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TRANSCRIPTIONAL REGULATION OF ALPHA-1 ADRENERGIC RECEPTORS

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1. ABSTRACT

Regulation of alpha$_1$-adrenergic receptors (alpha$_1$ARs) is a rapidly expanding field of study due to the involvement of these receptors in several human diseases. For example, upregulation of alpha$_{1a}$AR mRNA/protein with agonist exposure has been implicated in myocardial hypertrophy. In order to understand mechanisms underlying these findings, initial studies of human alpha$_{1a}$AR transcription have been completed and the primary promoter characterized. This review summarizes current knowledge regarding human alpha$_{1a}$AR gene transcription and compares characteristics with other adrenergic receptor genes.

2. INTRODUCTION

Alpha$_{1a}$ARs are G protein-coupled receptors which mediate myocardial inotropy/hypertrophy, smooth muscle contraction, and other sympathetic nervous system responses (1). After binding catecholamine agonist, alpha$_1$ARs couple predominantly via Gq to activate phospholipase C-beta, resulting in inositol triphosphate-mediated increases in intracellular calcium, as well as diacylglycerol-mediated activation of protein kinase C. Alpha$_{1a}$AR activation leads to cardiovascular smooth muscle contraction (2) and ultimately to hypertrophy and/or hyperplasia (3-5). cDNAs encoding three alpha$_1$-AR subtypes (alpha$_{1a}$, alpha$_{1b}$, alpha$_{1d}$) have been cloned, expressed in cells, and characterized pharmacologically (6, 7). The alpha$_{1a}$AR subtype predominates in human tissues (8-10), and has been shown to be important in symptomatology associated with benign prostatic hyperplasia (11, 12) and the pathophysiology of myocardial hypertrophy (13). Increased expression of alpha$_{1a}$ARs (mRNA and protein) has been demonstrated in neonatal rat myocardium upon agonist stimulation, concurrent with repression of alpha$_{1b}$ and alpha$_{1d}$AR subtypes (13). These findings underscore the importance of myocardial alpha$_{1a}$ARs in the pathophysiology of congestive heart failure where a high catecholamine state has been repeatedly demonstrated (14). In spite of evidence of increased alpha$_{1a}$AR mRNA and protein with sympathetic stimulation, mechanisms underlying alpha$_{1a}$AR up-regulation remain unclear. Therefore, we will focus on transcriptional regulation of alpha$_1$ARs in this review, with emphasis on the alpha$_{1a}$AR and comparison with other adrenergic receptor genes.

3. 5' UNTRANSLATED REGION AND PUTATIVE REGULATORY CONSENSUS SEQUENCES

In order to study alpha$_1$AR transcriptional regulation, the 5' untranslated region (5'UTR) of the gene must be characterized. Portions of the 5'UTR from alpha$_1$AR genes have been cloned by several investigators: human alpha$_{1a}$AR (6.2 kb, GenBank #U72653) (15), human alpha$_{1b}$AR (0.92 kb, GenBank #M99589) (16), rat alpha$_{1b}$AR (2.5 kb, GenBank #U83985) (17), and rat alpha$_{1d}$AR (0.4 kb, GenBank #L31771) (18, 19). Prokaryotic genes often include consensus sequences such as TATAA, CAAT, and GC boxes; these sequences facilitate the binding of RNA polymerase in the region of the transcription initiation site. Although TATA-less promoters have long been known to exist, initially it was thought that these types of promoters were restricted to housekeeping genes. Increasingly, TATA-less promoters have been identified for a wide range of genes, including those encoding adrenergic receptors. Thus far nine distinct adrenergic receptor subtypes have been identified (alpha1a, alpha$_{1b}$, alpha$_{1d}$, alpha$_{2a}$, alpha$_{2b}$, alpha$_{2c}$, beta$_1$, beta$_2$, beta$_3$). Taking species homologues into account,

