naphthalen-1-ylamino]-(2*S*)-2-propanol oxalate) on amplitude of spontaneous contractions. To evaluate a possible β_2-adrenoceptor-mediated effect, we studied the action of these compounds on human isolated bronchi. On the human isolated colon, SR 59119A, SR 59104A and isoprenaline reduced the initial amplitude of spontaneous contractions by 60%. The curves obtained in the presence of antagonists suggested an action mediated by β_3-adrenoceptor stimulation, since propranolol did not antagonize the action of SR 59119A and SR 59104A, whereas the combination of propranolol and SR 59230A significantly displaced the concentration–response curve of these agonists to the right. This study provides pharmacological evidence of modulation of human colonic motility, and especially of the amplitude of spontaneous contractions, by the atypical β-adrenoceptor, the β_3-adrenoceptor.

Keywords: β_3-Adrenoceptor; Colon, human; Colonic motility, human; Smooth muscle

1. Introduction

It is generally accepted that catecholamines, e.g., noradrenaline or adrenaline, can relax gastrointestinal smooth muscle by acting on post-junctional α- and β-adrenoceptors. However, it has been reported that this relaxation in response to catecholamines is partially resistant to blockade of classical α- and β-adrenoceptors in rat distal colon (McLaughlin and MacDonald, 1990), gastric fundus (McLaughlin and MacDonald, 1991), esophagus (De Boer et al., 1993) and jejunum (MacDonald et al., 1994) or in guinea pig taenia caecum (Koite et al., 1995). These effects have been attributed to an additional atypical β_3-adrenoceptor (Arch, 1989; Emorine et al., 1989, 1994). Molecular biology studies (Krief et al., 1993; Granneman et al., 1991, 1993) suggest, although not unanimously (Thomas and Liggett, 1993), that a form of the β_3-adrenoceptor is expressed in the human digestive system. A review summarized most of the studies on β_3-adrenoceptors and intestinal motility in the different animal species (Manara et al., 1995b).

* Corresponding author. Département de Pharmacologie, Faculté de Médecine Paris-Ouest, 15 rue de l'Ecole de Médecine, F-75270, Paris, France. Tel.: +33-1-4234-6871; Fax: +33-1-4407-1352.

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The role played by the β_3-adrenoceptor has been investigated by using specific agonists and antagonists. Several agonists exert relaxant effects on segments of the gastrointestinal tract in several species. These include BRL 37344 (*R*,*R* + *S*,*S*) [4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino] propyl] phenoxy] acetic acid) (McLaughlin and MacDonald, 1991; De Boer et al., 1993; Arch et al., 1984; Bond and Vanhoutte, 1992; Hoey et al., 1996), SR 58611A (Bianchetti and Manara, 1990; Croci et al., 1991; De Ponti et al., 1995), ICI D7114 (Holloway et al., 1991; Growcott et al., 1993; MacDonald and Lamont, 1993), ICI-215001 (Tesfamariam and Allen, 1994), SR 59104A (*N*-[(6-hydroxy-1,2,3,4-te-trahydronaphthalen-(2*R*)-2-yl)methyl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl) ethanamine hydrochloride) and SR 59119A (*N*-[(7-methoxy-1,2,3,4-tetrahydronaphthalen-(2*R*)-2-yl)methyl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride) (Croci et al., 1996).

SR 59230A (3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronaphthalen-1-ylamino]-(2*S*)-2-propanol oxalate) has been described as a β_3-adrenoceptor antagonist (Croci et al., 1996; Manara et al., 1995a, 1996; Galitzky et al., 1997). This compound exerts a potent action ($pA_2 = 8.82$) in the rat proximal colon, with high selectivity. In the human colon SR 59230A exerts a competitive antagonist effect against isoprenaline ($pA_2 = 8.31$) (De Ponti et al., 1996).

Since few studies have focused on the action of β_3-adrenoceptor agonists and antagonists on the human colon, we studied the effects of two recently developed β_3-adrenoceptor agonists with a new chemical structure, SR 59104A and SR 59119A (Croci et al., 1996), on amplitude of the spontaneous contractions of human intestinal smooth muscle and of their antagonism by SR 59230A.

