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Expression of β_3-adrenoceptor mRNA in rat tissues

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1 This study examines the expression of β_3-adrenoceptor messenger RNA (β_3-AR mRNA) in rat tissues to allow comparison with atypical β-adrenoceptors determined by functional and radioligand binding techniques.

2 A reverse transcription/polymerase chain reaction protocol has been developed for determining the relative amounts of β_3-AR mRNA in rat tissues.

3 Measurement of adipsin and uncoupling protein (UCP) mRNA was used to examine all tissues for the presence of white and brown adipose tissue which may contribute β_3-AR mRNA.

4 The β_3-AR mRNA is expressed at high levels in brown and white adipose tissue, stomach fundus, the longitudinal/circular smooth muscle of both colon and ileum, and colon submucosa. There was substantial expression of adipsin in colon submucosa and moderate expression in fundus, suggesting that in these regions at least some of the β_3-AR signal may be contributed by fat. Pylorus and colon mucosa showed moderate levels of β_3-AR mRNA with lower levels of adipsin. Ileum mucosa and submucosa showed low but readily detectable levels of β_3-AR.

5 Expression of adipsin in rat skeletal muscles coupled to very low levels of β_3-AR mRNA indicates that the observed β_3-AR may be due to the presence of intrinsic fat. β_3-AR mRNA was virtually undetectable in heart, lung and liver. These results raise the possibility that the atypical β-AR demonstrated by functional and/or binding studies in muscle and in heart is not the β_3-AR.

6 By use of two different sets of primers for amplification of β_3-AR cDNA, no evidence was found for differential splicing of the mRNA in any of the tissues examined.

7 The detection of β_3-AR mRNA in the gut mucosa and submucosa suggests that in addition to its established roles in lipolysis, thermogenesis and regulation of gut motility β_3-AR may subserve other functions in the gastrointestinal tract. The absence of β_3-AR mRNA in rat heart or its presence with adipsin in skeletal muscle suggests that atypical β-adrenoceptor responses in heart and skeletal muscle are unlikely to be mediated by β_3-AR.

Keywords: Messenger RNA; β_3-adrenoceptors; atypical β-adrenoceptors; adipsin; uncoupling protein; rat tissues

Introduction

Atypical β-adrenoceptors (β-ARs) have been demonstrated in a range of mammalian tissues by functional studies, radioligand binding and molecular biology techniques. In intact animals and *in vitro* studies, atypical β-ARs respond to novel lipolytic β-AR agonists such as BRL37344 and ICID7114, are resistant to blockade by conventional β-AR antagonists such as (-)-propranolol, and often display resistance to desensitization upon prolonged exposure to agonists (Arch & Kaumann,1993). Receptors with these functional characteristics have been described in brown and white adipose tissue (Harms *et al.*, 1977; Arch *et al.*, 1984, Hollenga & Zaagsma, 1989); skeletal muscle (Abe *et al.*, 1993; Liu & Stock, 1995), and multiple regions from the gastrointestinal tract (Coleman *et al.*, 1987; Bond & Clarke, 1988; Croci *et al.*,1988; Blue *et al.*, 1990; McLaughlin & MacDonald, 1990; Bianchetti & Manara, 1990; McLaughlin & MacDonald, 1991; Canfield & Paraskeva, 1992 ; Canfield & Abdul-Ghaffar, 1992; Roberts *et al.*, 1995; Cohen *et al.*, 1995). There is also some evidence for the participation of atypical β-ARs in responses from heart, lung and blood vessels (Kaumann, 1989; Arch & Kaumann, 1993; Kuratani *et al.*, 1994). The β_3-AR isolated from human, rat and mouse genomic or cDNA libraries and expressed in mammalian cells has been shown to possess the pharmacological properties expected of an atypical β-AR (Emorine *et al.*, 1989; Granneman *et al.*, 1991; Nahmias *et al.*, 1991; Blin *et al.*, 1994).

