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COMMENTARY

STRUCTURAL BASIS FOR FUNCTIONAL DIVERSITY OF β_1-, β_2- AND β_3-ADRENERGIC RECEPTORS

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The catecholamines adrenaline and noradrenaline maintain the homeostasis of the organism through the neuromediatory and hormonal actions they exert on the sympathetic nervous system and on a variety of peripheral functions. In spite of the ubiquity of adrenergic receptors, this regulation specifically targets particular cellular systems without affecting others. Since only two mediators are involved, this selectivity may be achieved through multiple receptors with distinct effector functions. The repertoire of receptors, however, may be more limited if the activity of each receptor can be modulated by different combinations of elements whose presence depends on cellular status.

It was initially thought that α- and β-adrenergic receptors (α- and β-ARs), further subdivided into α_1-, α_2-, β_1- and β_2-ARs, were sufficient to mediate the multiple effects of adrenaline and noradrenaline. A number of tissular and pharmacological properties of β-adrenergic ligands developed recently, however, are difficult to reconcile with this classification, and this suggests the involvement of additional receptor subtypes. These β-ARs, usually designated as "atypical," have mostly been described in adipose tissues [1], but their presence has also been observed in other organs such as heart, liver, and skeletal and digestive tract muscles [2–5]. Mainly because of the inadequacy of the ligands and/or methods used [6] and perhaps because of their low representation, these atypical receptors have remained difficult to identify unambiguously by classical approaches.

The molecular cloning of the first adrenergic receptor gene [7] provided new tools to address this question and yielded definitive support for the existence of additional adrenergic receptors. The genes coding for the pharmacologically defined α_1-, α_2-, β_1- and β_2-AR were isolated [8–11] and led to the cloning by homology of genes for additional α- and β-AR subtypes [12–14]. We evaluate here the structural characteristics of β_1-, β_2- and β_3-AR genes and proteins that may be the basis of the different activities of the three receptors which otherwise appear to respond to the same hormones and trigger the same second messenger pathway. We discuss the involvement of the β_3-AR in mediating various atypical effects of catecholamines in adipocytes and other tissues. In this context, the possible existence of other β-AR subtypes distinct from the currently defined β_1-, β_2- and β_3-AR will also be discussed.

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Genetic basis for the regulated expression of β-adrenergic receptor subtypes

Cellular activity is regulated at the gene level by hormonal and environmental factors. The existence of three β-AR genes, with distinct genetic regulatory properties, may allow the modulation of adrenergic responsiveness of specific cells implicated in a particular physiologic function. Such genetic regulation is often reflected by the presence, in the promoter regions of the genes, of conserved nucleotide sequences corresponding to binding sites for various factors and modulators of transcription. Thus, examination of the structures of β-AR gene promoters may provide clues on the functional differences existing between the three receptors.

Structures of the β_2- and β_3-AR promoters. The nucleotide sequence of the β_3-AR gene promoter region was determined [14] and compared to the corresponding region of the β_2-AR gene [11]. As shown in Fig. 1, both genes contain a reverse sequence for the CAAT box binding protein a few tens of nucleotides upstream from an A/T rich region reminiscent of a TATA box. A second CAAT box exists in the β_3-AR gene. Several sites for initiation of mRNA synthesis have been localized in the β_2-AR gene [11, 15], and transcription probably begins at homologous positions in the β_3-AR gene. An ATG translation initiation codon followed by a short open reading frame potentially encoding polypeptides of 19 or 16 amino acids is found between the mRNA start sites and the structural gene of the β_2- and β_3-AR respectively. Removal of this ATG codon from the β_2-AR gene increases receptor expression 10-fold [16]. Potential recognition sites for transcription factor NF1 and for proteins binding to the CACCC sequence are also common to both genes. Glucocorticoid and cAMP responsive elements (GRE and CRE) as well as sites for transcription factor Sp1 are found specifically in the β_2-AR promoter.

