Interspecies Differences in the Cardiac Negative Inotropic Effects of $\beta_3$-Adrenoceptor Agonists$^1$

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ABSTRACT

The aim of the present study was to compare the effects of three preferential (BRL 37344, SR 58611, CL 316 243) and a partial (CGP 12177) $\beta$-adrenoceptor ($\beta_3$-AR) agonists on the contractility of ventricular strips sampled from various mammalian species including humans. In the heart of the mammal, all $\beta_3$-AR agonists tested decreased contractility by 40 to 60% below control with an order of potency: BRL 37344 > CL 316 243 = SR 58611 $\gg$ CGP 12177. In the dog, the negative inotropic effects produced by $\beta_3$-AR stimulation were less pronounced than in humans, $\approx$30% below control. The order of potency of $\beta_3$-AR agonists was CGP 12177 > BRL 37344 = SR 58611 $\gg$ CL 316 243; i.e., very different from that observed in humans. In rat, only BRL 37344 was efficient to decrease contractility. In guinea pig, only CL 316 243 significantly reduced peak tension. In both species, the reduction in peak tension did not exceed 20 to 30%. Finally, in the ferret, none of the agonists tested induced a negative inotropic effect. In dog, the negative inotropic effects of CGP 12177 were not modified by nadolol, but were abolished by bupranolol, a $\beta_1$-AR antagonist. $\beta_3$-AR transcripts were detected in the dog but not in the rat ventricle by using a reverse transcription-polymerase chain reaction assay. We conclude that cardiac negative inotropic effects related to $\beta_3$-AR agonist stimulation vary markedly depending on the species. A comparable interspecies variation previously has been reported concerning the lipolytic effects of $\beta_3$-AR agonist stimulation. Our study demonstrates that the pharmacological profile of a $\beta_3$-AR agonist on the human myocardium cannot be extrapolated from usual animal models.

In 1989, the cloning of a gene encoding for a third $\beta$-adrenoceptor ($\beta$-AR) designated as the $\beta_3$-AR (Emorine et al., 1989) offered a putative explanation for those effects of catecholamines that could not be related to $\beta_1$- or $\beta_2$-AR stimulation. Since then, $\beta_3$-ARs have been characterized pharmacologically in a variety of tissues from human and laboratory mammals including dogs, cats, rabbits, guinea-pigs, and monkeys. $\beta_3$-ARs have been reported in white and brown adipose tissues, where they induced lipolytic and thermogenic effects (Arch et al., 1984; Zaagasma and Nahorski, 1990; Langin et al., 1991). They also have been identified in a number of gastrointestinal smooth muscle (Manara and Bianchetti, 1990; Koike et al., 1994), in the airways (Martin and Advenier, 1995), and in the blood vessels (Tavernier et al., 1992; Berlan et al., 1994; Shen et al., 1994), where they produced relaxation. However, in a given tissue, the pharmacological profile of the $\beta_3$-AR and the efficiency of the $\beta_3$-AR agonists were found to vary markedly depending on the species studied.

In the heart muscle, four populations of $\beta$-AR potentially modulate the cardiac function. The effects of $\beta_1$- and $\beta_2$-AR are well established both in humans and other mammals. Their stimulation produces positive chronotropic and inotropic effects. Concerning the two other $\beta$-ARs described more recently, less data are available. The atypical $\beta$-AR, termed by some authors as the $\beta_4$-AR, caused positive inotropic and chronotropic effects. This receptor, which has not yet been cloned, has been characterized pharmacologically in vitro in cardiac preparations from rats, guinea pigs, cats (for review, see Kaumann, 1989, 1997), and humans (Kaumann, 1996) and in situ in the pithed rat (Malinowska and Schlicker, 1996, 1997). We have demonstrated previously the presence of $\beta_3$-AR in the human ventricle. In human cardiac tissues,