Isolation of the β_3-AR gene has provided a highly specific means of detecting the presence of the corresponding mRNA in tissues known to possess atypical β-ARs. In all species examined, β_3-AR mRNA is readily detectable in both brown and white adipose tissue (Granneman *et al.*, 1991; Nahmias *et al.*, 1991; Granneman *et al.*, 1993; Krief *et al.*, 1993). In man, expression has also been found in gallbladder, colon, ileum, and heart (Krief *et al.*, 1993; Granneman *et al.*, 1993), although the presence of significant levels of mRNA for uncoupling protein (UCP) in both ventricles and atria suggested that the observed cardiac expresion of β_3-AR may be due to intrinsic fat deposits (Krief *et al.*, 1993). β_3-AR mRNA is also found in the human neuroblastoma cell line SK-N-MC (Granneman *et al.*, 1992). Expression of β_3-AR mRNA was not detectable by the polymerase chain reaction (PCR) in human skeletal muscle, lung, liver, kidney, thyroid or lymphocytes (Krief *et al.*, 1993). Northern analysis of RNA from mouse tissues failed to detect any expression of β_3-AR mRNA in non-adipose tissues, including ileum and colon (Nahmais *et al.*, 1991). In the rat, Northern analysis and ribonuclease protection assay detected a low level of expression in ileum but no other non-adipose tissue (Granneman *et al.*, 1991), although subsequent studies have demonstrated high levels of β_3-AR mRNA in rat fundus (Granneman & Lahners, 1994; Cohen *et al.*, 1995).

There are clearly mammalian tissues in which atypical β-ARs have been demonstrated by functional or radioligand binding studies, but where no β_3-AR mRNA has yet been detected by various hybridisation or PCR approaches. Such inconsistencies may occur because the functional effects are mediated by another atypical β-AR, or because these effects can be mediated by very low levels of the authentic β_3-AR. The latter possibility may arise in situations where a functional

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response is elicited by receptors on a small sub-population of cells. To address these alternatives, we have used PCR to examine a range of rat tissues for the presence of β_3-AR mRNA. Because β_3-AR mRNA is highly abundant in rat adipose tissue (Granneman *et al.*, 1991), markers were developed for both white and brown adipose tissue to check for fat contamination of other tissues. Expression of the serine protease adipsin is confined to adipose tissue and to the myelin sheath of nerves (tissues active in lipid metabolism; Cook *et al.*, 1987). The use of UCP as a marker for brown adipose tissue (BAT) has been described previously in human tissues (Krief *et al.*, 1993). In addition, we measured expression of transferrin receptor as an indicator of the amount of starting RNA and the efficiency of RT/PCR, based on the assumption that for a given tissue this mRNA is present at a reproducible level. Depending on the number of cycles used, PCR can detect levels of mRNA which are so low that they are unlikely to be of any functional significance. To overcome reservations of this type, PCR conditions were established under which amplification of each β_3-AR, adipsin, UCP and transferrin receptor mRNA from BAT showed a progressive increase with cycle number. Levels of mRNA were compared from each tissue with that seen in BAT. Our results indicate localization of β_3-AR mRNA at sites consistent with observation of atypical β-AR responses in some tissues.

Methods

Animal tissues

All tissues were obtained from adult male Sprague Dawley rats (250–300 g). Following removal, the tissues were frozen in liquid nitrogen and stored at −70°C. Hearts were dissected in Krebs-Ringer solution at 4°C to give left atria, right atria ± the region containing the sino-atrial node, left ventricle plus associated major blood vessels, and right ventricle containing myocardial tissue only. The gastrointestinal tract was separated into gastric fundus and pylorus, intact colon or ileum, and colonic and ileal mucosa, submucosa and longitudual/circular smooth muscle. Other tissues isolated intact included interscapular brown adipose tissue; epididymal white adipose tissue, lung, liver and soleus, gastrocnemius and plantaris muscles. All non-adipose tissues were carefully trimmed of fat prior to freezing.

