British Journal of Pharmacology (2000) 130, 1960–1966

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Functional, biochemical and molecular biological evidence for a possible β_3-adrenoceptor in human near-term myometrium

***,[1,5]M. Bardou, [2]C. Loustalot, [4]J. Cortijo, [3]B. Simon, [1]E. Naline, [5]M. Dumas, [4]S. Esteve, [6]T. Croci, [7]P. Chalon, [3]R. Frydman, [2]P. Sagot, [6]L. Manara, [4]E.J. Morcillo & [1]C. Advenier**

[1]Department of Pharmacology, Faculty of Medicine Paris-Ouest, 15 rue de l'Ecole de Médecine 75006 Paris, France; [2]Department of Gynecology, CHU du Bocage, 21 bd. de Lattre de Tassigny, BP 1542, 21000 Dijon, France; [3]CHU. Antoine Béclère, 157 rue de la Porte de Trivaux 92141 Clamart cedex France; [4]Department of Pharmacology, University of Valencia, Av. Blasco Ibañez 17, 46010 Valencia, Spain; [5]Laboratory of Cardiovascular Physiopathology and Pharmacology, Faculty of Medicine, 7 bd. Jeanne d'Arc, BP 87900, 21079 Dijon cedex, France; [6]Research Center Sanofi Midy, Via Piranesi, 38. 20137 Milan, Italy and [7]Sanofi Recherche, Labège Innopole, BP 137. Voie no1, 31676 Labège Cedex France

1 The possible existence of a β_3-adrenoceptor (β_3-AR) in human near-term myometrium was investigated by *in vitro* functional and biochemical studies and analysis of mRNA expression.

2 SR 59119A and SR 59104A and CGP 12177 (two selective agonists and a partial agonist, respectively, of the β_3-AR), salbutamol and terbutaline (β_2-AR agonists) each produced a concentration-dependent relaxation of the myometrial spontaneous contractions. There were no differences in pD_2 values for the relaxing potencies of terbutaline, salbutamol, CGP 12177 and SR 59119A. The rank order for their relaxing efficacies was SR 59119A > SR 59104A > terbutaline ≈ salbutamol ≈ CGP 12177 ($E_{max} = 52 \pm 7\%$, $42 \pm 12\%$ and ≈ 30% respectively).

3 Propranolol, a β_1- and β_2-AR antagonist, and ICI 118551, a β_2-AR antagonist (both at 0.1 μM), did not affect the SR 59119A-induced relaxation whereas SR 59230A, a selective β_3-AR antagonist (1 μM), significantly reduced the maximal relaxing effect of SR 59119A.

4 SR 59119A and salbutamol induced a significant increase in cyclic AMP levels that was antagonized by SR 59230A but not by propranolol for SR 59119A, and by propranolol but not by SR 59230A for salbutamol.

5 The β_3-AR mRNA was positively expressed in myometrium preparations in a reverse transcription polymerase chain assay.

6 The results presented provide the first evidence for the existence of the β_3-AR subtype in human near-term myometrium and suggest that the effects of SR 59119A might be mediated through an increase in cyclic AMP level.

British Journal of Pharmacology (2000) **130,** 1960–1966

Keywords: Human myometrium; near-term; β_3-adrenoceptor mRNA; β_3-adrenoceptor; tocolysis; cyclic AMP; SR 59119A; RT–PCR