2. Materials and methods

2.1. Tissue preparation

2.1.1. Human colon

Circular muscular strips (1 cm long, 0.4 cm wide) were taken from different pieces of macroscopically normal human colon from 25 patients undergoing left colectomy for colon cancer. The mucosa was removed and the strips were suspended isometrically (tension 1.5–2 g) in oxygenated modified Krebs solution at 37°C (composition in mM/l: NaCl, 118; KCl, 5.4, $CaCl_2$, 2.5, KH_2PO_4, 0.6, $MgSO_4$, 1.2, $NaHCO_3$, 25, Glucose, 11.7). The tissue was stored overnight at 4°C and the experiment was carried out the next day. After 1 h of equilibration, resting force was 1.81 ± 0.06 g ($n = 157$). Changes in the force of contraction were measured with Pioden strain gauges (UF-1), amplified (EMKA, France) and displayed on a recorder (Linseis L65514, Germany). In our experimental system, strips showed a sustained level of baseline tone and spontaneous rhythmic contractile activity.

2.1.2. Human bronchi

Human bronchial tissue (inner diameter 2–3 mm) was taken at a distance from the malignancy in lung of patients undergoing surgery for lung cancer. It was dissected free of parenchyma and transported to the laboratory in an ice-cold Krebs solution previously aerated with 95% O_2–5% CO_2. The tissue was stored overnight at 4°C and the experiment was carried out the next day. Rings from a segmental bronchus, under an initial load of 2 g, were suspended in Krebs solution (composition as above) at 37°C and aerated with 95% O_2–5% CO_2 (pH 7.40). After 1 h of equilibration, resting force was 1.53 ± 0.34 g ($n = 88$). Changes in force of contraction were measured isometrically with Pioden strain gauges (UF-1), amplified (EMKA, France) and displayed on a recorder (Linseis L65514, Germany).

2.2. Protocols

2.2.1. Human colon

After 1 h of equilibration, during which the colonic muscular strips were washed with Krebs solution every 15 min, cumulative concentration–response curves for isoprenaline, salbutamol, BRL 37344, SR 59104A and SR 59119A (10^{-8} to 3×10^{-5} M for all of them) were obtained by adding these compounds every 10–15 min until a plateau inhibition of contractions was reached. The effect of β-adrenoceptor agonists was expressed as a percentage of the maximal relaxation induced by theophylline (3×10^{-3} M) added at the end of the experiment. The agonist concentration producing 30% of the maximal response (EC_{30}) to theophylline was derived from log concentration–response curves.

To clarify which type of β-adrenoceptor was involved in these responses we used a non-selective β-adrenoceptor antagonist, propranolol (10^{-7} M, a concentration that blocks only 3% of the propranolol-resistant binding sites but 97% and 98% of β_1- and β_2-adrenoceptors (Roberts et al., 1995) and a selective β_3-adrenoceptor antagonist, SR 59230A (10^{-9}–10^{-7} M, Manara et al., 1995a, 1996; De Ponti et al., 1996; Galitzky et al., 1997). After a 30-min incubation with antagonists cumulative concentration–response curves for β-adrenoceptor agonists were made.

2.2.2. Human bronchi

After an equilibration period of 60 min, during which the rings were washed with Krebs solution every 15 min, half of the rings were contracted to 60–70% of maximal tension with histamine (10^{-6} M) and were then allowed to equilibrate until they reached a steady state, and the other half were not contracted.

Cumulative concentration–response curves for SR 59104A (10^{-8}–3×10^{-5} M), SR 59119A (10^{-8}–3×10^{-5}

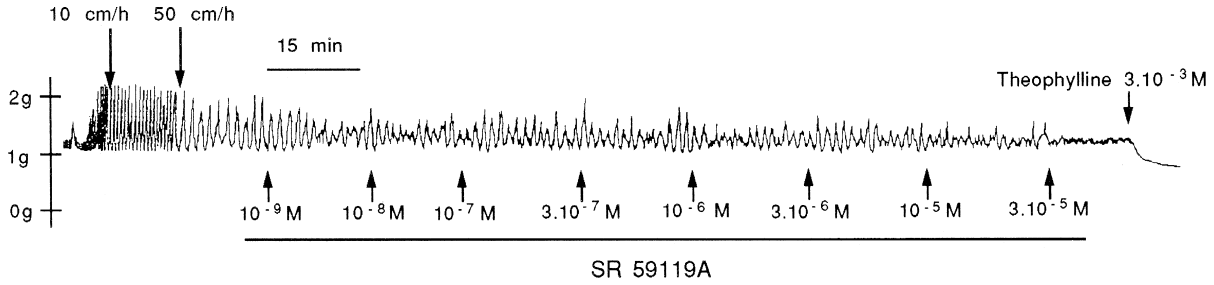

Fig. 1. Typical tracing showing the effects of SR 59119A on the mechanical activity of human colonic circular smooth muscle.