Table 1. Characteristics of 5'UTRs of Various Adrenergic Receptor Genes

Receptor	Available 5'UTR (kb)	%GC in first 1000 bp	TATA in first 1000 bp	CAAT in first 1000 bp
Alpha$_1$AR				
human alpha$_{1a}$	6.3	62	-	-
human alpha$_{1b}$	.90	58	-	+
rat alpha$_{1b}$	2.5	70	-	+
rat alpha$_{1d}$	.40	73	-	++
Alpha$_2$AR				
human alpha$_{2a}$	2.0	67	++++	-
rat alpha$_{2a/d}$	2.7	63	+	-
mouse alpha$_{2a}$	2.8	63	+	-
mouse alpha$_{2b}$	1.1	62	-	+
human alpha$_{2c}$	2.8	83	-	-
Beta$_1$AR				
human beta$_1$	3.1	67	-	-
rhesus beta$_1$	1.4	71	-	-
rat beta$_1$	1.2	72	-	-
Beta$_2$AR				
human beta$_2$	1.5	63	-	-
rat beta$_2$	3.7	60	-	-
turkey beta$_2$	.89	60	+	-
Beta$_3$AR				
human beta$_3$	1.3	55	-	-
rat beta$_3$	1.4	51	-	-

Gene sequences where ≥400 bp of 5'UTR are known. Criteria for TATA include 7/7 matches (TATA$^{A}/_{T}$A$^{A}/_{T}$). Criteria for CAAT include 7/9 matches (GG$^{C}/_{T}$CAATCT). The following adrenergic receptor genes (with their GenBank accession numbers in parenthesis) were analyzed: human alpha$_{1a}$ (U72653); human alpha$_{1b}$ (M99589); rat alpha$_{1b}$ (U83985); rat alpha$_{1d}$ (L31771); human alpha$_{2a}$ (M23533); rat alpha$_{2a/d}$ (RNU49747); mouse alpha$_{2a}$ (MMU29693); mouse alpha$_{2b}$ (M94583); human alpha$_{2c}$ (HSU72648); human beta$_1$ (X69168); rhesus monkey beta$_1$ (X75540); rat beta$_1$ (X75538); human beta$_2$ (M15169); rat beta$_2$ (L39264); turkey beta$_2$ (U13978); human beta$_3$ (M62473); and rat beta$_3$ (X77483).

seventeen distinct adrenergic receptor genes have been identified with >400 bp 5'UTR. Table 1 illustrates that less than half (seven out of seventeen) of the adrenergic receptor genes characterized contain TATA or CAAT box sequences. The human alpha$_{1a}$AR contains neither TATA nor CAAT boxes, although both human alpha$_{1b}$AR and rat alpha$_{1b}$AR genes contain at least one CAAT box. In comparison, the human alpha$_{2a}$AR contains four TATA boxes, rat and mouse alpha$_{2a}$AR genes each contain one, while all but one beta-AR (the turkey beta$_2$) contain none.

4. IDENTIFICATION OF TRANSCRIPTION INITIATION SITES

In order to gain insight into the molecular basis of gene expression under a variety of conditions, the transcription initiation site and basal promoter region (regulatory sequences directly upstream of the transcription initiation site) need to be defined. Recently, Razik *et al* determined (using 5' extension and RNase protection assays) that the human alpha$_{1a}$AR gene has at least five transcription initiation sites with the main transcription initiation site located 696 bp upstream of the ATG translation start codon (20). Multiple transcription initiation sites appear to be a common feature of alpha$_1$AR genes, with the rat alpha$_{1b}$AR containing three and the human alpha$_{1b}$AR at least two transcription initiation sites (16, 21-23). Other catecholamine receptors with multiple transcription initiation sites include the rat beta$_1$AR (24) and the human dopamine$_{1A}$ receptor (25).

5. USE OF REPORTER CONSTRUCTS TO DEFINE PROMOTERS

Characterization of basal promoter activity is often initially accomplished using reporter constructs containing serial deletions of the 5' flanking region to determine the smallest amount of sequence required for maximal basal reporter activity. Various reporters have been utilized over the last decade, with the two most common being chloramphenicol acetyl transferase (CAT) and luciferase. Luciferase reporter expression was recently utilized to define the general location of the human alpha$_{1a}$AR promoter; the functional promoter was found to reside within 125 bp upstream of the transcription initiation site in this gene (figure 1) (20). Once the general promoter region is identified, specific mutations can be performed to further define the importance of specific factors in basal transcription of a given gene.