Preparation of RNA

Frozen tissue was ground to a fine powder in a stainless steel mortar and pestle pre-cooled in liquid nitrogen. Total RNA was extracted by the method of Chomczynski & Sacchi (1987). To avoid any cross-contamination, the homogenizer probe was dismantled and washed thoroughly between each sample. The yield and quality of the RNA were assessed by measuring absorbance at 260 and 280 nm and by electrophoresis on 1.2% agarose gels. Total RNA from each tissue was treated with DNase to remove contaminating genomic DNA. The reaction mix contained 20 μg RNA, 100 mM sodium acetate (pH 7.0), 5 mM $MgSO_4$, 5 mM dithiothreitol, 36 u RNasin (Promega), and 10 u DNase I (Pharmacia) in a total volume of 40 μl. Following digestion at 37°C for 30 min, the solution was diluted to 400 μl with H_2O and extracted with an equal volume of phenol:chloroform (1:1). The RNA was precipitated with 1.0 ml of ethanol and 40 μl of 2 M sodium acetate. The yield and quality of DNase-treated RNA were determined as above.

Reverse transcription/PCR

cDNAs were synthesized by reverse transcription of 1.0 μg of each total RNA using oligo $(dT)_{15}$ as a primer. The RNA in a volume of 7.5 μl was heated to 70°C for 5 min then placed on ice for 2 min prior to the addition of reaction mix containing 1 × RT buffer (supplied by Promega), 1 mM dNTPs, 5 mM $MgCl_2$, 18 u RNasin (Promega), 20 u AMV reverse transcriptase (Promega), and 50 μg ml^{-1} oligo$(dT)_{15}$ in a volume of 12.5 μl. Following brief centrifugation, the reactions were incubated at 42°C for 45 min, then at 95°C for 5 min. The completed reverse transcription reactions were stored at −20°C and used for PCR without further treatment.

PCR amplification was carried out on cDNA equivalent to 100 ng of starting RNA, using oligonucleotide primers specific for β_3-AR, adipsin, UCP, and transferrin receptor (Table 1; synthesized at the Howard Florey Institute, Melbourne or by Bresatec, Adelaide). PCR mixes contained 1 u of Taq polymerase (Promega), the buffer supplied (10 mM Tris-HCl (pH 9), 50 mM KCl and 0.1% Triton X-100), 200 μM dNTPs, 3.5 mM $MgCl_2$, 2.5 pmol of forward primer, 2.5 pmol of reverse primer and cDNA in a volume of 10 μl. A single reaction mix containing all components except the cDNA was prepared for the entire PCR experiment and aliquoted to minimize variations between samples. As well as sample cDNAs, each PCR experiment included a negative control consisting of an RT reaction containing no added RNA, and a positive control corresponding to BAT3 cDNA. PCR was carried out in an FTS-1 capillary thermal sequencer (Corbett Research. Lidcombe, New South Wales, Australia). Following initial heating of samples at 95°C for 2 min, each cycle of amplification consisted of 30 s at 95°C, 30 s at an annealing temperature appropriate for the primers used, and 30 s at 72°C. Individual annealing temperatures were 65°C for β_3-AR, 56°C for adipsin, 53°C for UCP and 64°C for transferrin receptor. Following amplification, PCR products were electrophoresed on 1.3% agarose gels and transferred onto Hybond N$^+$ membranes by Southern blotting in 0.4 M NaOH/1 M NaCl. The membranes were rinsed for 5 min in 0.5 M Tris-HCl (pH 7.5)/1 M NaCl, then in 2 × SSC (0.3 M NaCl/30 mM sodium citrate).

