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Molecular Cloning and Expression of the Rat β_3-Adrenergic Receptor

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SUMMARY

Rat adipose tissues contain atypical β receptors that display certain pharmacological sensitivities that are similar to those found in the recently cloned human β_3 receptor. However, there are also certain pharmacological differences between the human atypical β_3 receptor and atypical receptors in rodent adipose tissues, which could indicate strong species differences, the existence of multiple atypical receptor subtypes, or both. To help decide among these possibilities, a rat β_3 receptor clone was obtained and expressed in Chinese hamster ovary cells. The predicted primary structures of the rat and human receptors are >90% similar. Despite this similarity, the pharmacological properties of the rat receptor differed from those reported for the human receptor but were similar to the properties exhibited by atypical receptors in rat adipose tissue. Specifically, the rat β_3 receptor had a high affinity for BRL 37344 and a relatively low affinity for norepinephrine and was partially activated by the β_1 and β_2 receptor antagonist CGP 12177. Northern blot analysis and nuclease protection assays of RNA from rat tissues indicate that the β_3 receptor is abundantly expressed only in adipose tissues.

The nature of the β adrenergic receptor subtypes in adipose tissue has been controversial. In addition to the well characterized β_1- and β_2-adrenergic receptors, rodent BAT and white adipose tissue contain atypical receptors that display pharmacological properties that are similar to those reported for the human β_3 receptor (1–3). For example, both the human β_3 receptor and atypical receptors in rat BAT are stimulated by the atypical agonist BRL 37344, and this activity is poorly antagonized by standard β receptor antagonists (1, 3–5). However, the atypical receptors controlling adenylyl cyclase activity in rat adipose tissue show several differences in pharmacology from that reported for the human β_3 receptor (4, 6, 7). These data indicate that there may be strong species differences with respect to the pharmacology of the β_3 receptor, that there may be multiple atypical receptor subtypes, or both.

In order to help differentiate among these possibilities, we have cloned a rat homolog of the β_3 receptor and have characterized its pharmacological properties in CHO cells. In addition, we have examined the expression of the β_3 receptor gene in various rat tissues.

Materials and Methods

Animals. Male Sprague-Dawley rats (Hilltop, Scottdale, PA) were used to obtain tissue mRNA for analysis and for cDNA library construction.

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Generation of β_3 cDNA probes. Probes for cloning the rat β_3 receptor cDNA and for measurement of tissue mRNA were obtained with the PCR. BAT RNA (10 μg) was reverse-transcribed with a β receptor-specific (3, 9, 10) oligonucleotide, primer A, 5′-GCGAATTCGAAGGCACTICIGAAGTCGGGGCTGCGGCAGTA-3′, which also contained an *Eco*RI restriction site on the 5′ end. This cDNA was then amplified with primer A and the human β_3-specific primer 5′-GCGCTGCGCCCGGACAGCTGTGGTCCTGG-3′ (3). PCR was performed as described previously (8). Samples were denatured for 2 min at 94°, annealed, and extended at 72° for 4 min. Thirty rounds of amplification were performed. One microliter of this reaction was further amplified, as described above, with the β_3-specific primer described above and a downstream primer, 5′-GCGAATTCGAAGAAGGGCAGCCAGCAGAG-3′, that is common (except for the added *Eco*RI site) to all β receptors (3, 9, 10). The β_3 receptor PCR product was cloned into the *Sma*I and *Eco*RI sites of the plasmid pGEM 3Z (Promega) and sequenced by the dideoxynucleotide chain-termination technique (Sequenase; United States Biochemical Corp.). The rat β_3 PCR product was found to be highly homologous to the human β_3 receptor gene (3) and, ultimately, identical to a rat cDNA clone encoding the rat β_3 receptor.