Abbreviations: AR, adrenoceptor

Introduction

The incidence of premature birth has risen over the past 15 years and remains relatively high in developed countries, despite preventative measures (Goldenberg & Rouse, 1998). Around 6% of all pregnancies end pre-term, and deaths of premature babies represent ~85% of perinatal mortality. A reduction in the number of premature deliveries depends on the early and specialized management of pregnancy and parturition, including the development of new tocolytic agents (Jannet *et al*., 1997). β_2-adrenoceptor agonists are widely used in the treatment of pre-term labour, but β_2-adrenoceptors undergo desensitization after prolonged stimulation, with a decrease in efficacy (Berg *et al*., 1985). Although β-adrenoceptors (Lands *et al*., 1967a,b) were originally subclassified into β_1- and β_2-adrenoceptors, another subtype, the β_3-subtype, has since been reported (Emorine *et al*., 1989; 1992; 1994). The β_3-adrenoceptor shares 40–50% amino acid sequence identity with β_1- and β_2-adrenoceptor (Granneman *et al*., 1993) and lacks recognition sites for the cyclic AMP-dependent protein kinase and β-adrenoceptor kinase implicated in the desensitization of β_2-adrenoceptor (Strosberg, 1993). β_3-adrenoceptor has been shown to mediate lipolysis in white adipose tissue and thermogenesis in brown adipose tissue (Arch *et al*., 1984; Lonnqvist *et al*., 1993; Zaagsma & Nahorski, 1990), to inhibit the contractile activity of ileum and colon (Bardou *et al*., 1998; Bond & Clarke, 1988; Manara & Bianchetti, 1990). In dogs, β_3-adrenoceptor produces sustained peripheral vasodilatation that is predominant in skin and fat (Berlan *et al*., 1994; Shen *et al*., 1994). In the heart, the existence of non β_1-, non β_2-adrenoceptors has been the subject of much debate. The heart β_3-adrenoceptor has been described by Gauthier *et al*. (1996) to be negatively coupled to adenylate cyclase through stimulation of a G_i protein, or to be coupled to a nitric oxide synthase pathway through a $G_{i/o}$ protein (Gauthier *et al*., 1998). A heart β_4-adrenoceptor (Kaumann, 1989; Kaumann & Molenaar, 1997; Kaumann *et al*., 1998; Molenaar & Summers, 1987), that induce positive inotropic effects and that is positively coupled to adenylate cyclase through stimulation of a G_s protein has been described. The heart β_4-adrenoceptor, as described by Kauman & Molenaar (1997) is more likely to be stimulated by atypical β-adrenoceptor agonists (like CGP 12177) than by β_3-adrenoceptor agonists (BRL 37344, SR 58611A for example). Myometrium has always been described as expressing

*Author for correspondence at: Faculté de Médicine Paris-Ouest, Laboratoire de Pharmacologie, 15, rue de l'Ecole de Médicine, F-75006 Paris, France; E-mail: Marc.Bardou@u-bourgogne.fr

predominantly β_2-adrenoceptor (Doggrell, 1995; Engstrom *et al*., 1999; Story *et al*., 1988; Sugrue *et al*., 1985) and the presence of a β_3-adrenoceptor in human near-term myometrium has never been studied. The purpose of the present study was to investigate the presence of the β_3-adrenoceptor and the effects of β_3-adrenoceptor agonists on contractions and cyclic AMP production in human myometrium obtained from women undergoing caesarean delivery.

Methods

Human myometrial tissue preparation

Myometrial tissue samples were obtained from 35 women (mean age 31 ± 4 years) with normal uncomplicated pregnancy near term (between the 38th and 40th weeks of gestation) but undergoing caesarean section. None of the women had been treated with β_2-adrenoceptor agonists prior to caesarean delivery. Tissue samples were excised from the longitudinal layer in the uterine body, at the antiplacental site, and were immediately placed in pre-oxygenated Krebs solution at 4°C (composition, mM: NaCl, 118; KCl, 5.4; $CaCl_2$, 2.5; KH_2PO_4 0.6, $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11.7) and transported to the laboratory. Tissues were dissected free from serosa and used fresh (functional studies) or quickly frozen at −80°C (biochemical and molecular studies). The use of human myometrial tissue for experiments was approved by the local ethical committees.

Functional study

Myometrial tissues were cut into 6–8 strips (8–10 mm long by 2–3 mm in cross section) from each muscle piece and were suspended isometrically under a resting tension of 2 g in a 10 ml organ bath containing Krebs solution (composition as above) at 37°C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide (pH 7.40). After 1 h during which the myometrial strips were washed every 15 min and the resting tension readjusted to 2 g, the strips were allowed to equilibrate for a further 1 h until they showed regular spontaneous rhythmic contractile activity. One end of each strip was connected to a force-displacement transducer and tension changes were measured with Pioden strain gauges (UF1), amplified (EMKA, Paris, France), and recorded on a pen-writing oscillograph (Linseis, L65514, Munich, Germany). Once contractions became regular in amplitude, inhibitory cumulative concentration-response curves (from 0.1–30 μM) were determined for each compound studied: salbutamol and terbutaline (β_2-adrenoceptor agonists), SR 59119A, SR 59104A (Bardou *et al*., 1998) and SR 58611A (all three being full β_3-adrenoceptor agonists) and CGP 12177, usually described as a β_1- and β_2-adrenoceptor antagonist and as a partial β_3-adrenoceptor agonist (Gauthier *et al*., 1996; Longhurst & Levendusky, 1999). Each concentration-response curve was constructed by adding the next concentration when contractions had reached a steady state (about 45 min). In additional experiments, concentration-response curves for SR 59119A and salbutamol were obtained after 45 min incubation with 1 μM of the β_3-adrenoceptor antagonist SR59230A (De Ponti *et al*., 1996; Malinowska & Schlicker, 1997; Manara *et al*., 1996) or 0.1 μM of either propranolol (β_1- and β_2-adrenoceptor antagonist), ICI 118551 (a selective β_2-adrenoceptor antagonist) or a combination of both SR 59230A and ICI 118551. Amplitude of contraction was recorded at steady state for all concentrations. Since 3–4 h was required for the construction of a cumulative concentration-response curve, only one complete curve was obtained in each strip. Each drug was tested on at least eight preparations, each one derived from a different patient, and time-matched control experiments were carried out using drug vehicles.