M) or the vehicle of these two compounds and for isoprenaline (10^{-8}–3×10^{-5} M) and salbutamol (10^{-8}–3×10^{-5} M) were obtained by adding these compounds every 3–20 min until a plateau was reached. The effects of β-adrenoceptor agonists were expressed as a percentage of the maximal relaxation induced by theophylline (3×10^{-3} M) added at the end of the experiment. The agonist concentration producing 30% of the maximal response (EC_{30}) to theophylline was derived from log concentration–response curves.

To establish whether the selective β_3-adrenoceptor antagonist SR 59230A was truly selective, it was used as pre-treatment (10^{-7} M). After an equilibration period of 30 min, cumulative concentration–response curves for the β-adrenoceptor agonists were plotted.

2.3. Substances

The substances used were: salbutamol sulphate; isoprenaline hydrochloride (Sigma, St. Louis, USA); propranolol (I.C.I. Pharma); theophylline sodium anisate (Bruneau, Paris, France).

SR 59104A, SR 59119A, BRL 37344 and SR 59230A were synthesised at Sanofi-Midy Research Centre (Milan, Italy).

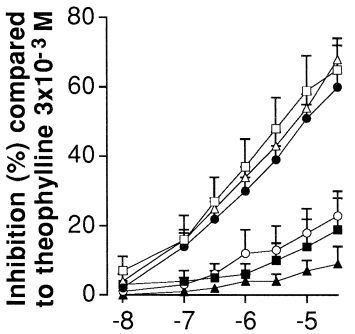

Fig. 2. Concentration–effect curves for isoprenaline (-□-, $n = 8/6$), SR 59104A (-●-, $n = 12/8$), SR 59119A (-△-, $n = 15/9$), salbutamol (-■-, $n = 6/3$), BRL 37344 (-○-, $n = 6/3$) and vehicle (-▲-, $n = 6/3$) on amplitude of spontaneous contractions of human isolated colon smooth muscle. Response are expressed as percentages of the inhibition caused by theophylline 3 mM. Means ± S.E.M. n = number of experiments/number of patients.

Salbutamol and isoprenaline were dissolved in distilled water. For SR 59119A, SR 59104A, SR 59230A and BRL 37344, the solvent was 0.2 ml of dimethylsulfoxide (DMSO) and 0.8 ml of distilled water for the 10^{-4} M concentration. From 10^{-5} to 10^{-8} M distilled water alone was used.