Library construction and screening. Library construction, screening, and cloning were performed using standard techniques (11). A cDNA library was constructed in LambdaGEM-4 (Promega) using poly(A)$^+$ RNA isolated from BAT of cold-exposed rats. This library contained approximately 3×10^6 recombinants, with an average insert size of 1.5 kb. Three hundred thousand recombinants were screened at high stringency (0.03 M NaCl, 3 mM sodium citrate, pH 7, at 65°) with the cloned rat β_3 PCR product labeled with [^{32}P]dCTP using random

ABBREVIATIONS: BAT, brown adipose tissue; CHO, Chinese hamster ovary; PCR, polymerase chain reaction; kb, kilobases; bp, base pairs; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

primers (11). Twenty-seven phage were isolated from the amplified library, and two plasmids (p108 and p109) of the same size (about 1.73 kb) were rescued. Sequencing of p108 and p109 from the 5′ ends indicated that they were identical and truncated with respect to the predicted initiation codon of the human β_3 receptor sequence (3). Screening of the remaining isolates by PCR failed to detect any full-length cDNAs, and primer extension experiments with tissue mRNA suggested that secondary structure, owing to high G-C content, may have limited the ability of the reverse transcriptase to synthesize cDNA through the missing 5′ region. Therefore, to obtain the remaining sequence, a Sprague-Dawley rat genomic library (Clontech) was screened with a p108 probe to obtain the rat genomic sequence. Four hundred forty-four base pairs of genomic sequence that overlapped with p108 β_3 cDNA identified it as the gene encoding the β_3 receptor. A full-length clone was then produced by cloning the genomic sequence from bases −104 to +390 (relative to translation initiation) into the *Acc*I site of p108. Both DNA strands were sequenced by the dideoxy chain-termination technique (11), and no discrepancies were found.

Transfection of CHO-k1 cells. The assembled β_3 receptor construct was cloned into pRC/CMV (Invitrogen), an expression vector containing the cytomegalovirus promoter and a neomycin resistance gene. This construct was transfected into CHO-k1 cells using the $CaPO_4$ method (11). Stably transfected cells were selected in the presence of Geneticin (800 μg/ml) and pooled for further analysis.

Adenylyl cyclase assay. Adenylyl cyclase activity was determined by the method of Salomon (12). Culture medium was removed and cells were washed in phosphate-buffered saline and then harvested in 25 mM HEPES (pH 8.0) buffer containing 2 mM $MgCl_2$ and 1 mM EDTA. Cell were homogenized and centrifuged at 48,000 × *g* for 15 min, to obtain crude membranes. Membrane pellets were resuspended and used directly or frozen at −80° until used. Freezing did not affect activity. Membranes (5–15 μg of protein) were preincubated at 4°, in a volume of 40 μl, with the specified drugs for 15 min. Adenylyl cyclase reactions were initiated by addition of substrate mixture and were terminated after 30 min at 30°. BAT membrane adenylyl cyclase activity was determined as previously described (4, 6), using membranes from 7-day-old rats. Concentration-response data were analyzed by nonlinear regression analysis with a one-site mass action equation for transfected CHO cells (Enzfitter, Elsevier Biosoft). A two-site model was used to analyze catecholamine-stimulated adenylyl cyclase in BAT, with the low affinity component representing stimulation by β_3 receptors (25).

Tissue mRNA analysis. The size of the β_3 receptor transcripts was determined by Northern blot analysis of rat poly(A)$^+$ RNA, as previously described (11, 13). The cDNA probe used corresponded to bp 228–665 of Fig. 1 and was labeled by random primers. Tissue mRNA distribution experiments were conducted on total RNA with a solution hybridization assay (11, 14). The radioactive cRNA probe used was transcribed *in vitro* from the cloned β_3 receptor PCR product (p110) with [^{32}P]CTP, using the T7 promoter. The probe corresponded to bp 746–917 in Fig. 1. Tissue or cellular RNA (5–50 μg) was co-precipitated with 3 × 10^4 cpm of the ^{32}P-labeled cRNA probe. Samples were hybridized at 55° for 12–18 hr and then diluted, and the nonhybridized probe was digested with 300 units of T-1 ribonuclease for 45 min at 37°. The [^{32}P]RNA probe that was protected from RNase digestion was electrophoretically resolved on a denaturing polyacrylamide gel containing 8 M urea. The gels were dried and exposed to Kodak XAR-5 film for 18–72 hr.