Detection and measurement of PCR products

The identity of the PCR products was verified by hybridization to independent probes (Table 1). Oligonucleotide probes (10 pmol) were end-labelled in a 10 μl reaction mix containing 15 μCi [γ-^{33}P]-ATP (2000 Ci mmol; Bresatec), 1 × One-Phor-All Plus buffer (Pharmacia), and 10 u T4 polynucleotide kinase (Pharmacia). Following incubation at 37°C for 30 min, reactions were heated at 90°C for 2 min and diluted to 100 μl with H_2O. The labelled probes were separated from unincorporated nucleotide by centrifugation through Chroma-spin 10 columns (Clontech), according to the manufacturers instructions. PCR products were fixed to nylon membranes by exposure to u.v. light for 2 min, and were then pre-hybridized for 2 h at 42°C in a buffer containing 5 × SSC, 0.5% SDS, 100 μg ml^{-1} herring sperm DNA, 5 × Denhardts solution and 0.1 mM ATP. Following addition of labelled oligonucleotide probe (equivalent to 5 pmol), hybridization was carried out at 42°C for 16 h. The filters were washed in 2 × SSC/0.1% SDS, twice at room temperature for 5 min, then twice at 42°C for 5 min. Radioactivity was detected with a Molecular Dynamics Phosphorimager (SI) after exposure to imaging plates for the times shown in Figure 2.

In the experiments described in Figure 1 and Table 2, the reverse primer was end-labelled prior to PCR. The reaction mix contained 250 pmol of oligonucleotide, 250 μCi [γ-^{33}P] ATP, 1 × One-Phor-All Plus buffer (Pharmacia), and 70 u T4 polynucleotide kinase (Pharmacia) in a volume of 100 μl. Incubation and purification of the oligonucleotide were as described above. PCR reactions were carried out with 2.5 pmol of the labelled reverse primer. Following transfer of the PCR products as above, Hybond N$^+$ membranes were dried at room temperature for 30 min, then apposed directly to phosphorimager plates. Exposure times for all quantitative experiments were 8 h.

Results

Design of oligonucleotide primers for PCR

In developing PCR methods for this study, it was found that the design of primers was of critical importance. The programme 'PRIMER' (Version 0.5, Whitehead Institute for Biomedical Research; 1991) was used to choose possible primer pairs, based on matching T_m values and other criteria such as length, GC content, and low complementarity. Primers were then checked against the Genbank database using the programme 'BLAST' (Altschul *et al.*, 1990) to ensure that there was no significant match with other known sequences. In particular, we chose β_3-specific primers with at least 25% mismatch to rat β_1-AR and β_2-AR sequences. Despite the above precautions, amplification of β_3-AR cDNA using 3 different sets of primers with T_m values of 60°C gave a high proportion of spurious products. This was exacerbated if the β_3-specific reverse primer was also used for the reverse transcription step, possibly because the effective annealing temperature during reverse transcription is only 42°C, leading to greater levels of mispriming. To obtain the maximum possible specificity, we carried out all reverse transcription reactions with oligo$(dT)_{15}$ as a primer, and in the case of β_3-AR cDNA amplification, chose PCR primers (b3.514 and b3.1030) with T_m values of 72°C and 70°C respectively (Table 1). The transferrin receptor primers were chosen using PRIMER and BLAST, also with high T_m values. The adipsin primers (rad.167 and rad.755) were found to give single amplified products at a PCR annealing temperature of 56°C. UCP primers were based on those used by Dr D. Ricquier and coworkers (Dr M.A. Cawthorne, personal communication). PCR using the β_3-AR, adipsin, UCP and transferrin receptor primers gave single products consistent with the expected sizes of 517 bp, 589 bp, 940 bp and 394 bp respectively.

Relationship between number of PCR cycles and amount of product formed

It was found previously that 35 cycles of PCR resulted in amplification of a substantial amount of β_3-AR product from tissues such as rat soleus muscle (Summers *et al.*, 1995). To compare expression in soleus muscle with that in BAT, PCR was carried out on cDNA from the equivalent of 200 ng RNA, with cycle numbers varying from 24 to 36 (data not shown). This experiment indicated that whereas BAT β_3-AR amplification reached a plateau at 31–32 cycles, the soleus muscle β_3-AR PCR product was still increasing at 36 cycles. This means that at 35 cycles the amount of soleus β_3-AR product is overestimated relative to that from BAT. In the present study, the amplification of BAT cDNA (equivalent to 100 ng starting RNA) was measured with each set of primers over a varying number of cycles (Figure 1). The experiment was done 3 times for amplification of β_3-AR to ensure that the time course was reproducible, but only once for adipsin, UCP and transferrin receptor. The amount of β_3-AR product increased up to 30 cycles, reaching a plateau at 34 cycles; 30 cycles was chosen for subsequent β_3-AR amplification, since this provides a maximal scale over which to measure expression in other tissues. A similar rationale was used to choose the cycle numbers for each set of primers, namely 24 cycles for adipsin, 30 for UCP and 32 for transferrin receptor.