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β₁-AR stimulation by catecholamines in the presence of nadolol (a β₁- and β₂-AR antagonist) or by preferential β₂-AR agonists induces a pronounced concentration-dependent, negative inotropic effect (Gauthier et al., 1996). By contrast, the effects of β₂-AR stimulation in the hearts from nonhuman mammalian species are unknown. This has important pharmacological implications because the vast majority of therapeutically developed for human use but selected in animal models. The present study was designed to address this issue. According to the adipocyte responses to β-AR agonists as reported by Lafontan (1994), the rat, dog, and guinea pig species were selected for this study. In addition, the ferret species also was selected because its ventricular myocardium exhibits contractile characteristics and adrenergic responses similar to those of the human myocardium (Cook et al., 1992). We found that β₂-AR stimulation produces very different effects in humans and in other mammal hearts and that the negative inotropic effects produced by β₂-AR agonists in human and dog ventricles are associated with the presence of β₂-AR transcripts. These findings are discussed in light of species- and tissue-specific effects.

Materials and Methods

Human Ventricular Biopsies. All protocols were approved by the Ethics Committee of the Centre National de la Recherche Scientifique (France). Twenty-four human endomyocardial biopsies were obtained from the right interventricular septum of cardiac transplant patients (20 men and 4 women; mean age, 51 ± 5 years) during right jugular vein catheterization performed routinely to detect possible rejection. None of the patients had evidence of cardiac rejection. All received immunosuppressive therapy (azathioprine, prednisolone, and cyclosporine). In addition, eight patients were given a calcium antagonist, and one patient was receiving a diuretic. Sixteen patients had no treatment known to possess cardiovascular effects. The effects of β₂-AR agonists in biopsies from patients treated with calcium antagonists were similar to those in biopsies from patients not receiving these drugs. Tissues were placed in a transport solution containing HEPES as the extracellular buffer and conveyed quickly to the laboratory.

Mammal Ventricular Tissues. The hearts of male Wistar rats (240–260 g), male guinea pigs (350–450 g), ferrets (0.8–1 kg), and male mongrel dogs (10–18 kg) were isolated under general anesthesia with sodium pentobarbital. Papillary muscles were dissected out from right and left ventricles.

Experimental Protocol. Preparations were placed in an experimental chamber and superfused at a flow rate of 5 ml/min with oxygenated (95% O₂; 5% CO₂) Tyrode’s solution warmed at 37°C. Tyrode’s solution for human tissues had the following composition: 116 mM NaCl; 5 mM KCl; 2.7 mM CaCl₂; 1.1 mM MgCl₂; 0.33 mM NaHPO₄; 24 mM NaHCO₃; and 5 mM glucose. For the other mammal cardiac tissues, this Tyrode’s solution was modified slightly in agreement with the literature. Tissues were allowed to recover for at least 60 min and then submitted to field stimulation at a pacing cycle length of 1700 ms. Stimulus pulse width was 1 to 2 ms, and amplitude was twice the diastolic threshold. Tension was recorded by using a mecanoelectric force transducer (Akers, AE 801; SensoNor, Horten, Norway), as described previously (Gauthier et al., 1994). Ventricular tissues were stretched stepwise (10-μm increments) to a length at which contraction force was maximal. Studies then were performed at 90% of maximal tension. After equilibration, cumulative concentration-response curves for the various β-AR agonists were determined by superfusion with increasing concentrations of the drugs. For all concentrations, tension was recorded at steady state on a digital storage oscilloscope (Gould 400; Gould, Les Ulis, France), a strip chart recorder (Gould 8188), and a digital tape recorder (DTR-1200; Biologic, Claix, France).

Statistics. Results are expressed as means ± S.E.M. of n number of experiments. The statistical significance of the effects of a drug was assessed by using one-way ANOVA followed by a Dunnett’s test.

To determine agonist potencies from the concentration-response curves, the EC₅₀ values were determined by fitting curves with the Boltzmann equation. pD₃ values then were calculated according to the equation pD₃ = −log(EC₅₀).