Results

The nucleotide and predicted amino acid sequences of the rat β_3 receptor are shown in Fig. 1. The consensus sequence for translation initiation (15) that was found in the assembled rat β_3 clone is followed by an open reading frame encoding a protein of 400 amino acids and a 3′ nontranslated sequence of about 750 bp. This deduced protein is 79% identical and 91% similar to the human β_3 receptor (Fig. 2). In contrast, the rat β_3 receptor

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                TAAGCCAGCGGGTCTGGGGGGAAAACTTCCCATCCCAGACGCGACACGAG
1                                                               45
ATG GCT CCG TGG CCT CAC AAA AAC GGC TCT CTG GCT TTC TGG TCA
MET Ala Pro Trp Pro His Lys Asn Gly Ser Leu Ala Phe Trp Ser
                                                                90
GAC GCC CCC ACC TTG GAC CCC AGT GCA GCC AAC ACC AGT GGG TTG
Asp Ala Pro Thr Leu Asp Pro Ser Ala Ala Asn Thr Ser Gly Leu
                                                               135
CCA GGG GTG CCA TGG GCA GCG GCA TTG GCT GGA GCA TTG CTG GCG
Pro Gly Val Pro Trp Ala Ala Ala Leu Ala Gly Ala Leu Leu Ala
                                                               180
CTG GCC ACG GTG GGA GGC AAC CTG CTG GTA ATC ACA GCT ATC GCC
Leu Ala Thr Val Gly Gly Asn Leu Leu Val Ile Thr Ala Ile Ala
                                                               225
CGC ACG CCG AGA CTA CAG ACC ATA ACC AAC GTG TTC GTG ACT TCG
Arg Thr Pro Arg Leu Gln Thr Ile Thr Asn Val Phe Val Thr Ser
                                                               270
CTG GCC ACA GCT GAC TTG GTA GTG GGA CTC CTC GTA ATG CCA CCA
Leu Ala Thr Ala Asp Leu Val Val Gly Leu Leu Val MET Pro Pro
                                                               315
GGG GCC ACA TTG GCG CTG ACT GGC CAC TGG CCC TTG GGC GCA ACT
Gly Ala Thr Leu Ala Leu Thr Gly His Trp Pro Leu Gly Ala Thr
                                                               360
GGC TGC GAG CTG TGG ACG TCA GTG GAC GTG CTC TGT GTA ACT GCC
Gly Cys Glu Leu Trp Thr Ser Val Asp Val Leu Cys Val Thr Ala
                                                               405
AGC ATC GAG ACC CTG TGC GCC CTG GCT GTA GAC CGC TAC CTA GCC
Ser Ile Glu Thr Leu Cys Ala Leu Ala Val Asp Arg Tyr Leu Ala
                                                               450
GTC ACC AAC CCT CTG CGT TAC GGC ACG CTG GTT ACC AAG CGC CGC
Val Thr Asn Pro Leu Arg Tyr Gly Thr Leu Val Thr Lys Arg Arg
                                                               495
GCC CGG GCG GCA GTA GTC CTG GTG TGG ATC GTG TCC GCC ACC GTG
Ala Arg Ala Ala Val Val Leu Val Trp Ile Val Ser Ala Thr Val
                                                               540
TCC TTT GCG CCC ATC ATG AGC CAG TGG TGG CGT GTA GGG GCA GAC
Ser Phe Ala Pro Ile MET Ser Gln Trp Trp Arg Val Gly Ala Asp
                                                               585
GCT GAG GCG CAA GAG TGT CAC TCC AAT CCG CGC TGC TGT TCC TTT
Ala Glu Ala Gln Glu Cys His Ser Asn Pro Arg Cys Cys Ser Phe
                                                               630