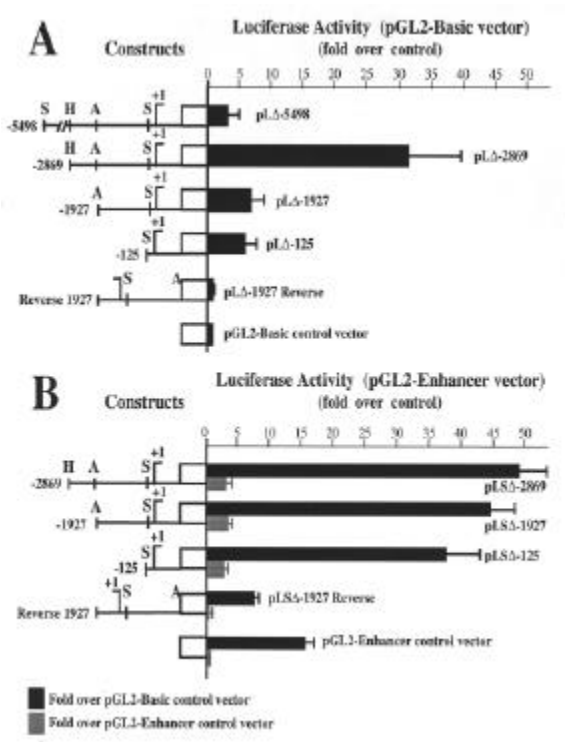

Figure 1. Luciferase reporter experiments. Constructs containing varying amounts of human alpha$_{1a}$AR 5'UTR fused to the promoterless luciferase gene were transiently transfected into SK-N-MC cells and resultant luciferase activity measured (n=5-12 independent transfections [each performed in duplicate for each construct]). The left half of the figure schematizes each construct (white box = promoterless luciferase), while the right half presents luciferase results. Luciferase activity is normalized for cotransfected chloramphenicol acetyl transferase activity (a separate plasmid utilized for normalization of transfection efficiency between experiments), and expressed as fold over control (black box, relative to pGL2 Basic in *A* and *B;* gray box, relative to pGL2 Enhancer in *B*) (mean SEM). The main transcription initiation site is defined as +1. ***A.*** Constructs in *A* utilize a pGL2-Basic vector (which does not contain a SV40 enhancer); luciferase results reveal the presence of an endogenous activator in human alpha$_{1a}$AR 5'UTR between -1927 and -2869, and a suppressor of basal transcription located upstream of -2869. ***B.*** Constructs in *B* utilize pGL2-Enhancer (which contains a SV40 enhancer in the vector). These results demonstrate that the first 125 bp upstream from the transcriptional initiation site is sufficient for basal transcription (contains entire promoter sequence). (Reproduced with permission from reference #20).

6. TRANSCRIPTION INITIATION IN TATA-LESS PROMOTERS

In the absence of TATA or CAAT binding sites in a promoter, initiation of transcription is often associated with Sp1 binding to GC rich regions (note the relatively high GC content of the adrenergic receptor genes shown in table 1) and the presence of initiator (Inr) consensus sequences (26-30). The region directly upstream from the transcription initiation start site in the human alpha$_{1a}$AR gene contains three GC boxes (G/TG/AGGCGG/TG/AG/AC/T); two of these GC boxes are found within the basic 125 bp promoter of the alpha$_{1a}$AR, and bind Sp1 (demonstrated by gel mobility shift assays) (20). Five Inr consensus sequences ([Py]$_3$[Py]$_2$ANT[Py]$_2$) are also located in the 650 bp region surrounding the major transcription initiation site of the human alpha$_{1a}$AR gene (15). This is consistent with previous findings that TATA-less promoters generally initiate transcription with RNA polymerase II at Inr sequences associated with Sp1 binding (31, 32).