Drugs. CGP 12177 ([+3-t-buty lamino-2-hydroxypropoxy]benzimidazol-2-one) was a gift from CIBA-Geigy (Basel, Switzerland), SR 58611 (N[2a]-7-carb-ethoxymethoxy-1,2,3,4-tetra-hydropyridyl]-2(1)-hydroxy-2(3-chlorophenyl) ethamine hydrochloride) was a gift from SmithKline Research (Montpellier, France), and CL 316 243 (5-[2(2-[2-chloroethoxy-2-hydroxyethyl]amino)propy]) 1,3-benzodioxole-2,2-dicarboxylate) was a gift from American Cyanamid Company (Pearly River, NY). Bupranolol was a gift from Schwartz Pharma (Mannheim, Germany). BRL 37344 ([+1-hydroxy-(3-chlorophenyl)ethyl]amino)propylphenoxyacetate) was obtained from Research Biochemicals International (Natick, MA), and nadolol was obtained from Sigma Chemical Co. (St. Louis, MO).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Assay. Total RNA was prepared from white adipocyte and cardiac samples by using a single-step guanidinium thiocyanate/phenol/chloroform extraction (Chomczynski and Sacchi, 1987). Total RNA was treated with DNRase I (Gibco/BRL, Cergy Pontoise, France). For myocardial samples, poly(A)⁺ RNA was purified by using the Dynabeads mRNA purification kit (Dynal, Skøyen, Norway). Isolated adipocyte total RNA (300 ng) and cardiac poly(A)⁺ RNA (10–50 ng) were treated with Superscript II RTase (Perkin-Elmer, Courtaboeuf, France), 2.5 mM MgCl₂, 10% dimethyl sulfoxide (v/v), and 2.5% (v/v) formamide. Sense (5'-GCC TCC AAC ATG CCC TA-3') and antisense (5'-GCC TGG GGC AGT AGA TG-3') primers common to the rat and canine β₂-AR DNA (Lenzen et al., 1998) were expected to yield a 480-bp amplicon. Another combination of sense (5'-GCC TGA CGG GCC GCT GCT TC-3') and antisense (5'-GCC ACC ACT TGC TCA TGA TGG GCG C-3') primers was used to amplify β₂-AR cDNA from dog myocardium. The expected length of the fragment was 243 bp. PCR products were separated by electrophoresis through 2% agarose ethidium bromide-stained gels. Gels were blotted onto nylon membranes (Hybond N⁺; Amersham, Little Chalfont, UK) that were hybridized at 65°C to the human β₂-AR cDNA. Blots were washed at a final stringency of 15 mM NaCl, 1.5 mM sodium citrate, and 0.1% SDS at 65°C and subjected to digital imaging (Molecular Dynamics, Sunnyvale, CA).

Results

Effects of β₂-AR Agonists in Rat and Guinea Pig Hearts. Figure 1 shows the effects of rat papillary muscle contractility of BRL 37344, SR 58611, CL 316 243, and CGP 12177 at a concentration range of 0.1 nM to 1 μM. Among the drugs tested, only BRL 37344 significantly decreased peak tension with a pD₃ of 7.67 ± 0.64 (n = 6). The maximum negative inotropic effect was obtained for a concentration of 0.1 μM (Fig. 1A), which decreased peak tension by 21.6 ± 6.6% below control value (P < .05; n = 6). At a higher concentration (1 μM), BRL 37344 restored the contractile force to the control level. This effect was associated with a 6.4 ± 2.3% reduction in time-to-peak (P < .05; n = 6). Other contractile parameters were not modified significantly when
compared with control values. The other β3-AR agonists tested did not produce significant effects on contractile function in rat ventricular tissues. In papillary muscles from the guinea pig, only CL 316 243 induced a concentration-dependent reduction in peak tension with a pD2 of 7.76 ± 0.85 (n = 5). At 1 μM, CL 316 243 decreased peak tension by 31.3 ± 2.4% (P < .05; n = 5) below control (Fig. 2A). This effect was associated with a 7.7 ± 1.5% reduction in time-to-peak (P < .05; n = 5). The other β3-AR agonists caused no inotropic effects (Fig. 2B).