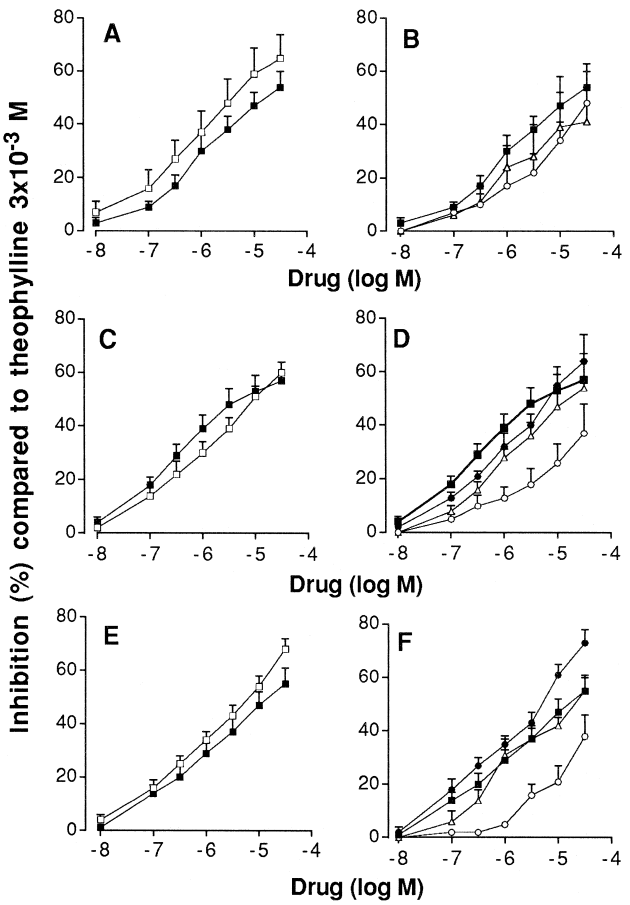

Fig. 3. Concentration–effect curves for isoprenaline (A), SR 59104A (C), SR 59119A (E) alone (-□-; A = 8/6; C = 13/9; E = 17/11), in the presence of propranolol (10^{-7} M) (-■-; A, B = 11/4; C, D = 8/3; E, F = 11/4) or in the presence of propranolol 10^{-7} M and SR 59230A 10^{-9} M (-●-; D = 5/5; F = 4/4), SR 59230A 10^{-8} M (-△-; B = 4/4; D = 5/5; F = 4/4), and SR 59230A 10^{-7} M (-○-; B = 10/5; D = 9/6; F = 11/8), (B, D, F) on the amplitude of spontaneous contractions of human isolated colon smooth muscle. Means ± S.E.M. n = number of experiments/number of patients.

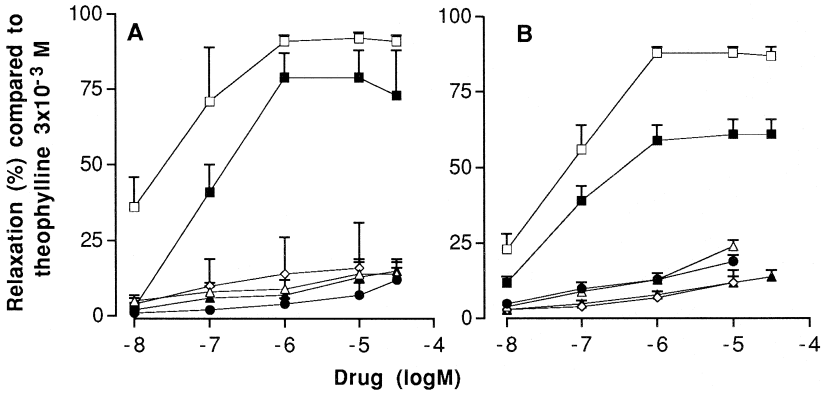

Fig. 4. Concentration–effect curves for isoprenaline (-□-, $n = 3/3$), SR 59104A (-●-, $n = 4/4$), SR 59119A (-△-, $n = 4/4$), salbutamol (-■-, $n = 3/3$), saline (-◇-, $n = 3/3$), and vehicle (-▲-, $n = 3/3$) on human isolated bronchus with basal tone (A) or contracted by histamine (10^{-5} M) (B). Means ± S.E.M. n = number of experiments/number of patients.

2.4. Statistical analysis

All results are expressed as means ± standard error ($x \pm$ S.E.M.). Differences among groups were analysed by a analysis of variance (ANOVA). Student's *t*-test on unpaired series was used to compare two groups. To avoid the bias of multiple comparisons a threshold of significance of 2% was chosen.

3. Results

3.1. Colon

3.1.1. Effects of agonists

We studied the effects of agonists, SR 59119A, SR 59104A, BRL 37344, isoprenaline and salbutamol, on the amplitude of spontaneous contractions of the isolated human colon. Fig. 1 provides a sample tracing. Fig. 2 indicates that SR 59119A, SR 59104A and isoprenaline reduced the amplitude of spontaneous contractions in a concentration-related manner, reaching 68% ± 4%, 60% ± 4% and 65% ± 9% at the maximum concentration tested (3×10^{-5} M), with EC_{30} values (in relation to the effect of theophylline 3×10^{-3} M) of (−log M) 6.15 ± 0.14 ($n = 15$), 5.88 ± 0.21 ($n = 12$) and 5.64 ± 0.3 ($n = 8$), respectively. BRL 37344 and salbutamol had no noteworthy activity. Analysis of variance showed that the effects of isoprenaline, SR 59119A and SR 59104A were not different between each other. Neither were those of BRL 37344, salbutamol and the vehicles of SR 59119A and SR 59104A. Then we considered substances as two distinct groups. The substances in the first group (isoprenaline, SR 59119A and SR 59104A) were significantly more effective than those of the second group (BRL 37344, salbutamol and the vehicles) ($P < 0.02$); this was true from the concentration of 10^{-7} M.