Biochemical study

Myometrial strips were equilibrated in Krebs solution (composition as above) continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide at 37°C (pH 7.40) for 50 min, and then exposed to antagonists (0.1 μM propranolol or 1 μM SR 59230A), or their vehicle for 20 min, followed by addition of agonists (salbutamol or SR 59119A, each at 10 μM) for 5 min. In paired uterine strips, time-matched control experiments were carried out for a total incubation period of 75 min to determine the basal cyclic nucleotide content of unstimulated tissues. At the end of the incubation period, the tissues were processed as previously outlined by Buhimschi *et al*. (1995). The samples were immediately transferred into liquid nitrogen and homogenized in ice-cold 10% trichloroacetic acid. The homogenate was centrifuged at $10{,}000 \times g$ for 15 min at 4°C. The pH of the supernatant was neutralized by the addition of excess calcium carbonate followed by low-speed centrifugation. Aliquots of the supernatant were tested for cyclic AMP and cyclic GMP by enzyme immunoassay kits (RPN 225 and RPN 226, respectively; Amersham Pharmacia Biotech Ltd, Little Chalfont, U.K.) following the instructions of the manufacturer, without acetylation.

Analysis of functional and biochemical studies

In functional experiments the effect of each relaxant agent, including its maximal effect (E_{max}), was expressed as a percentage of the initial amplitude of spontaneous contractions. Drug potency is expressed as pD_2 values (negative logarithm of the molar concentration of the drug to produce half of its maximal effect). pD_2 values were calculated using Microsoft Excel 97. Data are expressed as mean ± s.e.mean. Differences among groups were analysed by analysis of variance (ANOVA) followed by the Bonferoni-corrected *t*-test or by Student's *t*-test for paired or unpaired data, as appropriate.

In functional studies, the β_3-adrenoceptor antagonist SR 59230A depressed the maximal response to SR 59119A. In order to evaluate the potency of this antagonist, we have calculated a pK_B value and its s.e.mean by applying the following equation as previously described by Paquet *et al*. (1999).

$$K_B = [B]/(\text{slope} - 1)$$

in which slope is that of the double-reciprocal plot of equieffective concentrations of agonist (A) in the absence (1/A) or in the presence (1/A′) of the antagonist (B) and [B] represents the antagonist concentration.

Molecular study: β_3-adrenoceptor transcript analysis by RT–PCR

Total RNA from human uterus tissue was prepared by using a single-step acid guanidium isothiocyanate-phenol-chloroform method as previously described by Chomczynski & Sacchi (1987). To avoid contamination with genomic DNA, total RNA was treated with DNase I (Gibco/BRL, Cergy Pontoise, France). Digestion was carried out at room temperature for

15 min and stopped by addition of 20 mM EDTA, pH 8.0, and incubated at 65°C for 10 min.

Isolated human myometrial polyA$^+$ RNA was treated with Superscript II RNAse H$^-$ reverse transcriptase (Gibco/BRL, Cergy Pontoise, France) and oligo(dT)12–18 primers as described previously by Gauthier *et al.* (1996). cDNA synthesis was performed using 5 μg of total RNA incubated in a 20 μl reaction containing 50 mM Tris-HCl (pH 8.3), 3 mM DTT, 10 mM KCl, 0.5 mM dNTP, 40 u. RNAsin, 200 u superscript reverse transcriptase (Gibco/BRL, Cergy Pontoise, France) for 1 h at 37°C. A control without reverse transcription was performed to verify that amplification did not proceed from residual genomic cDNA.