GCC TCC AAT ATG CCC TAC GCG CTG CTC TCC TCC TCC GTC TCC TTC
Ala Ser Asn MET Pro Tyr Ala Leu Leu Ser Ser Ser Val Ser Phe
                                                               675
TAC CTT CCC CTC CTT GTG ATG CTC TTC GTC TAT GCT CGA GTG TTC
Tyr Leu Pro Leu Leu Val MET Leu Phe Val Tyr Ala Arg Val Phe
                                                               720
GTC GTA GCT AAG CGC CAG CGG CGT TTG CTG CGC CGG GAG CTG GGC
Val Val Ala Lys Arg Gln Arg Arg Leu Leu Arg Arg Glu Leu Gly
                                                               765
CGT TTT CCG CCC GAG GAG TCT CCG CGG TCT CCG TCG CGC TCT CCA
Arg Phe Pro Pro Glu Glu Ser Pro Arg Ser Pro Ser Arg Ser Pro
                                                               810
TCC CCT GCC ACA GTC GGG ACA CCC ACG GCA TCG GAT GGA GTG CCC
Ser Pro Ala Thr Val Gly Thr Pro Thr Ala Ser Asp Gly Val Pro
                                                               855
TCC TGC GGG CGG CGG CCT GCG CGC CTC CTA CCG CTC GGG GAA CAC
Ser Cys Gly Arg Arg Pro Ala Arg Leu Leu Pro Leu Gly Glu His
                                                               900
CGC GCC CTG CGC ACC TTG GGT CTC ATT ATG GGC ATC TTC TCT CTG
Arg Ala Leu Arg Thr Leu Gly Leu Ile MET Gly Ile Phe Ser Leu
                                                               945
TGC TGG CTG CCC TTC TTT CTG GCC AAC GTG CTG CGC GCA CTC GTG
Cys Trp Leu Pro Phe Phe Leu Ala Asn Val Leu Arg Ala Leu Val
                                                               990
GGG CCC TCC CTA GTT CCC AGC GGA GTT TTC ATC GCC CTG AAC TGG
Gly Pro Ser Leu Val Pro Ser Gly Val Phe Ile Ala Leu Asn Trp
                                                              1035
TTG GGC TAT GCC AAC TCT GCC TTC AAC CCG CTC ATC TAC TGC CGC
Leu Gly Tyr Ala Asn Ser Ala Phe Asn Pro Leu Ile Tyr Cys Arg
                                                              1080
AGC CCG GAC TTT CGC GAC GCC TTC CGT CGT CTT CTG TGC AGC TAC
Ser Pro Asp Phe Arg Asp Ala Phe Arg Arg Leu Leu Cys Ser Tyr
                                                              1125
GGT GGC CGT GGA CCG GAA GAG CCA CGC GTG GTC ACC TTC CCA GCT
Gly Gly Arg Gly Pro Glu Glu Pro Arg Val Val Thr Phe Pro Ala
                                                              1170
AGC CCT GTT GCG TCC AGG CAG AAC TCA CCG CTC AAC AGG TTT GAT
Ser Pro Val Ala Ser Arg Gln Asn Ser Pro Leu Asn Arg Phe Asp
                                                              1218
GGC TAT GAA GGT GAG CGT CCA TTT CCC ACA TGA AGGACCATGGAGATC
Gly Tyr Glu Gly Glu Arg Pro Phe Pro Thr ---
                                                              1277
TAGCAAGGAGCCTGACTTCTGGAGAAATTTTTTTTTTAAGACAGAAAGACAAGCAACGTC
                                                              1333
CATGGATGCAAACCTTTTATACAGCCCTTGATTTCTGCTCAGAGTGAGTTCCCAGG
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Fig. 1. Nucleotide and predicted amino acid sequences of the rat β_3 receptor. Nucleotide sequence from bases −35 to +227 was derived from genomic DNA, and the remainder from cDNA.