7. MULTIPLE PROMOTERS

Multiple promoters are present in several alpha$_1$AR genes. Gao, *et al* demonstrated three distinct alpha$_{1b}$AR mRNA species in rat liver by Northern blot analysis corresponding to three distinct promoters and transcription initiation sites (22). Each promoter was found to contain distinct regulatory consensus sequences and transcription factor protein binding profiles, with the majority of transcription arising from the second promoter. Partial hepatectomy in the rat results in decreased overall expression of the rat liver alpha$_{1b}$AR, particularly of the dominant species of mRNA corresponding to the second promoter (22). These events appear to be linked to a decrease in the transcription factor C/EBP during hepatocyte regeneration (33). Hence differential activation of specific promoters occurs depending on the physiologic state of the animal. While it is tempting to speculate that the purpose of multiple promoters in a gene may be to ensure transcription in a variety of physiologic and pathologic states (e.g. "fed vs. fasting" in liver), there is as yet insufficient information to confirm this hypothesis; hence these findings may provide avenues for further investigation in a wide variety of genes. In addition, it is interesting to note that the alpha$_{1a}$AR mRNA is the only alpha$_1$AR subtype mRNA present in human liver (9). Hence alpha$_1$AR hepatic regulation may differ in human versus rat liver.

8. CELL-SPECIFIC EXPRESSION OF ADRENERGIC RECEPTOR mRNAs

Gene expression is known to vary depending on the type of tissue examined. One explanation for this finding may be the presence of cell specific complements of transcription factors. In the human alpha$_{1a}$AR, expression occurs in many native human tissues, although expression is limited to only a few human cell lines. While human SK-N-MC neuroblastoma, DU145 prostate cancer, and Chang liver hepatoma cell lines all contain alpha$_1$AR expression, DU145 and Chang liver cells contain only the alpha$_{1b}$AR subtype, while SK-N-MC cells contain alpha$_{1d}$ > alpha$_{1a}$, with no alpha$_{1b}$AR (10). After transfecting alpha$_{1a}$AR expressing cells with luciferase reporter

Table 2. *Cis*-Regulatory Elements in Adrenergic Receptor Genes.[a]

		Elements and Their Consensus Sequences					
		AP-1 [b]	AP-2 [c]	CRE [d]	GRE [e,f]	ERE [g]	IRE [h]
Adrenergic Receptor	Avail. 5'UTR	$T^{G}/_{T}AGTCA$	$CC^{C}/_{G}C^{A}/_{G}GGC$	Multiple Consensus Sequences	$AGAACAN_3$ TGTTCT	$AGGTCAN_3$ TGACCT	$T^{G}/_{A}TTTG$ CCTTTGA GTGGAAA
$Alpha_1AR$							
human $alpha_{1a}$	6.25	2	2	6	6	3	12
human $alpha_{1b}$	.92		1	1			
rat $alpha_{1b}$	2.5	1	1	2	1		6
rat $alpha_{1d}$	.4		2			1	
$Alpha_2AR$							
human $alpha_{2a}$	2.0		2	2	1		
rat $alpha_{2a/d}$	2.7		1	2			5
mouse $alpha_{2a}$	2.8		1	2	1	1	3
mouse $alpha_{2b}$	1.1		2			1	2
human $alpha_{2c}$	2.8		7	3	1	2	4
$Beta_1AR$							
human $beta_1$	3.1		2	5	2	1	3
monkey $beta_1$	1.4		2	3	2		2
rat $beta_1$	1.2	1		5	1	1	
$Beta_2AR$							
human $beta_2$	1.5			2	1		4
rat $beta_2$	3.7	1		3	4	4	12
turkey beta	.89	1	2				2
$Beta_3AR$							
human $beta_3$	1.3			3		1	1
rat $beta_3$	1.4		1	1	1		3