Two regions of the β_3-AR gene display close to 55% sequence homology with part of the promoter

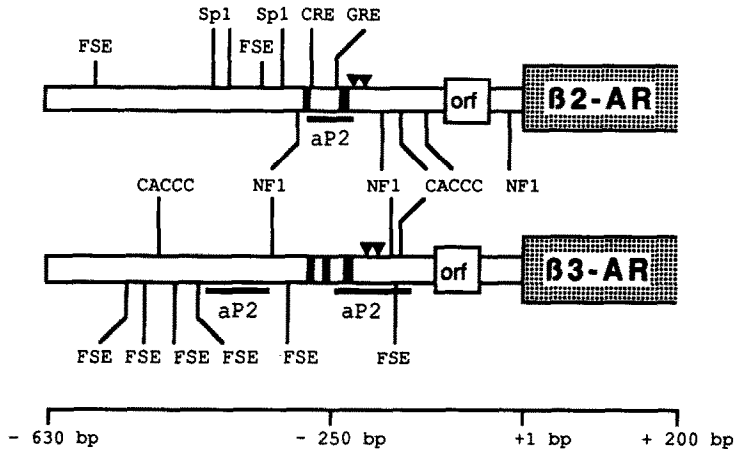

Fig. 1. Schematic structure of the β_2- and β_3-AR promoters. The β_2-AR and β_3-AR stippled boxes represent the coding region of each gene. In the promoter regions, CAAT and TATA boxes (black and hatched boxes, respectively), and sites for the initiation of mRNA synthesis (inverted triangles), are shown. The open reading frames (orf), coding for short polypeptides, that are found between mRNA start sites and β-AR structural genes are represented by open squares. The position of potential binding sites for various factors and modulators of mRNA transcription are indicated (NF1, CACCC, Sp1, CRE, GRE and FSE). The regions presenting sequence homologies with the promoter of the adipocyte P2 lipid binding protein (aP2) gene are highlighted with solid lines.

of the gene for the adipocyte P2 (aP2) lipid binding protein [17]. Similar sequence homologies also exist within the β_2-AR promoter region but are restricted to shorter fragments (Fig. 1). 3T3-F442A adipocytes express several nuclear factors which bind specifically to the aP2 promoter fragment [18]. Some of these factors recognize a 14 nucleotide long fat specific element (FSE) which also occurs within promoters of several genes that participate in adipose differentiation [19, 20]. Approximations of such motifs (65–75% homology) are found in the two β-AR promoters; six in the β_3- and two in the β_2-AR gene (Fig. 1). Recognition sites for other adipocyte factors have been shown to exist in the aP2 promoter [18], and may also occur in the β_3- and/or β_2-AR.

Multifactorial control of β-AR subtype mRNA synthesis. The structural similarities and differences between the promoters of the β_2- and β_3-AR genes should be reflected by specific actions of regulatory factors on the level of expression of each β-AR gene. During tissue development, several factors and regulators of transcription interact to modulate the level of expression of various genes. Some factors may be cell and differentiation dependent and thus allow selective regulation of adrenergic sensitivity in accordance with cellular function.

Basal expression of β-AR genes involves ubiquitous factors such as those for CAAT and TATA elements and may be further regulated by several modulators of transcription. For many other genes, where regulatory elements (e.g. NF1, GRE and CRE) are often present as close inverted repeats, efficient modulation of mRNA synthesis requires dimerization of transcription factors [21–23]. An interesting observation is that in the β_2- and β_3-AR genes these same types of sequences appear as monomers, not as inverted repeats. These features of the β_2- and β_3-AR promoters, and the occurrence of an additional ATG codon upstream from that for the receptors, may thus result in low levels of β_2- and β_3-AR basal expression. Because many transcription factors can interact synergistically to generate their effects [21–23], it is possible that cooperation of two heterologous factors, instead of dimerization of a unique factor, is required for efficient modulation of β-AR gene expression. Such modulation would then require the cooperation of specific factors whose presence depends on tissue origin and environmental stimuli.

In an initial study of the coordinated expression of the three β-AR genes during the adipose conversion of 3T3-F442A cells [24], we have shown that the preadipocytic form of these cells expresses low amounts of β-AR mRNA solely of the β_1 character. Upon differentiation, β_1-AR mRNA levels increase about 5-fold and similarly high amounts of β_3-AR mRNA are attained. Low levels of β_2-AR mRNA also appear but remain a minority. When dexamethasone is supplied to preadipocytes and maintained along the differentiation process, the β_1- and β_3-AR messages are totally depressed but the β_2-AR expression is stimulated about forty times above that of preadipocytes. In contrast, when dexamethasone is supplied to fully mature adipocytes, it stimulates β_2-AR mRNA levels by only a factor of 2–3 although it still depresses β_1- and β_3-AR mRNA expression. In smooth muscle tissues, glucocorticoids also stimulate 2- to 3-fold the expression of β_2-AR mRNA [25, 26].