is 52% and 49% similar to rat β_1 (16) and rat β_2 (17) receptors, respectively.

The rat and human proteins are most highly conserved in the predicted transmembrane regions, where they are 98% similar. The rat β_3 receptor contains conserved amino acids that are believed to be important in the binding of catecholamines (18, 19), including Asp^{80}, Asp^{114}, Ser^{106}, and Ser^{109}. The rat and human β_3 receptors are also highly similar in the regions that are believed to confer GTP-binding protein-coupling specificity (20, 21), including the beginning and end of the third cytoplasmic loop and the beginning of the cytoplasmic tail. Consensus sequences for *N*-linked glycosylation are found at Asn^8 and Asn^{26}.

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RAT   - MAPWPHKNGSLAFWSDAPTLDPSAANTSGLPGVPWAAALA 40
        :::::: : ::: : : ::: : .:::::::::::: ::::
HUMAN - MAPWPHENSSLAPWPDLPTLAPNTANTSGLPGVPWEAALA 40

              I                                  
        GALLALA---TVGGNLLVITAIARTPRLQTITNVFVTSLA 77
        :::::::   ::::::::: ::: :::::::.:::::::: 
        GALLALAVLATVGGNLLVIVAIAWTPRLQTMTNVFVTSLA 80

           II                                    
        TADLVVGLLVMPPGATLALTGHWPLGATGCELWTSVDVLC 117
        .::::.::::.:: :::::::::::::::::::::::::: 
        AADLVMGLLVVPPAATLALTGHWPLGATGCELWTSVDVLC 120

              III                                
        VTASIETLCALAVDRYLAVTNPLRYGTLVTKRRARAAVVL 157
        :::::::::::::::::::::::::::::.::::: ::.:::: 
        VTASIETLCALAVDRYLAVTNPLRYGALVTKRCARTAVVL 160

           IV                                    
        VWIVSATVSFAPIMSQWWRVGADAEAQECHSNPRCCSFAS 197
        ::.:::.:::::::::::::::::::::: :::::::::.::: 
        VWVVSAAVSFAPIMSQWWRVGADAEAQRCHSNPRCCAFAS 200

                    V                             
        NMPYALLSSSVSFYLPLLVMLFVYARVFVVAKRQRRLLRR 237
        :::: :::::::::::::::::::::::::::: :: :::: 
        NMPYVLLSSSVSFYLPLLVMLFVYARVFVVATRQLRLLRG 240

        ELGRFPPEESPRSPSRSPSPATVGTPTASDGVPSCGRRPA 277
        :::::::::::: .:::: .:: ::: .  .:::.:::::: 
        ELGRFPPEESPPAPSRSLAPAPVGTCAPPEGVPACGRRPA 280

                         VI                       
        RLLPLGEHRALRTLGLIMGIFSLCWLPFFLANVLRALVGP 317
        ::::: ::::: ::::::: :.:::::::::::::::: :: 
        RLLPLREHRALCTLGLIMGTFTLCWLPFFLANVLRALGGP 320

                      VII                         
        SLVPSGVFIALNWLGYANSAFNPLIYCRSPDFRDAFRRLL 357
        ::::   :.:::::::::::::::::::::::::: :::::: 
        SLVPGPAFLALNWLGYANSAFNPLIYCRSPDFRSAFRRLL 360

        CSYGGRGPEEPRVVTFPASPVASRQNSPLNRFDGYEGERP 397
        :  : : : ::   . ::   .   .           :  
        CRCGRRLPPEPCAAARPALFPSGVPAARSSPAQPRLCQR- 399