PCR reactions were performed with 2 μl of reverse transcriptase products in 50 μl reaction containing 50 mM Tris-HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 1.75 mM $MgCl_2$ 10% DMSO, 0.3 mM each primer, and 3.5 μg of TAQ and PWO DNA polymerases (Boehringer Mannheim, Germany). The amplification sequence consisted of 30 cycles of 94°C for 0.5 min, 58°C for 1 min, 68°C for 2.5 min, preceded by a denaturing step at 95°C for 1 min and followed by treatment at 68°C for 10 min. To rule out the possibility of amplifying genomic DNA, PCR was carried out with no prior RT of the RNA in some experiments.

The amplicons were generated using specific primers derived from the cDNA sequence (sense 5′-CGC GTA GGG GCC GAC GC antisense 5′-CCT GGG CTG CGC TGG GCT). The expected length of the fragment was 650 bp with this sense and antisense combination. PCR products were separated by electrophoresis through 1% agarose ethinium bromide-stained gels.

Drugs and solutions

The drugs and chemicals used and their sources were: SR59119A (N-[(7-methoxy-1,2,3,4-tetrahydronaphthalen-(2R)-2-yl)methyl]-(2R)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride), SR 59104A (N-[(6-hydroxy-1,2,3,4-tetrahydronaphthalen-(2R)-2-yl)methyl]-(2R)-2-hydroxy-2-(3-chlorophenyl)ethanamine hydrochloride), SR 58611A (ethyl{(7S)-7-[(2R)-2-(3-chlorophenyl)-2-hydroxyethylamino]-5,6,7,8-tetrahydronaphthalen-2-yloxy}acetate hydrochloride) and SR 59230A (3-(2-ethylphenoxy)-1-[(1S)-1,2,3,4-tetrahydronaphthalen-1-ylamino]-(2S)-2-propanol oxalate) were gifts from Sanofi-Synthelabo Research Centre (Milan, Italy), (±)-CGP12177A ((±)-4-(3-t-butylamino)-2-hydroxypropoxy)-1,3-dihydro-2H-benzimidazol-2-one); salbutamol sulphate, terbutaline, ICI 118551 hydrochloride (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride) (Sigma, St Louis, MO, U.S.A.); theophylline sodium anisate (Delalande, Quétigny, France). Drugs were dissolved in distilled water, absolute ethanol or 20% polyethyleneglycol 300 as appropriate, and diluted in Krebs solution as required. Drug concentrations are given as final bath concentrations.

Results

Relaxation response to β-adrenoceptor agonists

The two β_3-adrenoceptor agonists, SR 59119A (Figure 1) and to a lesser extent SR 59104, induced a dose-dependent inhibition of *in vitro* contractions of human near-term myometrium at concentrations ranging from 0.1–30 μM (Figure 2, Table 1). The maximum effect obtained at a concentration of 30 μM for SR 59119A and SR 59104A was

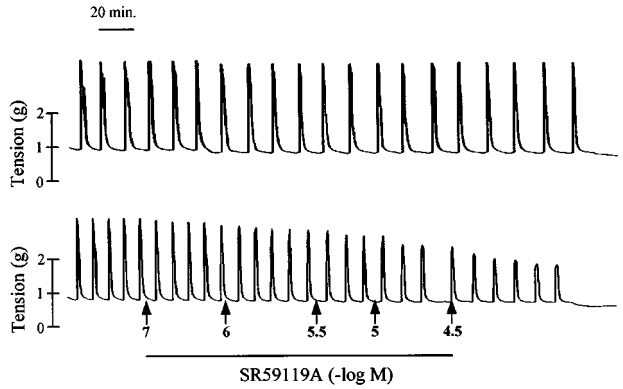

Figure 1 Representative recording of the effect of SR 59119A on spontaneous contraction of human near-term myometrium (lower trace) and time-matched control (upper trace).

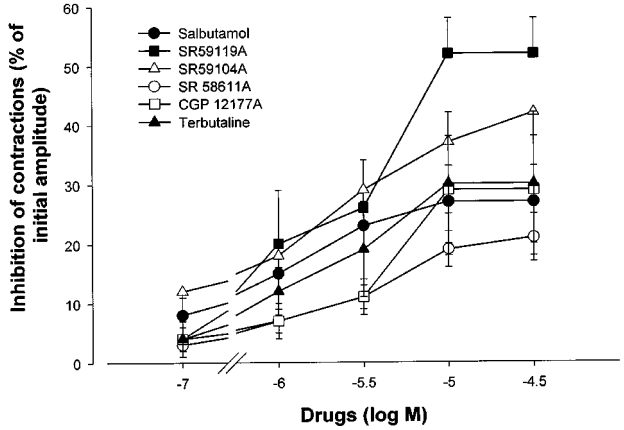

Figure 2 Effect of three full β_3-adrenoceptor agonists SR 59119A, SR 59104A and SR 58611A, of a partial β_3-adrenoceptor agonist CGP 12177, and of two β_2-adrenoceptor agonists salbutamol and terbutaline, on spontaneous contraction of human near term contractions. Results are expressed as mean ± s.e.mean. Inhibition of contractions is expressed as a percentage of initial amplitude of contractions.

