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SPECIAL REPORT

Functional evidence of atypical β_3-adrenoceptors in the human colon using the β_3-selective adrenoceptor antagonist, SR 59230A

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The role of β_3-adrenoceptors in human colonic circular smooth muscle was assessed *in vitro* by use of the β_3-selective antagonist SR 59230A. Isoprenaline, in the presence of the selective β-adrenoceptor antagonists CGP 20712A (β_1) and ICI 118551 (β_2), both at 0.1 μM, concentration-dependently relaxed the preparation ($pEC_{50} = 5.22$). This effect was potently and competitively antagonized by SR 59230A with a pA_2 of 8.31, while its **R,R** enantiomer SR 59483A gave an apparent pK_B of 6.21. Relaxation was likewise produced by CGP 12177A ($pEC_{50} = 6.05$), but not by BRL 37344. Although only one of these β_3-selective agonists was effective, the remarkably high potency of SR 59230A as a stereospecific antagonist of non-β_1 non-β_2 relaxation of human colonic muscle by isoprenaline provides strong functional evidence of β_3-adrenoceptors in that tissue.

Keywords: β_3-Adrenoceptors; human colon; smooth muscle relaxation; gastrointestinal motility; antispasmodics; SR 59230A

Introduction Comprehensive animal data attest to the involvement of β_3-adrenoceptors in the control of gut motility (for a review see Manara *et al.*, 1995b). Molecular biology studies (Krief *et al.*, 1993; Granneman *et al.*, 1993) suggest, although not unanimously (Thomas & Liggett, 1993), that a human-specific form of these receptors is expressed in the human digestive system and there is conflicting preliminary functional evidence of β_3-adrenoceptors in the human intestine monitored *in vitro* for mechanical responses (McLaughlin *et al.*, 1988; 1991). The recent introduction of the first β_3-selective adrenoceptor antagonist, SR 59230A (Manara *et al.*, 1995a), prompted the present study to ascertain whether functionally relevant β_3-adrenoceptors occur in the human gut. We studied *in vitro* β-adrenoceptor agonist-induced relaxation of human colonic muscle strips, which was prevented with high affinity by the novel compound SR 59230A.

Methods Approval for the experiments was given by the Department's Ethics Committee. Circular muscle strips (1 cm long, 0.3 cm wide) of macroscopically normal sigmoid colon with the mucosa removed from 17 patients (7 females, 10 males; mean age 63.3 ± 2.5 years) undergoing sigmoidectomy for colon cancer, were suspended isotonically (tension 15–20 mN) in oxygenated, modified Krebs solution at 37°C (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.1, $NaHCO_3$ 25, ascorbic acid 0.11) containing 0.5 μM desipramine, 30 μM hydrocortisone hemisuccinate (Lepetit, Milan, Italy) and 1 μM phentolamine. In our experimental conditions, strips exhibited a good level of baseline tone: their relaxation was expressed as a percentage of the maximal effect of isoprenaline or, when comparing different agonists, of papaverine, 100 μM, added at the end of each experiment. β-Adrenoceptor agonists were added cumulatively. Unless otherwise specified, drugs other than SR compounds were purchased from Sigma (St. Louis, MO, U.S.A.): (±)-isoprenaline hydrochloride; CGP 20712A (1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl) phenoxy]-2-propanol methan-esulphonate, Ciba-Geigy, Basel, Switzerland); ICI 118551 (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropyl-aminobutan-2-ol, ICI, Milan, Italy); SR 59230A (3-(2-ethylphenoxy)-1[(1S)-1,2,3,4-tetrahydronaphth-1-ylamino]-(2S)-2-propanol oxalate); SR 59483A (the **R,R** isomer of SR 59230A); BRL 37344, (**R,R** + S,S) [4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl] amino]propyl] phenoxy]acetic acid, synthesized at Sanofi Midy (Milan, Italy) and CGP 12177A, (±)-4-(3-*t*-butyl-amino-2-hydroxypropoxy)benzimidazol-2-one hydrochloride) (Ciba-Geigy, Basel, Switzerland).