        FPT 400

        LDG 402
```

Fig. 2. Alignment of the rat and human β_3 receptor amino acid sequences. The human sequence is from Ref. 3. The predicted membrane-spanning regions are *overlined*. :, Identical residues; ·, conserved substitutions.

There are few major differences between the rat and human (3) amino acid sequences. However, it is notable that three amino acids present in the first transmembrane-spanning region of the human receptor are absent in the rat. It is conceivable that the absence of these amino acids contributes to the pharmacological differences between the rat and human receptors reported below. Perhaps the greatest divergence between the rat and human β_3 receptors occurs in the cytoplasmic tails. Nevertheless, like the human β_3 receptor, the cytoplasmic tail of the rat receptor is notably deficient in serine and threonine residues, which are potential phosphorylation sites for the β-adrenergic receptor kinase (22). In addition, β_3 receptors of both species have no consensus sequence for phosphorylation by protein kinase A.

CHO-k1 cells were stably transfected with the rat β_3 receptor construct in order to study the pharmacological properties of the rat homolog. In principle, the affinities of various compounds for the rat β_3 receptor could be determined by radioligand binding techniques. However, because the affinity of standard β antagonists (e.g., pindolol and alprenolol) for the rat β_3 receptor is extremely low (see below), the use of available β-adrenergic radioligands was not feasible for characterizing the rat β_3 binding site. Of the potential ligands tested, CGP 12177 exhibited the highest affinity in functional assays. However, we were unable to detect specific binding of [^{3}H]CGP 12177 to CHO-rat β_3 membranes, presumably because the affinity of the interaction was too low to be detected by filtration binding techniques. Therefore, pharmacological characterization was performed using receptor activation of adenylyl cyclase.

Norepinephrine, epinephrine, isoproterenol, and the atypical β agonist BRL 37344 each maximally activated adenylyl cyclase in membranes of CHO cells expressing the rat β_3 receptor (Fig. 3, *left*). The potency order of these full agonists was BRL 37344 > isoproterenol > norepinephrine ≥ epinephrine (Table 1). In contrast, nontransfected CHO cells did not respond to any of the agonists tested, up to a concentration of 100 μM.

We found that several compounds that are classified as antagonists of β_1 and β_2 receptors were partial agonists in CHO-rat β_3 cells (Fig. 3, *right*). The most potent and efficacious of these was CGP 12177, which stimulated activity with a K_{act} of about 500 nM and had an intrinsic activity of 0.5, relative to isoproterenol (Table 1). Alprenolol was as potent as CGP 12177 but was less than half as effective in stimulating adenylyl

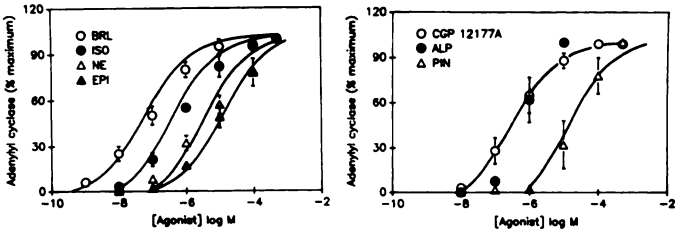

Fig. 3. Activation of adenylyl cyclase by various full (*left*) and partial (*right*) agonists. *BRL*, BRL 37344; *ISO*, isoproterenol; *NE*, norepinephrine; *EPI*, epinephrine; *ALP*, alprenolol; *PIN*, pindolol.

TABLE 1

Effects of various adrenergic compounds on adenylyl cyclase activity in CHO-rat β_3 cells and rat brown fat

Values are means ± standard errors of 3 to 10 experiments. Brown fat data for CGP 12177 and BRL 37344 are from Refs. 6 and 4.

Agonists and partial agonists	CHO-rat β_3 cells		Rat brown fat	
	K_{act}	Intrinsic activity (relative to norepinephrine)	K_{act}	Intrinsic activity
	μM		μM	
BRL 37344	0.08 ± 0.03	1.1	0.73 ± 0.17	1.3
CGP 12177	0.52 ± 0.34	0.5	2.8 ± 0.6	0.5
Isoproterenol	0.65 ± 0.07	1.0	16 ± 2	1.0
Alprenolol	0.79 ± 0.10	0.2		
Norepinephrine	5.8 ± 4.0	1.0	41 ± 6	1.0
Epinephrine	7.0 ± 1.1	0.9		
Pindolol	18.6 ± 2.4	0.2		

Compounds with no significant effects in CHO-rat β_3 cells	Basal activity		
	Compound alone	+Norepinephrine (10 μM)	+BRL 37344 (0.1 μM)
	%		
Water (control)	100	339 ± 38	333 ± 41
Metoprolol (10 μM)	103 ± 10	408 ± 61	370 ± 46