Dexamethasone thus has a much stronger potency to stimulate β_2-AR expression in preadipocytes than in differentiated adipocytes or smooth muscle tissues. As suggested by the presence in the β_2-AR promoter region of a GRE in the vicinity of aP2 promoter-like sequences, this could reflect synergistic cooperation of glucocorticoid receptors with

preadipocyte specific factors and/or other factors active on the β_2-AR promoter. Positive interactions between glucocorticoid receptors and transcription factors NF1 and Sp1 or those binding to CACCC and CAAT boxes have already been observed [22, 27].

Cooperation of several factors binding to the aP2 promoter-like sequences of the β_3-AR gene could be sufficient to strongly promote β_3-AR expression during adipose differentiation. No sequences matching those proposed for negative regulatory GRE [21] were detected in the β_3-AR gene, but the nucleotide sequences involved in this type of regulation have not been studied extensively. The inhibitory effects of dexamethasone on β_3-AR expression could also indirectly result from its action on other genes whose products, in turn, control the transcription of the β_3-AR gene.

The preceding observations demonstrate the differential genetic control of the β_2- and β_3-AR genes. Little is known about the β_1-AR promoter and the precise mode of regulation of the expression of β-AR genes remains to be determined. Some regulatory elements may specifically modulate the expression of a given β-AR gene, whereas other factors would be active on the three. Even in this latter case, the possibility of positive or negative interactions among various modulators of β-AR gene expression could lead to quantitative differences in the expression of each β-AR gene. Such mechanisms could allow various specialized cells to independently modulate their adrenergic sensitivity in response to changes in hormonal and environmental conditions.

G-protein coupling of β-AR subtypes and modulation of adrenergic sensitivity

Differential regulation of β-AR function may also depend on the relative ability of the three receptor subtypes to activate one or several G-protein(s) upon agonist binding. This ability is, in turn, modulated by various protein kinases involved in homologous or heterologous desensitization of adrenergic responsiveness [28]. Domains of the β_2-AR interacting with stimulatory G-proteins (G_s) and kinases have been defined functionally [29–32] and are extremely well conserved between β_2-ARs of various mammals. Comparison with the corresponding regions of the β_1- and β_3-AR may thus give insight into G-protein coupling properties and susceptibility to kinases of individual β-AR subtypes.

Interactions of β-AR subtypes with G-proteins. Overall amino acid sequence identity between the three receptors (Fig. 2) is about 50% and may reach 90% in transmembrane regions that participate in catecholamine binding [33–35]. In the β_2-AR, segments of the second and third intracellular loop and of the C-terminal tail are involved in receptor coupling to G_s, and in agonist-promoted activation of adenylate cyclase [29]. These stretches, at the boundaries between cytoplasmic loops and transmembrane domains, are particularly conserved among the three β-ARs. Although homologies also exist with corresponding regions of other receptors coupled to G-proteins (α-adrenergic, muscarinic, serotoninergic and dopaminergic), the sequence conservation is higher inside the β-AR family. These conserved features of β-ARs probably reflect the fact that the three receptors bind to catecholamines and trigger a common second messenger pathway via coupling to G_s.

G-proteins, however, also belong to a heterogeneous multigenic family and each β-AR displays specific features in regions participating in G-protein coupling (Fig. 2). For example, the C-terminus of the third cytoplasmic loop of the β_1- and β_3-AR contains, respectively, one (position 311) and two (positions 279 and 284) proline residues whereas none occurs in the β_2-AR. Among mammalian G-protein coupled receptors for which sequence data are available, only the muscarinic M2 receptor displays such residues at homologous positions. In the same region, just before the sixth transmembrane domain, the β_3-AR contains a cysteine (position 292) instead of a lysine or arginine residue conserved in the other β-ARs. Interestingly, another cysteine residue (position 153) unique to the β_3-AR occurs in the second intracytoplasmic loop which, in the β_2-AR, is also implicated in G-protein coupling. In the regions just following the fifth and seventh membrane domains, several charged amino acids are also proper to each receptor subtype.