Effects of β3-AR Agonists in the Ferret Heart. In ferret, none of the β3-AR agonists tested induced a negative inotropic effect as shown in Fig. 3B. Inversely to the rat and guinea pig hearts, BRL 37344 induced in the ferret ventricle a marked, positive inotropic effect at concentrations greater than 10 nM. At 1 μM, peak tension was increased by 240.0 ± 91.5% (P < .05; n = 5) as compared with control (Fig. 3A). At this concentration, the positive inotropic effect varied markedly depending on the preparation. This effect was associated with an abbreviation of the twitch. At 1 μM, BRL 37344 decreased total twitch duration by 12.7 ± 2.3% (P < .05; n = 5) and time-to-peak by 8.5 ± 5.0% (P < .05, n = 5), half-contraction time by 12.7 ± 6.6% (P < .05; n = 5), and half-relaxation time by 19.5 ± 3.0% (P < .05; n = 5).

Effects of β3-AR Agonists in Dog Hearts. The cardiac effects of β3-AR agonists in dog are illustrated in Fig. 4. In this species, the β3-AR agonist that induced the most efficient negative inotropic effect was CGP 12177, with a maximum effect obtained at 0.1 μM (Fig. 4A). At this concentration, CGP 12177 decreased peak tension by 26.9 ± 4.0% (P < .05; n = 5). The pD2 for CGP 12177 was 9.33 ± 0.13 (n = 5). Other contractile parameters were not modified significantly. At higher concentrations, CGP 12177 increased peak tension. Both BRL 37344 and SR 58611 induced similar concentration-dependent negative inotropic effects (Fig. 4B) with pD2 values of 8.78 ± 0.33 (n = 6) and 8.67 ± 0.24 (n = 6), respectively. At 0.1 μM, BRL 37344 reduced peak tension by 26.3 ± 4.6% (P < .05, n = 6), total twitch duration by 8.4 ± 3.0% (P < .05, n = 6), and time-to-peak by 5.0 ± 3.1% (P < .05, n = 6). At higher concentrations of BRL 37344, the control contractile force was restored. At 1 μM, SR 58611 decreased peak tension by 33.7 ± 5.7% (P < .05, n = 6) without significant alteration in the twitch kinetics. Finally, CL 316 243 induced no significant modification in cardiac contractility in the dog species (Fig. 4B). Therefore, in the canine ventricular myocardium, the rank order of potency of β3-AR agonists to decrease peak tension was CGP 12177 > BRL 37344 = SR 58611 >> CL 316 243.

Figure 5 shows the concentration-response curves for CGP 12177, the most potent β3-AR agonist in the dog heart. Concentration-response curves were established in the absence or presence of β-AR antagonists. The negative inotropic effect of CGP 12177 was not modified by pretreatment with 10 μM nadolol, a β1- and β3-AR antagonist with low affinity for the β2-AR (Bond and Clarke, 1988; Emorine et al., 1989; Sugawara et al., 1992; Galitzky et al., 1993). By contrast, in the presence of 1 μM bupranolol, which combines β1-, β2-, and β3-AR antagonist properties (Langin et al., 1991; Galitzky et
In the human ventricular myocardium, all the β<sub>3</sub>-AR agonists tested produced a concentration-dependent, negative inotropic effect (Fig. 6). BRL 37344 decreased peak tension at concentrations ranging from 0.1 nM to 1 μM. The maximum effect was observed at 1 μM, which decreased peak tension by 55.7 ± 3.7% (P < .05, n = 8) as compared with control (Fig. 6A). This effect was associated with an abbreviation of the twitch. At this concentration, BRL 37344 decreased total duration of the twitch by 12.5 ± 2.7% (P < .05, n = 8), time-to-peak by 11.3 ± 1.6% (P < .05, n = 8), half-contraction time by 11.4 ± 2.4% (P < .05, n = 8), and half-relaxation time by 17.5 ± 4.9% (P < .05, n = 8). The pD<sub>2</sub> for BRL 37344 was 8.75 ± 0.20 (n = 8).