3.1.2. Effect of antagonists

Propranolol (10^{-7} M) did not alter significantly the effects of the β_3-adrenoceptor agonists SR 59104A and SR 59119A, or of isoprenaline on the amplitude of contractions (Fig. 3A, C and E). However, in the presence of propranolol, the selective β_3-adrenoceptor antagonist SR 59230A at the concentration of 10^{-7} M significantly shifted the concentration–response curves for the three

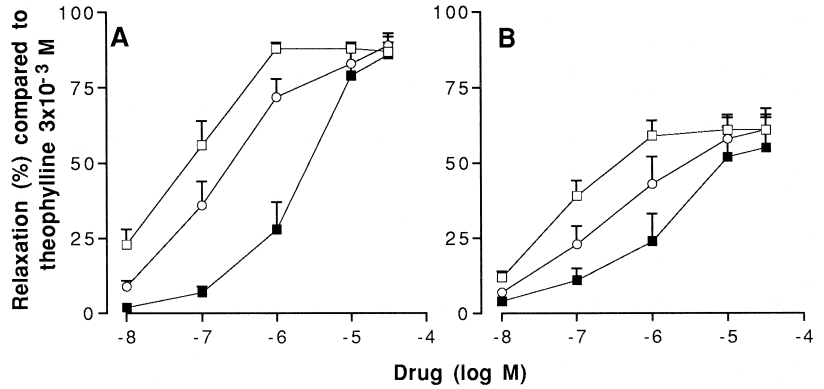

Fig. 5. Concentration–effect curves for isoprenaline (A) and salbutamol (B), alone (-□-; A = 17/16; B = 20/16), in the presence of SR 59230A 10^{-7} M (-○-; A = 9/9; B = 8/8), and in the presence of propranolol 10^{-7} M (-■-; A = 6/6; B = 5/5) on the human isolated bronchus contracted by histamine (10^{-5} M). Means ± S.E.M. n = number of experiments/number of patients.

agonists to the right—by 1–2 log units (Fig. 3B, D and F). The pK_b calculated according to Kenakin (1987) for SR 59320A at the concentration of 10^{-7} M was 8.36 ± 0.06 ($n = 9$), 8.63 ± 0.18 ($n = 11$) and 7.26 ± 0.39 ($n = 10$) for SR 59104A, SR 59119A and isoprenaline, respectively.

3.2. Human bronchi

The β_3-adrenoceptor agonists SR 59119A and SR 59104A had no relaxant effect on human bronchi with basal tone (Fig. 4A) or after they were precontracted with histamine (Fig. 4B). We did not test BRL 37344 in this model, since Martin et al. (1994) attribute its action on human bronchi to the β_2-adrenoceptor. The effect of the vehicle was the same as that of saline.

As was to be expected, propranolol caused a significant right-ward shift of the concentration–response curve for isoprenaline and salbutamol, whereas SR 59230A had no significant effect (Fig. 5A and B).

4. Discussion

Animal studies with supposedly selective agonists for β_3-adrenoceptors have shown these agents to have relaxant effects on several segments of the digestive tract (McLaughlin and MacDonald, 1990, 1991; De Boer et al., 1993; MacDonald et al., 1994; Koite et al., 1995; Manara et al., 1995b; Bond and Vanhoutte, 1992; Hoey et al., 1996; Bianchetti and Manara, 1990; Croci et al., 1991, 1996; De Ponti et al., 1995; MacDonald and Lamont, 1993).

There are still only few reports regarding human tissues. MacDonald et al. (1996) found a relaxant effect of isoprenaline, adrenaline and noradrenaline on the spontaneous activity of human colon circular smooth muscle. From experiments with propranolol and phentolamine they concluded, although tentatively, that there was primarily an α_1- and β_1-adrenoceptor mediated effect. Kelly et al. (1997) described an inhibitory effect of isoprenaline and CGP 12177A (a β_3-adrenoceptor agonist) on human taenia coli muscle strips. The curves obtained in the presence of several antagonists CGP 20712A (β_1-adrenoceptor antagonist) ICI 118551 (β_2-adrenoceptor antagonist) and propranolol (β_1- and β_2-adrenoceptor antagonist) led them to suggest that relaxation of the human colon involves a mixed population of β_1 and β_3-adrenoceptors. In a recent study, De Ponti et al. (1996) concluded that isoprenaline had a β_3-adrenoceptor-mediated effect on the human colon since its effects were inhibited by SR 59230A, a selective antagonist of this receptor.