These differences may thus support, beside common coupling to G_s, preferential interactions of a given β-AR subtype with one (or several) additional G-protein(s). Modulation of the activity of adenylate cyclase and additional effector systems as ion channels or phospholipases has already been observed for cardiac β-ARs [36] and for M1 and M2 muscarinic receptors [37, 38]. Such specific properties of individual β-ARs may thus allow different subtypes to preferentially regulate individual enzymatic pathways, even while other β-ARs are expressed by the same cell.

Interaction of β-AR subtypes with protein-kinases. Desensitization of β-AR responsiveness, leading to uncoupling from G-proteins, internalization and eventually down-regulation of receptors, is thought to be mediated by phosphorylation by several kinases [28].

The Ser^{262} residue in the third intracytoplasmic loop of the β_2-AR is required [30] for rapid heterologous uncoupling of the receptor from the G-protein by the cAMP-dependent protein kinase (PKA). At corresponding positions, the β_1-, but not the β_3-AR, displays a canonical site for PKA phosphorylation constituted of a Ser residue (position 312) surrounded by several basic amino acids (Fig. 2). Although not followed by a basic amino acid, the Ser^{390} residue in the C-terminal region of the β_3-AR could represent such a PKA phosphorylation site. The Ser^{346} residue in the C-terminal domain of the β_2-AR also occurs in such a configuration but this latter is not involved in rapid heterologous desensitization of the receptor [30].

Homologous desensitization of the β_2-AR involves a specific kinase, the β-AR kinase (β-ARK), which phosphorylates several Ser and Thr residues in the C-terminal end of the receptor [39]. The β_1-AR also displays such a Ser/Thr rich C-terminal region. On the other hand, the β_3-AR has a short C-terminal region where only few hydroxylic residues occur. In addition, in both β_1- and β_2-AR, putative target residues for the β-ARK are found in the vicinity of