SR 58611 and CL 316 243 induced comparable concentration-dependent negative inotropic effects (Fig. 6B) with pD<sub>2</sub> values of 7.96 ± 0.30 (n = 6) and 8.13 ± 0.18 (n = 5), respectively. At 1 μM, SR 58611 reduced peak tension by 47.5 ± 7.6% (P < .05; n = 6), total duration by 10.6 ± 2.7% (P < .05; n = 6), time-to-peak by 9.6 ± 2.8% (P < .05; n = 6), half-contraction time by 7.5 ± 3.3% (P < .05; n = 6), and half-relaxation time by 7.1 ± 4.2% (P < .05; n = 6). At 1 μM, CL 316 243 decreased peak tension by 43.9 ± 4.9% (P < .05, n = 5) without concomitant modification in other contractile parameters.

CGP 12177, the partial β<sub>3</sub>-AR agonist, also decreased peak tension, but at higher concentrations as compared with preferential β<sub>3</sub>-AR agonists and also to a lesser extent (Fig. 6B). The pD<sub>2</sub> for CGP 12177 was 6.47 ± 0.15 (n = 5). The maximum effect was obtained for a concentration of 100 μM, which decreased peak tension by 37.9 ± 5.7% (P < .05; n = 5) below the control level. The twitch kinetics were not modified by this compound. Thus, the rank order of potency in the human ventricle was BRL 37344 > CL 316 243 = SR 58611 >> CGP 12177.
Detailed analysis of β3-AR pharmacology and species-related variations in the myocardium.

**Detection of β3-AR mRNA in Rat and Dog Ventricular Myocardium.** To determine whether the presence of a β3-AR-mediated negative inotropic effect was associated with the expression of β3-AR transcripts, we used a RT-PCR assay. RT-PCR was performed with primers located in the first exon of the β3-AR gene. RNA was treated with DNase I to prevent contamination by genomic DNA. We previously reported expression of β3-AR mRNA in human myocardium (Gauthier et al., 1996). Using a similar protocol, two amplicons of expected sizes were obtained with different combinations of primers in dog myocardium (Fig. 7). β3-AR mRNA expression was detected in four different animals. Hybridization to human β3-AR cDNA confirmed the identity of the amplified products. No amplification was observed in rat myocardium (n = 3). As a control of the assay, reverse-transcribed β3-AR mRNA was readily amplified by PCR in rat and dog white adipocytes (Fig. 7).

**Discussion**

The present study reveals the complexity of β3-AR pharmacology in the myocardium as illustrated by the pronounced interspecies variability and the heterogeneous pharmacological profiles of β3-AR agonists in a given species. In human, all the β3-AR agonists tested induced a marked negative inotropic effect. In dog, the negative inotropic effects of three β3-AR agonists were less pronounced than in the human heart and virtually absent for CL 316 243. In addition, the rank order of potency of the various β3-AR agonists differs markedly in the human and dog hearts. In rat and guinea pig, a significant decrease in peak tension (20–30%) was observed with only one agonist: BRL 37344 for the rat and CL 316 243 for the guinea pig. Finally, in ferret, none of the agonists induced negative inotropic effects. Based on these findings, we have classified species within three groups of responders defined as hyper-responders (human and dog), hyposponders (rat and guinea pig), and non-responders (ferret) to β3-AR agonist stimulation in the myocardium (see Table 1). For the group of hyper-responders, the functional effects induced by β3-AR agonists were associated with the presence of β3-AR transcripts.