[a]Adrenergic receptor genes containing ε400 bp 5'UTR were analyzed for the indicated transcription factors using consensus sequences found in MacVector 5.0 (Oxford Molecular Group PLC, Campbell, CA) as well as updated sequences from the literature [Hai, 1989 #21; Bridges, 1992 #22; O'Brien, 1995 #20; Thomas, 1992 #19; Kozak, 1984 #23; Faisst, 1992 #25; Lucas, 1992 #24]; only full consensus matches for each element were accepted; [b]AP-1[Angel, 1987 #30]; [c]AP-2 [Imagawa, 1987 #28; Mitchell, 1987 #29]; [d]cAMP response element (CRE), [Hai, 1989 #21], CRE sequences used for analysis are defined as follows: TGACGTCA (35), (TGACTCCA, CGAGGTCA, GTCGTCA) [Thomas, 1992 #19], (TACGTCA, TGACGTA, TGAGGTCT); [e]glucocorticoid response element (GRE); [f]androgen response elements usually overlap with GRE sites, however a novel androgen binding site has recently been described [Zhou, 1997 #36]--this sequence was absent in all adrenergic receptor genes examined; [g]estrogen response element (ERE) [Lucas, 1992 #24]; and [h]insulin response element (IRE) [O'Brien, 1995 #20; Bridges, 1992 #22]. (Modified from ref #15 with permission).

constructs containing the human $alpha_{1a}$AR primary 125 bp promoter region, it was found that SK-N-MC cells drive higher basal luciferase activity than DU 145 or Chang liver cells (20). These experiments support the suggestion that factors and proteins necessary for transcription of the human $alpha_{1a}$AR are cell specific.

9. MODULATION OF GENE EXPRESSION VIA *CIS*-ACTING ELEMENTS

Basal promoter activity, though essential to define, does not fully define transcriptional regulation of a gene. Regions other than the promoter exist in the 5'UTR which modulate basal adrenergic receptor gene expression. In fact, binding of transcription factors distant from the transcription initiation site have been shown to induce conformational changes thought to affect RNA polymerase II stability in the promoter region. In the human $alpha_{1a}$AR gene, both enhancer and repressor sequences have been shown to reside far upstream from the transcription initiation site (enhancer 1927-2869 bp, repressor 2869-5498 bp; figure 1) (20). Several putative positive and negative response elements exist in these regions, respectively, although further investigation is required to identify specific activators and suppressors.

In addition to transcription factor-mediated enhancement and repression of basal promoter activity, gene transcription can be induced by hormones and drugs. Hormones and drugs can directly modulate gene

transcription by binding to consensus sequences in the 5'UTR of a gene, or indirectly modulate expression of other transcription factors. In the adrenergic receptor family, the direct role of modulatory hormones is suggested by the presence of multiple hormone binding sites in specific genes including cAMP response elements (CRE; binding sites for the CRE binding protein CREB), glucocorticoid response elements (GRE), estrogen response elements (ERE), and insulin response elements (IRE) (table 2) (15). Supporting this contention in the human $alpha_{1a}$AR gene is evidence that $beta_3$AR stimulation of cAMP production in rat brown adipose tissue upregulates $alpha_{1a}$AR expression (34). Furthermore, human $alpha_{1a}$AR mRNA and protein expression levels are elevated by increasing intracellular cAMP with forskolin/IBMX treatment (20). Direct and indirect modulation of mRNA expression by hormones and drugs is an exciting area of research which may provide novel targets for therapeutic intervention in various diseases.

10. PERSPECTIVES

In summary, understanding regulation of human $alpha_1$ARs is important, especially in light of subtype specific upregulation of the $alpha_{1a}$AR with agonist in myocardial hypertrophy. Transcriptional regulation appears to play a large role in this process and may reveal clues to receptor subtype-specific regulation in health and disease. Studies of basal and inducible transcription of $alpha_{1a}$AR and $alpha_{1b}$AR subtypes are already underway (as presented in this review), giving insight into factors important in regulating each of these genes. Further research on the effect of specific diseases on $alpha_1$AR subtype transcription is now possible, potentially providing new targets for therapeutic intervention.

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