CGP 20712A (10 μM)	112 ± 25	369 ± 47	360 ± 13
ICI 118,551 (10 μM)	95 ± 7	302 ± 41	279 ± 28
Dopamine (100 μM)	93 ± 8		

cyclase. Pindolol weakly stimulated adenylyl cyclase activity, with very low potency.

Table 1 summarizes the effects of various adrenergic agents on adenylyl cyclase activity in CHO-rat β_3 cells and compares these data with results in BAT. Specifically included among the compounds tested were several that have been reported to discriminate between the human β_3 receptor (3) and atypical receptors in BAT (4, 6, 25). The relative potencies of the various agonists in CHO-rat β_3 cells are very similar to those found in BAT. Further, CGP 12177 is a partial agonist of similar intrinsic activity in both CHO-rat β_3 cells and BAT. Additionally, the β receptor antagonists metoprolol, CGP 20712A, and ICI 118, 551 have no significant interaction with the rat β_3 receptor or atypical receptors in BAT (4, 6), yet they have been reported to be antagonists of the human β_3 receptor (3).

There is very little information regarding the tissue distribution of β_3 receptor transcripts. The initial report describing the tissue distribution of β_3 mRNA used a human DNA probe that exhibited a great degree of nonspecific hybridization (3) (see Discussion). We, therefore, investigated the β_3 mRNA in tissues by both Northern blot analysis and a highly specific nuclease protection assay. Of the tissues examined, only BAT and white adipose tissue contained high levels of β_3 transcripts (Fig. 4). Low levels of expression (about 5% of adipose tissue) were detected in the ileum, whereas no expression was found in the other tissues examined. Northern blot analysis confirmed the results of the nuclease protection assay and further indicated that β_3 transcripts in white fat consist of a major mRNA species of 2.1 kb and a minor species of about 4.4 kb.

Discussion

The nature of the β receptors controlling adipose tissue function has been controversial (1, 7). Rat adipose tissues clearly contain β receptors with an atypical pharmacological profile (1, 4, 5). However, the pharmacological profile of atypical receptors in rodent adipose tissue differs from that reported for the human β_3 receptor (3, 4, 6, 7). These data indicate that there may be species differences with respect to the pharmacology of the β_3 receptor, that there may be multiple atypical receptor subtypes, or both. In order to differentiate among these possibilities, we have cloned and expressed a rat homolog of the β_3 receptor.

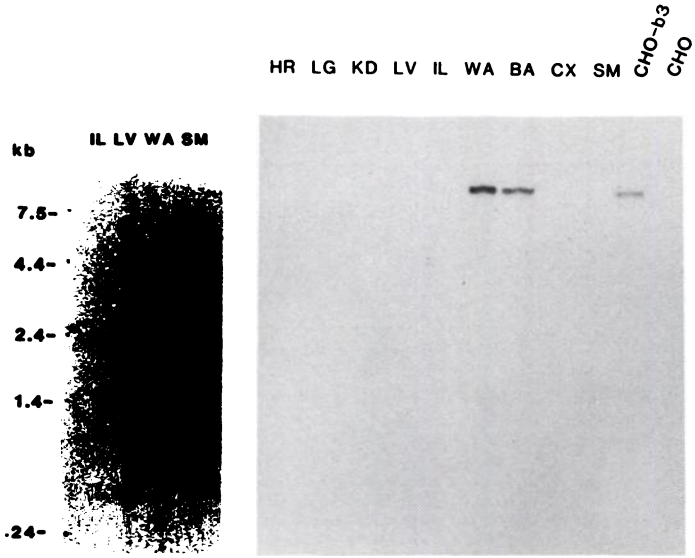

Fig. 4. Tissue distribution of β_3 mRNA in rat tissues. *Left*, Northern blot analysis of poly(A)$^+$ RNA. *IL*, ileum, 17.8 μg; *LV*, liver, 11.9 μg; *WA*, white adipose tissue, 1.3 μg; *SM*, skeletal muscle, 0.6 μg. *Right*, RNase protection assay of total RNA. *HR*, cardiac left ventricle, 50 μg; *LG*, lung, 50 μg; *KD*, kidney, 50 μg; *IL*, ileum, 50 μg; *WA*, white adipose tissue, 15 μg; *BA*, BAT, 15 μg; CHO-b_3 cells, 5 μg; CHO cells, 5 μg; *CX*, cerebral cortex.

Our work indicates that the pharmacological sensitivities of the cloned rat β_3 receptor are similar to those exhibited by atypical receptors in rat BAT. Comparisons of the relative potencies of agonists provide the best basis for assessing agonist action among cells that may exhibit large differences in the level of receptor expression (23, 24). As illustrated in Table 1, the relative potencies of agonists for stimulation of adenylyl cyclase in CHO-rat β_3 cells are virtually identical to those observed for atypical receptors in rat BAT. Additionally, CGP 12177 is a partial agonist, with 50% intrinsic activity, in membranes of both BAT and CHO-rat β_3 cells (4, 6). Finally, the typical β receptor antagonists CGP 20712A and ICI 118,551 do not block atypical receptors in BAT (4) or CHO-rat β_3 cells (Table 1). These observations strongly indicate that the rat β_3 receptor accounts for most, if not all, of the atypical properties of β receptors observed in adipose tissue.