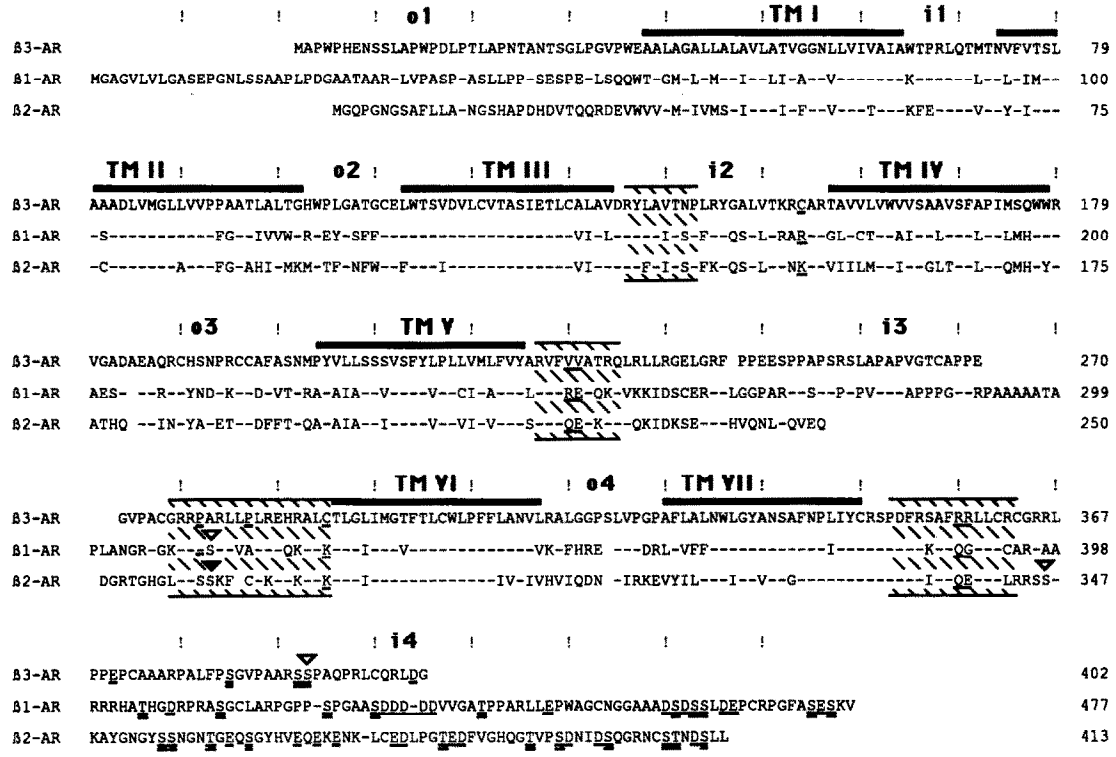

Fig. 2. Structural properties of the β_1-, β_2 and β_3-AR proteins. Amino acid sequences of the human β_1- and β_2-AR have been aligned with those of the human β_3-AR. The position in the corresponding sequence of the amino acid at the end of each line is indicated and an exclamation point (!) marks every tenth amino acid residue. Hyphens indicate residues of the β_1- and β_2-ARs identical to those found at corresponding positions in the β_3-AR. To maximize homologies, gaps, represented by empty spaces, have been introduced in the three sequences. The seven transmembrane segments (TM I to TM VII) are highlighted by solid bars and alternate with extracellular (o1 to o4) and intracellular (i1 to i4) domains. Hatched areas represent regions thought to interact with G-proteins. In these regions, amino acids that could support coupling of individual β-AR subtype to particular G-proteins are underlined. The PKA phosphorylation site required for heterologous desensitization of the β_2-AR is marked by a black inverted triangle, and such potential sites in the β_1- and β_3-AR by open inverted triangles. In the C-terminus, double underlines show the serine and threonine residues which, as is the case for the β_2-AR, could be phosphorylated by the β-ARK that mediates homologous desensitization. Single underlines show the acidic residues occurring in their vicinity.

several acidic residues, whereas the Ser residues of the β_3-AR tail are not.

Although no role has yet been attributed to tyrosine protein kinases in desensitization of β-AR, it is noticeable that only the β_2-AR displays Tyr residues in its C-terminal domain. It is also noteworthy that β_2-AR mRNA expression can be modulated by cAMP [40, 41] and that a cAMP responsive element occurs in the promoter region of the β_2-AR gene but not in that of the β_3-AR.

Based on these differences, it may be hypothesized that the β_3-AR is not subject to desensitization, or that the basic mechanisms are distinct from those leading to attenuation of β_1- and β_2-AR responsiveness. Indeed, evidence exists for the presence in human adipose cells of β-ARs resistant to prolonged agonist exposure [42]. Since G-protein coupling and receptor desensitization are intimately linked [29–31], this observation further supports the possibility that β_3-ARs interact with G-proteins distinct from those coupled to β_1- and β_2-ARs.

Is there further heterogeneity of the β-ARs?

The pharmacologic properties of the human β_3-AR, as determined in Chinese hamster ovary cells transfected with the cloned gene (CHO-β_3 cells), are clearly distinct from those of the β_1- and β_2-AR [14]. In fact, they are more similar to those of atypical receptors which mediate catecholamine effects in rodent tissues such as fat cells, skeletal muscle, digestive tract smooth muscle, heart, and liver [1, 2, 6]. The presence of β_3-AR mRNA in these same types of tissues [14] and in 3T3-F442A adipocytes, and the homologies between the promoter of the β_3-AR gene and that of the aP2 lipid binding protein, further support this pharmacologic correlation.

Nevertheless, minor pharmacologic differences [1] may be interpreted as evidence for the existence of other β-ARs closely related to the cloned β_3-AR. For example, CGP 12177, a potent β_1- and β_2-AR antagonist, activates fat metabolism in rat adipocytes