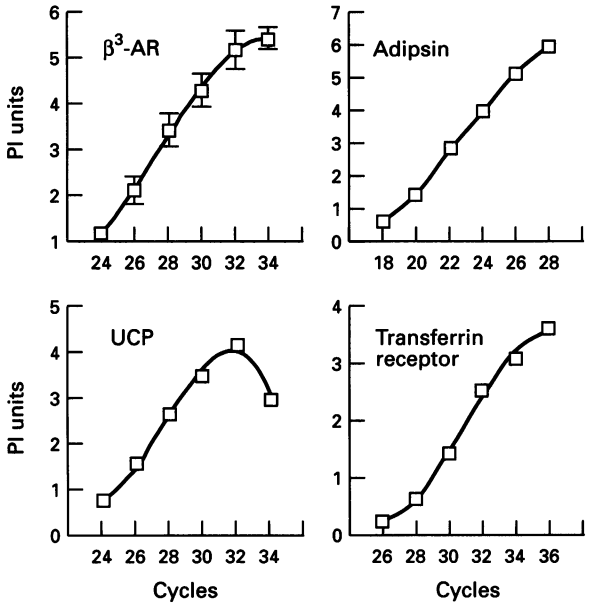

Figure 1 Time course of cDNA amplification. The reverse primers were labelled prior to PCR so that the amount of product formed could be quantitated directly. Following amplification for the number of cycles shown, the radiolabelled PCR products were electrophoresed and transferred to nylon membranes. Signals were imaged and quantitated using ImageQuaNT software (Molecular Dynamics). PI units are the total pixel values (volumes) generated by analysis of bands under Volume Report. Points on the graph for amplification of β_3-AR represent the mean±s.e. mean from three separate PCR experiments. Lines were fitted using a polynomial function.

Table 1 Oligonucleotides used as PCR primers and hybridization probes

Name	*Length*	*Strand*	*Sequence* (5'→3')	T_m (°C)	*Location*
Primers					
b3.514	25	for	TAGTCCTGGTGTGGATCGTGTCCGC	72.2	S73473: 514–538
b3.1030	25	rev	GCGATGAAAACTCCGCTGGGAACTA	70.0	S73473: 1006–1030
b3.1237	25	rev	CGCTCACCTTCATAGCCATCAAACC	68.7	S73473: 1213–1237
rad.167	19	for	ATGAGCAGTGGGTGCTGAG	60.0	M92059: 167–185
rad.755	20	rev	AGAACGTTTTCAATCCACGG	60.0	M92059: 736–755
rUCP.180	20	for	ATGGTGAGTTCGACAACTTC	55.1	M11814: 180–199
rUCP.1102	20	rev	TATGTGGTGCAGTCCACTGT	57.5	M11814: 1083–1102
rTR.204	30	for	CAGGTAGTAACATTGACCCAGTGGAGGCTC	70.8	M58040: 204–233
rTR.598	30	rev	GATCGTCTGCACAGGTATAGAAGGCAATCC	70.7	M58040: 569–598
Probes					
rb3.pro	21	+	ACGGCATCGGATGGAGTGCCC	72.7	S73473: 840–860
rad.pro1	20	+	CCCTTGCAACGCGAGGACCG	72.4	M92059: 400–419
rUCP.pro	27	—	GACTTTGGCGGTGTCCAGCGGGAAGGT	77.8	M11814: 270–296

[a] T_m determined by 'PRIMER' (Version 0.5, Whitehead Institute).
[b] Genbank accession number and nucleotide numbers within corresponding entry.