Whether norepinephrine stimulates β_3 receptors in BAT was recently questioned by studies showing that norepinephrine-stimulated responses were potently inhibited by β_1-selective antagonists, but BRL 37344-stimulated activity was not (4, 6). The present work, however, clearly demonstrates that norepinephrine does stimulate adenylyl cyclase via the rat β_3 receptor. The reason norepinephrine acts principally through β_1 receptors in tissues containing both β_1 and β_3 receptors is probably due to the relatively low affinity of norepinephrine for the rat β_3 receptor (Table 1). The K_{act} of norepinephrine for β_3 receptor-stimulated adenylyl cyclase activity in adipose tissue membranes is about 40 μM, whereas its affinity for the β_1 receptor is about 100 times higher (4, 25). Thus, activation of adenylyl cyclase by catecholamines conforms to the β_1 subtype when both receptors are present in sufficient amounts to fully activate adenylyl cyclase.

Although the pharmacological sensitivities of the cloned rat β_3 receptor were very similar to those of atypical receptors in BAT, they were different from those reported for the human β_3 receptor (3). For example, the human β_3 receptor was reported to have a high and nearly equal affinity for BRL 37344, norepinephrine, and isoproterenol (3). In contrast, the potency of norepinephrine at the rat β_3 receptor is about 8 times less than that of isoproterenol and 75 times less that of BRL 37344. In addition to differences in rank order of agonist potencies, we have found that numerous adrenergic compounds that have been reported to interact with the human β_3 receptor (3) have no activity at the rat receptor, and vice versa. For example, CGP 12177 and alprenolol were each partial agonists of the rat β_3 receptor, yet neither was found to interact with the human β_3 receptor (3). Conversely, ICI 118,551, CGP 20712, and metoprolol each have been reported to block the human β_3 receptor, yet none of these compounds blocks or stimulates activity in CHO-rat β_3 cells. Finally, the potency and efficacy of pindolol for stimulation of adenylyl cyclase were remarkably low.

We also investigated β_3 receptor gene expression in various tissues with a sensitive, highly specific, nuclease protection assay, with a rat-specific probe. This analysis indicates that

the β_3 receptor is abundantly expressed only in adipose tissues. This is consistent with a recent commentary indicating that the human β_3 gene contains numerous fat-specific promoter elements (26). Of other tissues that are believed to contain atypical receptors (27, 28), only low levels of expression were found in the ileum and no transcripts were detected in skeletal muscle. These results contrast with a previous report (3) that appeared to indicate that the liver contains the greatest abundance of β_3 transcripts. Furthermore, the size of the major species of β_3 mRNA found in the present study (2.1 kb) was smaller than that previously reported. The reason for the discrepancy is uncertain; however, the sequence of the human probe that was used in the earlier work (encoding the cytoplasmic tail and containing a portion of the 3′ nontranslated region) has only 30% homology with the rat β_3 cDNA and thus would not be expected to hybridize specifically to rat β_3 mRNA.

In summary, the present study indicates that rats express a homolog of the human β_3 receptor in an adipose tissue-specific fashion. The cloned rat receptor is stimulated by the atypical agonist BRL 37344 and by relatively high concentrations of catecholamines. Despite the high degree of homology of the primary structures, the pharmacological properties of the rat β_3 receptor differed from those previously reported for the human homolog. However, the rat β_3 receptor appears to account for many of the atypical pharmacological properties of rat adipose tissue β receptors.

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