In the dog, the negative inotropic effect of CGP 12177, the most potent β3-AR agonist identified in this species, was not modified by pretreatment with nadolol (a β1- and β2-AR antagonist), indicating that negative inotropy was not mediated by β1- and β2-AR. Inversely, bupranolol, which possesses β3-AR antagonist properties, abolished CGP 12177 negative inotropic effect. These data are in agreement with our previous data obtained in human ventricular biopsies. The negative inotropic effect of BRL 37344 was not modified by pretreatment with nadolol, but was shifted to the right by bupranolol (Gauthier et al., 1996). SR 59230, a compound described as a selective β3-AR antagonist in some tissues (De Ponti et al., 1996), did not antagonize at 0.1 μM the effect of SR 58611 in the human ventricle (data not shown). In the dog, CGP 12177 at the highest concentrations (1–100 μM) was observed with only one agonist: BRL 37344 for the rat and CL 316 243 for the guinea pig. Finally, in ferret, none of the agonists induced negative inotropic effects. Based on these findings, we have classified species within three groups of responders defined as hyper-responders (human and dog), hyposponders (rat and guinea pig), and non-responders (ferret) to β3-AR agonist stimulation in the myocardium (see Table 1). For the group of hyper-responders, the functional effects induced by β3-AR agonists were associated with the presence of β3-AR transcripts.

**Table 1**

Classification of species according to the intensity of their response to the β3-AR agonist stimulation

<table>
<thead>
<tr>
<th>Category</th>
<th>Hyper-responders</th>
<th>Hyporesponders</th>
<th>Non- or Weak Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardium</td>
<td>Human</td>
<td>Rat</td>
<td>Ferret</td>
</tr>
<tr>
<td>Rodents</td>
<td>Dog</td>
<td>Guinea pig</td>
<td>Human</td>
</tr>
<tr>
<td>Hibernating mammals</td>
<td>Rabbit</td>
<td>Guinea pig</td>
<td>Baboon Macaque</td>
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<tr>
<td>Adipose tissue&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Marmoset</td>
<td></td>
<td>Macaque</td>
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</table>

<sup>a</sup> From Lafontan, 1994.
restored contractile force close to the control level. In addition, when the β1-, β2-, and β3-AR were inhibited by bupranolol, a slight increase in contractility was observed. This latter effect could be attributed to stimulation of a putative β3-AR as reported previously by Kaumann (1996, 1997). The existence of β3-AR in the heart was deduced from the effects of partial agonists such as CGP 12177, cyanopindolol, and pin
dolol, which exert positive inotropic effects in vitro in atrial tissues from rat, guinea pig, cat, and human (Kaumann, 1989, 1996, 1997) and cause tachycardia in vivo in the rat (Malinowska and Schlicker, 1996, 1997). Positive chronotropic effects also have been reported with selective β3-AR agonists such as BRL 37344 and CL 316 243 in dog and rat (Tavernier et al., 1992; Shen et al., 1994, 1996). However, this effect was not related to a direct stimulation of cardiac β3-AR but, rather, to a reflex mechanism because it was abolished after sinoaortic denervation in conscious dogs (Tav
ernier et al., 1992) or after β1- and β3-AR blockade (Shen et al., 1996). In our study, another β3-AR agonist, BRL 373444, when used at high concentrations, produced in some species an increase in contractile force or restored contractility to the control level. This effect likely resulted from a nonspecific activation of the β1- and β3-AR at high concentrations (Muzz
in et al., 1992; Ida et al., 1996; Oriowo et al., 1996).

In the ventricular myocardium of human and dog, the β3-AR stimulation produced a negative inotropic effect in stark contrast to β1- and β2-AR stimulation. In other tissues such as adipocytes, β3-AR but also β1-AR and β2-AR stimulation produces lipolysis. However, as in the heart, the lipolytic effects of β3-AR stimulation markedly vary depending on the species. As also reported in Table 1, three groups were defined previously by Lafontan (1994) in the adipose tissue: 1) a group of hyperresponders composed of rodents and hi
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2) a group of hyporesponders, including rabbit, dog, and marmoset monkey, in which the three classes of β-AR are equally involved in the lipolytic effect of catecholamines; and 3) a third group composed of guinea pig, baboon and macaque monkeys, and human, in which β3-AR agonists induce a very weak response or even no response at all. Strong species-related differences also have been reported in other tissues. In the canine bronchi, selective β3-AR agonists induced relaxation whereas these drugs produce almost no effect in human, guinea pig, and sheep bronchi (Martin and Advenier, 1995). In the colon, motility inhibition by β3-AR agonists is more pronounced in the dog than in the rat or the guinea pig (Manara et al., 1995). In the vessels, β3-AR agonists produce a much stronger vasodilator effect in dog than in rat. By contrast, β3-AR agonists produce no vasodilator effects in nonhuman primates (Shen et al., 1996). Thus, interspecies variability in β3-AR pharmacology not only concerns the heart but also other tissues. It is important to note that a species classified as a hyperresponder to car
diac β3-AR agonist stimulation may be a weak responder to β3-AR agonist stimulation in adipocyte (e.g., human). Con
versely, rodents that are hyperresponders to adipocyte β3-AR agonist stimulation are hyporesponders to cardiac β3-AR ag
onist stimulation. Thus, interspecies variability differs de
pending on the organ.