Table 1 Maximal effect (E_{max}) and potency (pD_2) values for salbutamol, terbutaline and selective β_3-adrenoceptor agonists in human pregnant myometrium near term

	n*	E_{max}†	pD_2
Salbutamol	15	27 ± 6%‡	6.00 ± 0.20
Terbutaline	9	30 ± 12%	6.06 ± 0.29
SR 59119A	11	52 ± 7%	5.65 ± 0.14
SR 59104A	8	42 ± 12%	5.98 ± 0.18
SR 58611A	9	21 ± 4%‡	5.91 ± 0.22
CGP12177	9	29 ± 9%‡	5.83 ± 0.21

*n = number of experiments, each experiment was conducted on tissue from a different patient; †E_{max} indicates the maximal effect for each agent as a percentage of inhibition of initial amplitude of spontaneous contractions; ‡$P < 0.05$ from E_{max} values for SR 59119A.

52 ± 7% and 42 ± 12% respectively, a value greater than that obtained for salbutamol and terbutaline that was 27 ± 6% and 30 ± 12% respectively, although significance was reached only for SR 59119A vs salbutamol. SR 58611A and the partial agonist CGP 12177A were only marginally effective but were as potent as all the other drugs tested in the present study (Figure 2 and Table 1). The pD_2 values did not differ significantly.

The dose-response curve of SR 59119A and salbutamol were then performed in the presence of 0.1 μM propranolol, a concentration that blocks 97 and 98% of β_1- and β_2-adrenoceptors respectively, but only 3% of β_3-adrenoceptors (Roberts *et al.*, 1995), 0.1 μM ICI 118551, a selective β_2-adrenoceptor antagonist, 1 μM SR 59230A, a selective β_3-adrenoceptor antagonist, and in the presence of a combination of ICI 118551 and SR 59230A. A 45-min pretreatment with each of the antagonists had no effect on the amplitude of contractions. However, the inhibition of contractions obtained in the presence of SR 59119A was not antagonized by either propranolol or ICI 118551 but was statistically reversed, in terms of efficacy, in the presence of SR 59230A (E_{max} 35±6% in SR 59230A experiments compared with 52±6% in control experiments, $P<0.05$, with a pK_B value of 6.57±0.32), without further antagonism by the combination of the β_2- and the β_3-adrenoceptor antagonists ICI 118551 and SR 59230A (Figure 3A, Table 2). The pD_2 value for SR 59119A was not significantly modified by SR 59230A. This might be partly explained because the maximal effect was decreased by the antagonists. The inhibitory effect on myometrial contractions of salbutamol was significantly reduced by propranolol (E_{max}=9±3% in propranolol experiments vs 27±6% in control experiments, pK_B value of 8.22±0.24) but was not antagonized by SR 59230A. The antagonism produced by ICI 118551 failed to reach statistical significance but gave a pK_B value of 7.44±0.35, and further antagonism was not observed with the combination of ICI 118551 and SR 59230 (Figure 3B and Table 2).

Table 2 Maximal effect for SR 59119A and salbutamol in the absence (control) and presence of propranolol, ICI 118551, SR 59230A or combination of SR 59230A and ICI 118551

	n	*SR 59119A*	*Salbutamol*
Control	11	52±6%	27±6%
Propranolol (0.1 μM)	11	48±11%	9±3%‡
ICI 118551 (0.1 μM)	11	54±6%	17±4%
SR 59230A (1 μM)	11	35±6%‡	27±7%
Combination	11	42±8%‡	14±3%

n=number of experiments, each experiment was conducted on tissue from a different patient; †E_{max}: maximal effect as a percentage of that produced by theophylline (3×10^{-3} M); ‡$P<0.05$ from control experiments.

Cyclic AMP and cyclic GMP myometrial content after stimulation with salbutamol and SR 59119A.