A β_3-adrenoceptor-mediated effect on the human taenia coli was also suggested (Roberts et al., 1997) on the basis of comparison of the effects of propranolol and isoprenaline, and the finding of β_3-mRNA. Propranolol, CGP 12177A and ICI 118551 showed only weak affinity in antagonising the action of isoprenaline (pK_b respectively 7.93, 7.71 and 7.54).

The present findings provide further pharmacological evidence that the atypical β-adrenoceptor, the β_3-adrenoceptor, may be involved in modulating human colonic motility, in vitro. Under our in vitro conditions, the β_3-adrenoceptor agonists with a new chemical structure, SR 59119A and SR 59104A, and isoprenaline had a similar inhibitory action on the spontaneous contractions of human colonic smooth muscle strips directed towards the circular cells. Under similar conditions, BRL 37344, which is one of the most potent β_3-adrenoceptor agonists in relaxing in vitro intestinal preparations from different animal species, had no effect on the human colon. This agrees with the reports of MacDonald et al. (1996), Kelly et al. (1997) and De Ponti et al. (1996), who found BRL 37344 had no relaxant effects in humans, in contrast to its effects in other species. We cannot offer any definite explanation why this β_3-adrenoceptor agonist is not active. One possibility is that there may be species-related receptor subtypes, a suggestion that would be consistent with the numerous differences in agonist affinity and efficacy noted in cultured cells transfected with genes coding for the human or rodent β_3-adrenoceptor (Granneman et al., 1993; Blin et al., 1994).

Our results suggest there are some β_3-adrenoceptor-mediated inhibitory effects on the amplitude of spontaneous contractions since salbutamol had little effect. The dose–response curves for isoprenaline and SR 59119A and SR 59104A obtained in the presence of antagonists support this suggestion. Pre-incubation of muscle strips for 1 h with a non-selective β_1/β_2-adrenoceptor antagonist, propranolol (10^{-7} M), did not affect the response to SR 59119A, SR 59104A and isoprenaline, whereas, it was antagonised after pre-incubation with propranolol plus a selective β_3-adrenoceptor antagonist, SR 59230A, both at the above concentrations.

Another question is, why SR 59230A had a greater inhibitory effect on the action of SR 59119A and SR 59104A than on that of isoprenaline? A residual action of isoprenaline at β_1- and/or β_2-adrenoceptors, in spite of the presence of propranolol, might explain this finding.

There are as yet no pharmacological data for human tissues regarding the binding and affinity of the two new β_3-adrenoceptor agonists, SR 59119A and SR 59104A, for various receptors. Croci et al. (1996), however, found they had weak affinity for 5-HT_{1A} receptors (K_i, 25 nM for SR 59104A and 162 nM for SR 59119A) and for 5-HT_{1B} receptors (K_i, 80 nM and 1 μM). Neither of these compounds, at concentrations up to 1 μM, displaced the specific radioligands for different receptors including 5-HT_3, $5HT_4$, 5-HT_{1C}, 5-HT_{1D} receptors, 5-HT-uptake, α_1- and α_2-adrenoceptors, histamine H_1 receptors, muscarinic, $GABA_A$ and $GABA_B$, opioids and dopamine D_1 and D_2 receptors. Thus SR 59104A et 59119A show a high degree of selectivity.

Our findings for human bronchi suggest a β_3-adrenoceptor-mediated effect on the colon, because the β_3-adrenoceptor agonists, SR 59119A and SR 59104A, did not reduce bronchial tone, so probably they have no β_1- and/or β_2-adrenoceptor-mediated actions. This would agree with Martin et al. (1994), who found that SR 58611A and BRL 37344, both β_3-adrenoceptor agonists, had no effect on human bronchi in vitro. Croci et al. (1996) also reported that SR 59104A and SR 59119A had no relaxant effect on guinea pig trachea. However, in view of the species differences, our study of human bronchi was necessary and showed, in fact, that the β_3-adrenoceptor antagonist SR 59230A has no significant β_2-adrenoceptor antagonist action at 10^{-7} M.

To summarise, therefore, this study shows that SR 59119A and SR 59104A, two β_3-adrenoceptor agonists with a new chemical structure, reduced the amplitude of spontaneous contractions of colonic muscle strips directed towards the circular cells, presumably by acting on β_3-adrenoceptors. Although it is hard to extrapolate these in vitro findings to the in vivo situation, they are of interest in that they cast light on the action of new selective β_3-adrenoceptor agonists on digestive tract smooth muscle.

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