Several explanations could account for interspecies differ
ences related to β3-AR agonist stimulation. One concerns

β3-AR expression. In the dog, the negative inotropic effect
induced by β3-AR agonists is associated with the expression of β3-AR transcripts. These results agree with those obtained in human ventricle (Gauthier et al., 1996). By contrast, we did not detect β3-AR mRNA in rat ventricular myocardium as shown previously in the rat right ventricle (Evans et al., 1996). The expression level may not be the sole factor that governs the response to β3-AR stimulation. Sequences of β3-ARs show either deletions or substitutions of key amino acids between species. The human, monkey, and bovine β3-ARs have a higher interspecies homology in their amino acid sequence than rodents. In particular, in the first transmembrane region, a three-residue (valine-leucine-alanine) de
letion is observed in the smaller but not in the larger mammals (Strosberg and Petri-Rouxel, 1996). However, deletion of these three residues from the human β3-AR gene does not restore the “rodent-like” pharmacological profile, suggesting that these residues are not critically involved in interspecies specificity (Gros et al., 1998). In addition, the human β3-AR is structurally different from its known homologs in other species. These differences concern transmembrane regions that are considered critical for ligand binding and G protein interaction (Strosberg and Petri-Rouxel, 1996). Clearly, further investigations are needed to clarify the consequences of the difference in β3-AR structure across species. Another hypothesis is based on the exon-intron boundary of the β3-AR gene. The β3-AR gene contains two introns (Granneman et al., 1992; Lelias et al., 1993) in opposition to β1- and β2-AR genes, which are intronless. This structure leads to splice variants. The B and C isoforms contain 12 and 6 additional amino acids, respectively, at their C terminus in comparison with the A isoform (Granneman et al., 1992; Lelias et al., 1993). In rat adipocytes, a unique isoform is expressed that is close to the B isoform, whereas in human brown adipocytes, the C isoform is predominant (Lelias et al., 1993; Van Spronsen et al., 1993). It could be hypothesized that the physiological response to β3-AR stimulation differs depending on the isoform expressed in a given species (Levasseur et al., 1995). Interestingly, the response to β3-AR stimulation is variable in a given species depending the tissue. Again, human is a hyperresponder to β3-AR stimulation in the heart but a weak responder in the adipose tissue. This discrepancy could result from expression of different isoforms in the heart and the adipose tissue. Further investigations are needed to deter
mine the expression levels of the three isoforms in the vari
ous tissues, as well as their coupling pathway and their physiological roles. Indeed, differences in structure or iso
forms between species and tissues could be responsible for a poor or high coupling of the β3-AR or lead to a different coupling pathway. In adipose tissue, the three β-ARs are linked to adenyl cyclase through G proteins (Blin et al., 1993; Strosberg and Petri-Rouxel, 1996), whereas in the hu
man ventricular muscle the β3-AR, but not the β1- and β2-AR, is coupled to Gq proteins (Gauthier et al., 1996) and stimul
ates a nitric oxide synthase, leading to an increase in nitric oxide production and intracellular cGMP (Gauthier et al., 1998).

The present study demonstrates that caution should be taken in using animal models for the development of thera
peutic compounds active on the human β3-AR and that any novel drug targeted to β3-AR should be evaluated on cardiac human tissue at least for safety reasons.
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References


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