Influence of propranolol and SR 59230A SR 59119A and salbutamol (each at 10 μM) induced a significant increase in cyclic AMP level compared with the control (Figure 4). The trend towards a greater increase in cyclic AMP level after stimulation with SR 59119A compared with salbutamol failed to reach statistical significance. The effect of SR 59119A on cyclic AMP stimulation was not modified after pretreatment with propranolol (0.1 μM) whereas it was strongly antagonized ($P<0.05$) by SR 59230A (1 μM). In contrast, the effect of salbutamol was not antagonized by SR 59230A but was abolished after pretreatment with propranolol, $P<0.05$ (Figure 4).

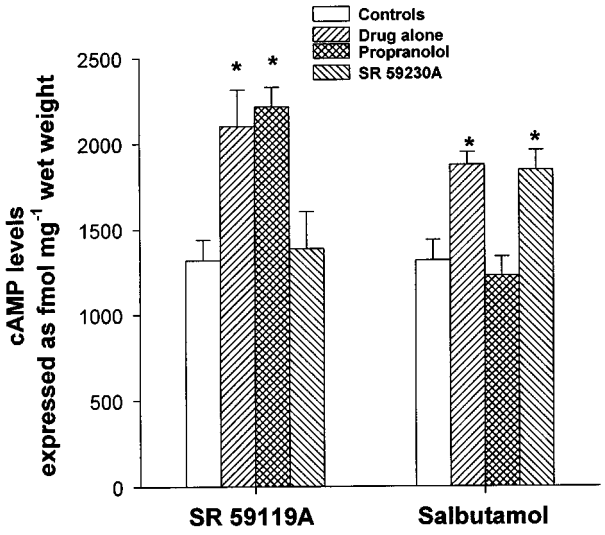

Figure 4 Effects of SR 59119A and salbutamol (both at 10 μM) on cyclic AMP production expressed as fmol mg^{-1} wet weight and influence of either propranolol (0.1 μM) or SR 59230A (1 μM). Results are expressed as mean±s.e.mean. Asterisks indicate a cyclic AMP level that was statistically different from baseline value (*$P<0.05$).

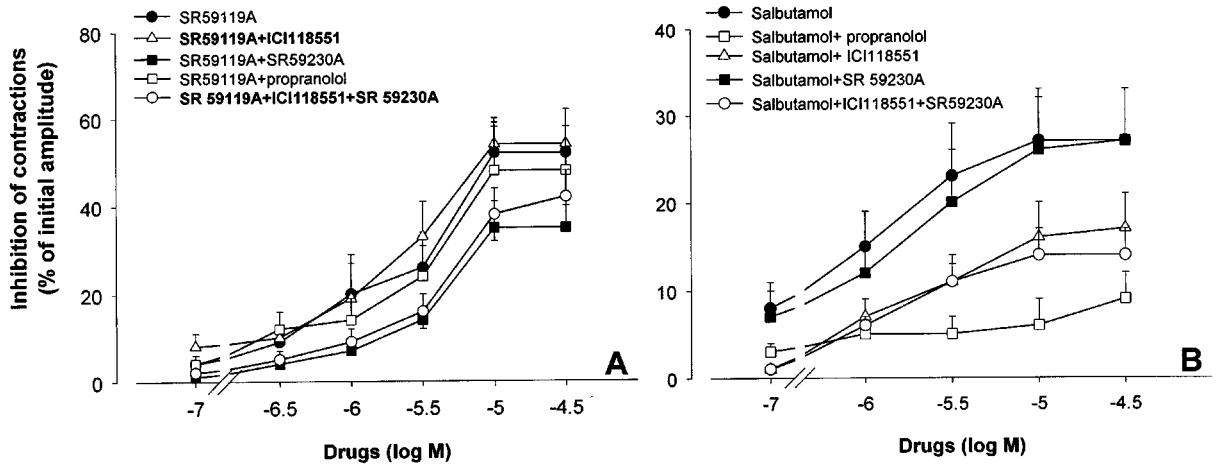

Figure 3 Effects of SR 59119A (A) or salbutamol (B), alone or after pretreatment with either propranolol (0.1 μM), ICI 118551 (0.1 μM), SR 59230A (1 μM) or combination of ICI 118551 (0.1 μM) and SR 59230A (1 μM) on spontaneous contraction of human near-term contractions. Results are expressed as mean±s.e.mean. Inhibition of contractions is expressed as a percentage of initial amplitude of contractions.