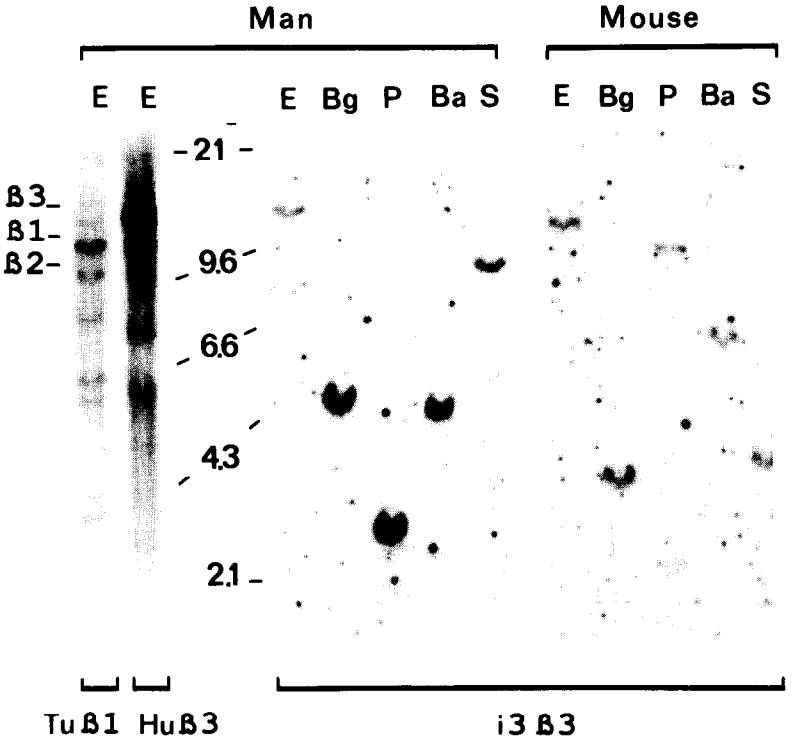

Fig. 3. Analysis of the human and murine genome for sequences homologous to β-AR genes. Southern blots of human and mouse genomic DNA digested with restriction enzymes Eco RI (E), Bgl II (Bg), Pst I (P), Bam HI (Ba) and Sac I (S) were hybridized separately to probes comprising the sequences coding for the entire turkey β_1- (Tuβ1) or human β_3-AR (Huβ3), or for the third intracytoplasmic loop of the human β_3-AR (i3β3). The Tuβ1 and Huβ3 probes hybridize to the human β_1-, β_2- and β_3-AR genes as well as to several other sequences. In contrast, in the genome from human, mouse and rat (not shown for rat), the subtype selective i3β3 probe detects a single sequence corresponding to the β_3-AR gene. The position and the length (in kilo-base pairs) of size markers are indicated.

[43], but has no effect on cAMP accumulation in CHO-β_3 cells [14] nor on lipolysis in human fat cells.* This compound, however, is a fairly potent inducer of cAMP accumulation in 3T3-F442A adipocytes† which express the product of the murine β_3-AR gene. It is thus probable that several differences between the cloned β_3-AR and atypical tissular β-ARs are species related. Indeed, a single amino acid substitution in the β_2-AR is sufficient to generate partial agonist activity from antagonists [44] or to modify coupling properties of the receptor [45]. On the other hand, some discrepancies could reflect differences in the β-AR signalling pathway of human fat deposits as compared to that of other mammals.

During our preliminary characterization of the β_3-AR, we have described, in rat adipose tissues, muscle and liver, two molecular forms of mRNA hybridizing to a β_3-AR specific probe [14]. Both transcripts, however, certainly proceed from the rat homologue of the cloned gene since the probe used in this experiment, which is highly specific for the β_3-AR, hybridizes to a single sequence in human, rat and mouse genomes (Fig. 3). On the other hand, analyses at relatively low stringency of human genomic DNA, with less selective probes encompassing the entire coding regions of the turkey β_1- and human β_3-AR genes, indicate the presence of homologous sequences distinct from the β_1-, β_2- and β_3-AR genes (Fig. 3). The probes used in this experiment contain the sequences coding for the seven transmembrane regions that constitute the hormone binding sites of the receptors. One of the additional sequences may represent the 5-hydroxytryptamine$_{1A}$ receptor which binds adrenergic ligands with low affinity and whose gene has been isolated using the hamster β_2-AR gene [46]. The other sequences could correspond to receptors for related hormones such as dopamine or serotonine. It is also conceivable that some represent receptors for an unidentified endogenous adrenergic agonist. Such a ligand has already been suggested to control magnesium metabolism through atypical adrenergic receptors [47]. A similar situation has been encountered for imidazoline receptors which were inferred from atypical effects of α-adrenergic ligands [48].

According to the genetic data, other adrenergic receptors may thus exist. Since the corresponding genes are detected only with the non-selective β-AR probes, it is foreseeable that these receptors will be as structurally distinct from the three β-ARs as these are from one another. On the other hand, these

* Lafontan M, personal communication, cited with permission.

† Fève B and Pairault J, unpublished results.

other receptors could, as is the case for the β_3-AR, display low affinity for standard β-AR antagonists which were indeed developed to specifically target β_1- and β_2-ARs. In this perspective, the β_3-AR could represent such a receptor for this putative endogenous catecholamine.

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