Identification of PCR products

To verify that amplification was specific to each target mRNA, a full set of PCR reactions was carried out with unlabelled primers and the PCR products were then probed with oligonucleotides independent of either primer (Table 1). As shown in Figure 2, bands corresponding to β_3-AR and adipsin were observed in a wide range of rat tissues, whereas UCP was seen primarily in BAT. RNA used in these experiments was treated with DNase to remove any traces of contaminating genomic DNA. As an additional verification that the signals obtained for β_3-AR were derived exclusively from mRNA, we carried out a parallel PCR experiment using the b3.514 forward primer and a reverse primer (b3.1237, Table 1) which corresponds to the coding region immediately 3′ to intron 1 of the rat β_3-AR gene. The major PCR bands seen for all positive tissues are consistent with the predicted size of 724 bp, and the intensities parallel those obtained using the b3.1030 reverse primer. Fragments derived from contaminating genomic DNA would have been 1120 bp due to inclusion of the first intron (Granneman *et al.*, 1992).

Levels of β_3-AR, adipsin, UCP and transferrin receptor mRNA in rat tissues

In order to compare the levels of β_3-AR and adipsin mRNA among rat tissues, RNA was extracted from a number of animals. PCR was carried out with labelled reverse primers, and the amount of product was quantitated by phosphorimaging. Each PCR experiment included BAT3 cDNA from a single batch reverse transcription as a positive control. This enabled correction for differences between experiments due to factors such as the specific activity of the labelled primers and the activity of a particular batch of Taq polymerase. All values from a particular experiment were expressed as a percentage of that obtained for BAT3 in that experiment, but no other corrections were made. As shown in Table 2, this protocol gave reasonable consistency on tissues from up to 6 animals. We did not apply any corrections for the level of transferrin receptor mRNA, as this varies by approximately 6 fold among the tissues. We believe that this variation reflects genuine differences in expression of the transferrin receptor gene rather than differences in the input cDNA, since the levels of transferrin receptor mRNA within a given tissue and also between related tissues were in generally good agreement.

In addition to BAT and white adipose tissue (WAT), expression of β_3-AR mRNA was found in all gastrointestinal tissues examined. The finding of β_3-AR mRNA in fundus at a level comparable to that in adipose tissue is consistent with the results of Cohen *et al.* (1995). Regions with even higher β_3-AR mRNA expression were the longitudinal and circular smooth muscle of colon and ileum, while low levels of mRNA were observed in pylorus, colon mucosa and ileum mucosa and submucosa. Most parts of the gastrointestinal tract had low levels of adipsin mRNA, with the exception of colon submucosa. The high level of β_3-AR mRNA seen in this region may be due largely to the presence of fat associated with nerves or blood vessels. The three skeletal muscles had detectable