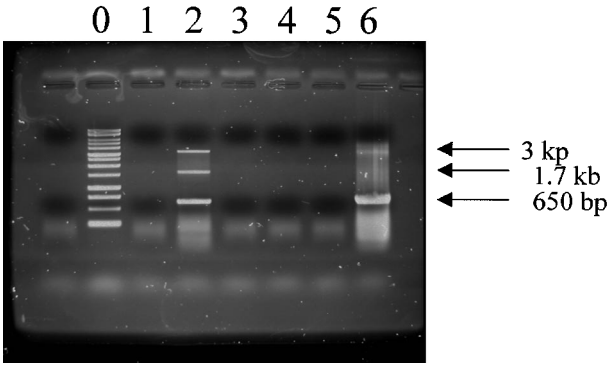

Figure 5 Agarose gel electrophoresis of RT–PCR products of β_3-adrenoceptors using RNA extracted from human near-term myometrium. Lane 0, size marker, lines 1 and 2 cell line SK-N-MC used as positive control, lanes 3 and 4 human bronchi, lanes 5 and 6 human near-term myometrium. Lanes 1, 3 and 5 are without reverse transcription, lanes 2, 4 and 6 are with reverse transcription.

The cyclic GMP levels were about 20 fold lower than the corresponding cyclic AMP levels, and they were not increased either by SR 59119A or by salbutamol (data not shown).

Isolation of mRNA by RT–PCR To determine whether the presence of a β_3-adrenoceptor-mediated inhibition of human near-term myometrium contractions was associated with the expression of β_3-adrenoceptor transcripts we used an RT–PCR assay. RNA was treated with DNase I to prevent contamination by genomic DNA.

As shown in Figure 5, β_3-adrenoceptor mRNA expression was detected, as a 650 bp fragment, in human myometrium (lane 6, $n=5$). Hybridization to human β_3-adrenoceptor cDNA confirmed the identity of the amplified products. No amplification was found in human bronchi (lanes 3 and 4, $n=5$). As a control for the assay, reverse transcribed β_3-adrenoceptor mRNA was readily amplified by PCR in SK-N-MC cell line known to express the β_3-adrenoceptor (Esbenshade *et al*., 1992).

PCR products without prior RT of the RNA did not reveal any positive bands (Figure 5).

Discussion

Various studies have shown that β_2-adrenoceptors exist in human gravid myometrium and that their stimulation produces an increase in cyclic AMP level and relaxation (Andersson *et al*., 1980; Berg *et al*., 1985). But treatment with β_2-adrenoceptor agonists is often accompanied by a decrease in efficacy and of binding sites for β_2-adrenoceptor agonists (Andersson *et al*., 1980; Berg *et al*., 1985; 1987). This is to our knowledge the first study that demonstrates the presence of functional β_3-adrenoceptor in human near term myometrium. The functional and biochemical effects observed in the present study are very likely to be mediated through stimulation of the β_3-adrenoceptor. Indeed, in a previous report we have shown that on spontaneous contractions of human colon, the inhibitory effect of SR 59119A, the most efficient agonist in this study, was insensitive to inhibition of 0.1 μM of propranolol (Bardou *et al*., 1998). The human colon is known to express β_3-adrenoceptors (Bardou *et al*., 1998; Berkowitz *et al*., 1995; Krief *et al*., 1993). These results confirm the selectivity of this β_3-adrenoceptor agonist. The pharmacological evidence for the presence of β_3-adrenoceptor in human myometrium was also supported by the use of β-adrenoceptor antagonists. The inhibitory effects of SR 59119A on human myometrial contractions were antagonized by the selective β_3-adrenoceptor antagonist SR 59230A but not by the β_1- and β_2-adrenoceptor antagonist propranolol or by the selective β_2-adrenoceptor antagonist ICI 118551. The selectivity of the β_3-adrenoceptor antagonist used in this study, SR 59230A, has been reported in previous studies (De Ponti *et al*., 1996; MacDonald & Watt, 1999; Malinowska & Schlicker, 1997; Manara *et al*., 1996). Thus SR 59230A antagonized the SR 58611A-induced relaxation of rat thoracic aorta (Trochu *et al*., 1999) and bound with high affinity to β_3-adrenoceptor cloned in Chinese hamster ovary cells even if it has been described, in a single study, to be able to bind to both β_1- and β_2-adrenoceptor (Candelore *et al*., 1999). The relatively weak inhibitory effect of salbutamol found in the present study was not affected by SR 59230A but was antagonized by propranolol and, although not reaching statistical significance, also by ICI 118551. Similar results have been reported previously in human bronchi, which are known to express both β_1- and β_2-adrenoceptor but not β_3-adrenoceptor (Mak *et al*., 1996), where SR 59230A failed to antagonize the *in vitro* relaxation induced either with isoprenaline or with salbutamol (Bardou *et al*., 1998).