Results are expressed as means ± s.e.mean. pA_2 values were calculated from constrained plots (best straight line with a slope of unity) after verifying that the experimental data fitted a theoretical Schild plot (slope not significantly different from unity). Where only one antagonist concentration was used, 'apparent' pK_B values were derived from the equation: $pK_B = -\log$ [antagonist concentration/(CR − 1)], where CR is the concentration ratio of the agonist in the presence and absence of antagonist.

Results Isoprenaline concentration-dependently relaxed circular muscle strips with a pEC_{50} value of 6.46 ± 0.01: maximal relaxation was 84 ± 8% of that induced by 100 μM papaverine. Isoprenaline-induced relaxation was antagonized to a similar extent by a combination of the selective β-adrenoceptor antagonists CGP 20712A (β_1) and ICI 118551 (β_2) at two different concentrations (each 0.1 μM or 1 μM): isoprenaline pEC_{50} values were 5.22 ± 0.03 and 5.03 ± 0.02, respectively (Figure 1a). Thus, the lower concentration of antagonists was used to block β_1- and β_2-sites when testing whether SR 59230A and its **R,R** enantiomer, SR 59483A, further prevented the isoprenaline-induced relaxation. In these experimental conditions, the residual relaxing ability of isoprenaline was hardly reduced by 1 μM SR 59483A with an apparent pK_B of 6.21 (Figure 1a), whereas it was potently and competitively antagonized with a pA_2 of 8.31 ± 0.06 by SR 59230A (Figure 1b), which up to 1 μM did not show any agonist activity *per se*.

Two other β-adrenoceptor agonists were tested without adding antagonists, in view of their presumed β_3-selectivity. BRL 37344 had no effect in any of four strips from four patients up to 100 μM, although 10 μM isoprenaline relaxed the same preparations even in the presence of CGP 20712A and ICI 118551 (both at 0.1 μM). CGP 12177A relaxed three strips from two subjects with a pEC_{50} value of 6.05 ± 0.06. Maximal relaxation, achieved with 10 μM CGP 12177A, was 56 ± 5% of that induced by papaverine.

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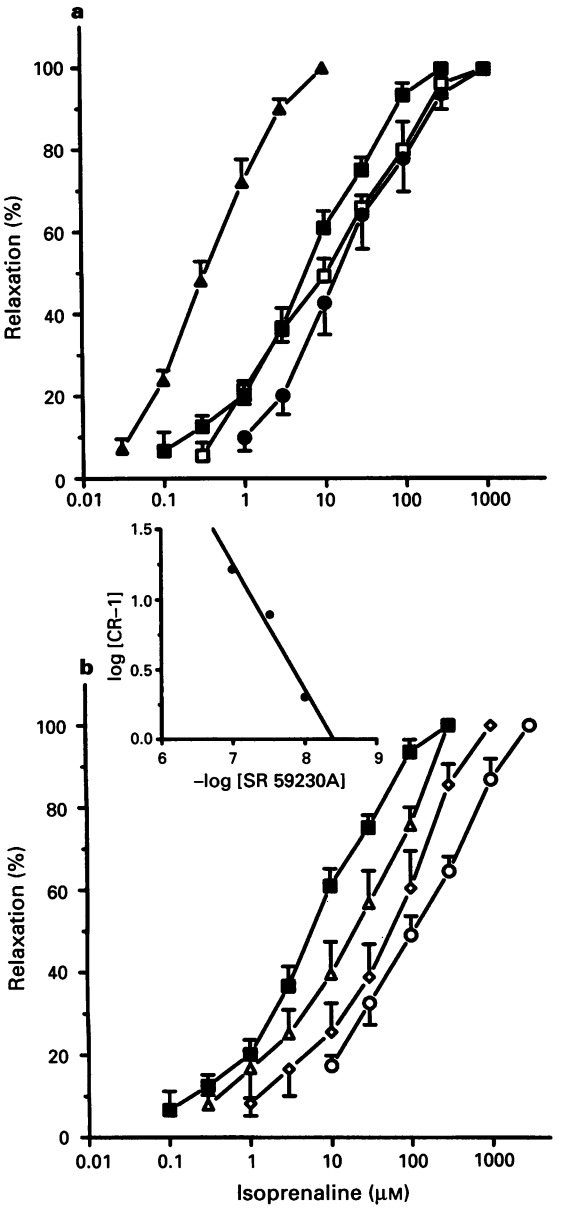