The functional part of our study fulfils the four criteria defined by Kaumann & Molenaar (1996) for the β_3-adrenoceptor: (1) the receptor has been selectively stimulated by β_3-adrenoceptor-selective agonists; (2) the receptor has been stimulated by a non-conventional partial agonist; (3) the receptor has been resistant to blocking by antagonists possessing only high affinity for β_1- and β_2-adrenoceptor; (4) the receptor has been blocked by β_3-adrenoceptor-selective antagonists.

In our study, SR 59119A and salbutamol were responsible for a significant increase in cyclic AMP level but failed to increase the cyclic GMP level. The stimulation of cyclic AMP production after exposure to SR 59119A was not modified by pre-treatment with propranolol but was virtually abolished by SR 59230A, providing confirmation of the β_3-adrenoceptor-mediated nature of this stimulation. The relationship between cyclic AMP and smooth muscle relaxation has been well documented and shows that agents that increase the synthesis of cyclic AMP, e.g. β-adrenoceptor stimulants, as well as agents that inhibit the degradation of cyclic AMP, such as phosphodiesterase inhibitors, all decrease smooth muscle contraction (Bardou *et al*., 1999; Komas *et al*., 1991; Torphy *et al*., 1991). In our study, the functional inhibition of spontaneous contractions and the stimulation of cyclic AMP production induced by SR 59119A appears to be mediated through stimulation of β_3-adrenoceptors. Therefore, this increase in cyclic AMP level is probably responsible for the inhibitory effect on contraction since it has previously been demonstrated that inhibitors of phosphodiesterase type 4 (such as rolipram) are very potent as *in vitro* inhibitors on human near-term myometrium spontaneous contractions (Bardou *et al*., 1999; Leroy *et al*., 1989). It has recently been shown that in human heart β_3-adrenoceptor stimulation might be responsible for a negative inotropic effect mediated through stimulation of a nitric oxide synthase pathway and stimulation of cyclic GMP production (Gauthier *et al*., 1998). These conflicting results confirm the contrasting effects of β_3-adrenoceptor agonists in various tissues.

In the functional study, SR 59119A was more efficient than salbutamol whereas both agonists induced similar increases in cyclic AMP production. An explanation of this discrepancy is

that β_2-adrenoceptor has been shown to undergo acute desensitization after exposure to β_2-adrenoceptor agonists whereas β_3-adrenoceptor does not (Nantel *et al*., 1995). In biochemical experiments samples were exposed for 5 min to agonists whereas the time needed to construct a full concentration-response curve in the functional study was about 3 h. Nantel *et al*. (1995) have shown that in CHW and L cells expressing either β_2-adrenoceptor or β_3-adrenoceptor, cyclic AMP content reached a maximum level during the first hour of exposure to isoprenaline and then decreased more rapidly in cells expressing the β_2-adrenoceptor (Nantel *et al*., 1995). Moreover, this long-term desensitization does not occur in CHO cells transfected with the human cloned β_3-adrenoceptor (Nantel *et al*., 1994). Furthermore, it has been shown that β_3-adrenoceptor mRNA expression was increased by BRL 35135, a selective β_3-adrenoceptor agonist (Emilsson *et al*., 1998).

Finally, pharmacological evidence for myometrial β_3-adrenoceptor was strengthened by detection of β_3-adrenoceptor transcripts in the human myometrium using a reverse transcription polymerase chain assay. As adipocytes are rare in myometrial tissue, it is unlikely that expression of β_3-adrenoceptor is due to the presence of this type of cell.

Although the therapeutic potential of the findings reported in this study remains to be established, the use of selective β_3-adrenoceptor agonist might be of interest in the pharmacological treatment of premature labour and in other conditions where inhibition of uterine contractility is indicated, since data from randomized trials indicate that tocolytic drugs, if used frequently usually fail to induce a strong and sustained uterine relaxation (Goldenberg & Rouse, 1998).

The authors are indebted to the surgical teams of the Services of Obstetrics and Gynaecology at the University Hospitals, Antoine Béclère Hospital (Clamart, France), CHU du Bocage Hospital (Dijon, France) and La Fe Hospital (Valencia, Spain) for making the myometrial tissue available to us. Special thanks go to Mrs Ruth Bardou for her careful reading of this manuscript.

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(Received January 6, 2000
Revised April 28, 2000
Accepted May 18, 2000)