Figure 1 Effect of isoprenaline on human colonic circular muscle strips. (a) Concentration-response relationships for isoprenaline alone (▲; $n=7$ preparations from 6 patients) and in the presence of: CGP 20712A and ICI 118551 combined, each 0.1 μM (■; $n=5$ preparations from 5 patients) or 1 μM (□; $n=3$ preparations from 3 patients); CGP 20712A, ICI 118551 combined (each 0.1 μM) and SR 59483, 1 μM (●; $n=4$ preparations from 4 patients). (b) Competitive antagonism of isoprenaline-induced relaxation by SR 59230A. All experiments were carried out with CGP 20712A and ICI 118551 combined (each 0.1 μM) in the incubation medium. Isoprenaline concentration-response curves were obtained in the presence of the following concentrations of SR 59230A: 0 μM ($n=5$ preparations from 5 patients: same data as in (a), ■), 0.01 (△), 0.03 (◇) and 0.1 (○) μM ($n=4$ preparations from 4 patients). The inset shows the Schild plot (slope = − 0.90). Points represent means ± s.e.mean and are expressed as percentages of maximal relaxation induced by isoprenaline.

Discussion We employed the first antagonist selective for β_3-adrenoceptors to obtain functional evidence of their presence in the human colon. *In vitro* relaxation of colonic muscle strips by isoprenaline was substantially mediated by β_1- and/or β_2-adrenoceptors in view of its antagonism by CGP 20712A and ICI 118551 combined. Higher concentrations of isoprenaline were still effective in the presence of an excess of these antagonists, but not when SR 59230A was also added to the incubation medium. Isoprenaline antagonism by SR 59230A was competitive and potent, with a calculated pA_2 close to that reported earlier in the rat colon (Manara *et al.*, 1995a). This finding, whose specificity was strengthened by the much lower affinity of the **R,R** enantiomer of SR59230A, definitely supports the presence of functional β_3-adrenoceptors in the human colon. The observed stereoselectivity is remarkable also in view of the currently considered distinctive feature of β_3-adrenoceptors (as compared to β_1- and β_2-adrenoceptors), consisting of their limited ability to distinguish between the enantiomers (low eudismic index) of conventional β-blockers (Manara *et al.*, 1995b).

While SR 59230A had virtually the same high potency in human and rat preparations, we confirmed the negative results obtained by other investigators in the human isolated colon with BRL 37344 (McLaughlin *et al.*, 1991), which is one of the most potent β_3-adrenoceptor agonists for relaxing several intestinal *in vitro* preparations from different animal species (Manara *et al.*, 1995b). BRL 37344 is also a potent 'lipolytic' β_3-adrenoceptor agonist when tested on rat, but not on guinea-pig or human adipocytes (Carpené *et al.*, 1994). Unlike BRL 37344, CGP 12177A (a β_1-/β_2-adrenoceptor antagonist and β_3-adrenoceptor partial agonist) relaxed the human colon at least as potently as isoprenaline tested in the presence of CGP 20712A and ICI 118551 (i.e. when it could not act on either β_1- or β_2-adrenoceptors). The lack of response of our intestinal preparation to BRL 37344 but not to CGP 12177A suggests that the former is inadequate for the human native β_3-adrenoceptor. This is fairly consistent with the numerous differences in agonists' affinity and efficacy that have been noted in cultured cells transfected with genes coding for human or rodent β_3-adrenoceptors (Liggett, 1992; Granneman *et al.*, 1993; Blin *et al.*, 1994), although the results of this kind of study are not always easy to reconcile with those of functional tests on tissues expressing native receptors (Manara *et al.*, 1995b), possibly because of the high level of expression of receptors in transfected cells.

In conclusion, we found that human colonic smooth muscle was relaxed by isoprenaline in the presence of selective antagonists preventing its action on β_1- and β_2-adrenoceptors and by the β_3-adrenoceptor agonist, CGP 12177A; and that a selective β_3-adrenoceptor antagonist displayed high affinity in stereospecifically preventing this effect of isoprenaline. There is thus strong functional evidence of β_3-adrenoceptors in the human gut and hence of a potential physiological role of these receptors in man.

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