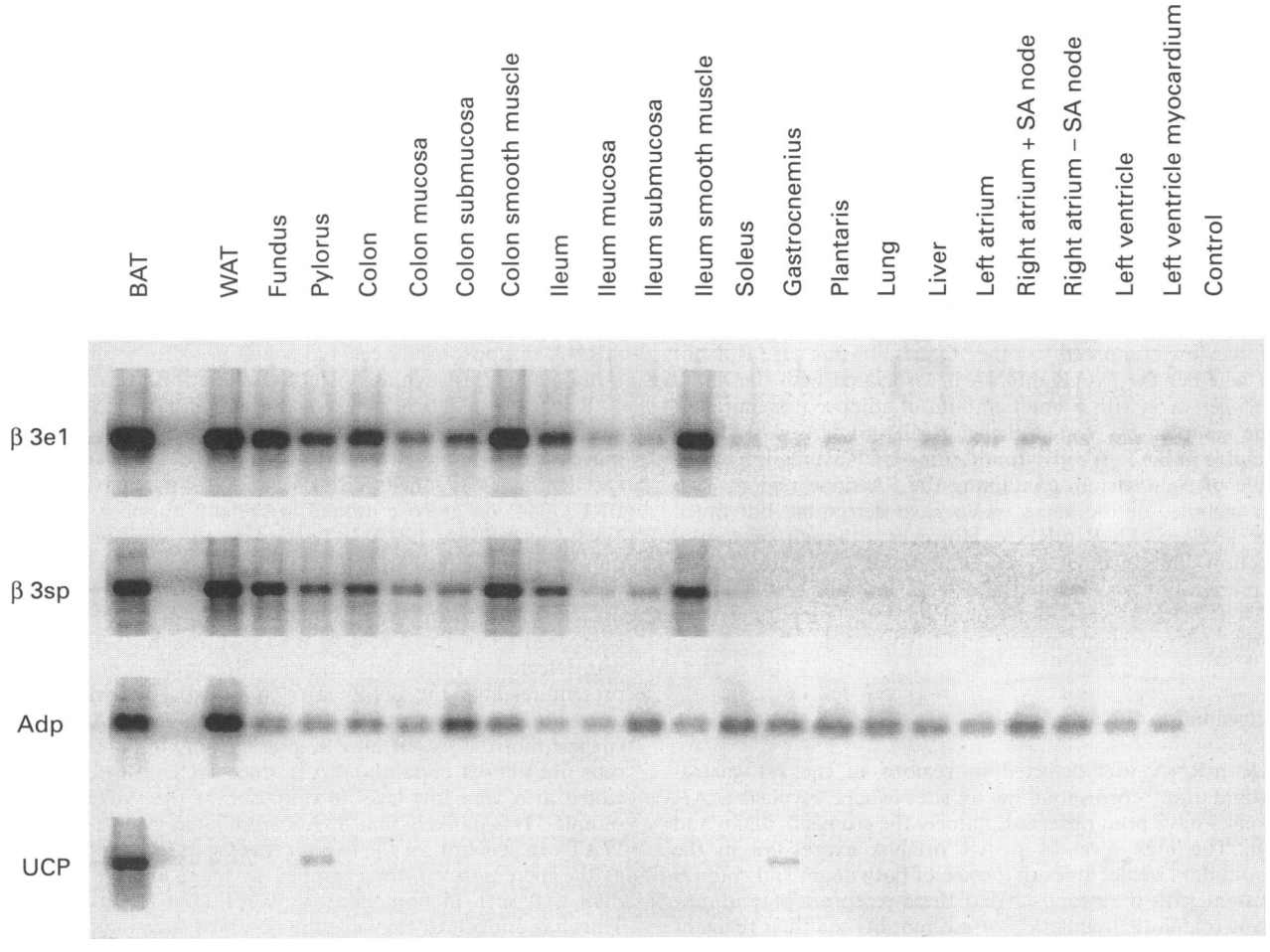

Figure 2 Expression of β_3-AR, adipsin, and UCP in rat tissues. RT/PCR was done using the following unlabelled primers: β3 el, b3.514 and b3.1030; β3 sp, b3.514 and b3.1237; Adp, rad.167 and rad.755; UCP, rUCP.180 and rUCP.1102. Following electrophoresis and transfer to nylon membranes, the identity of the PCR products was verified by hybridization to independent probes (Table 1). Sizes of the products were determined from ethidium bromide stained gels by comparison with 100 bp DNA ladder (Pharmacia). The blots were exposed to phosphorimager plates for 5 days prior to scanning. Products produced using each set of primers within one PCR experiment were blotted onto a single membrane, and no modifications of the images between samples were made.

Table 2 Levels of mRNA in rat tissues

Tissue	*β_3-AR*		*Adipsin*		*UCP*	*TR*	
BAT	61±16	(4)	79±15	(4)	98(2)	57±24	(4)
WAT	104±12	(4)	105±14	(4)	ND	45±11	(4)
Fundus	74±6	(3)	15±5	(3)	ND	157±4	(3)
Pylorus	14±3	(3)	6.0±3.3	(3)	0.81(2)	178±8	(3)
Colon	41±8	(3)	6.4±2.9	(3)	ND	130±36	(3)
Colon mucosa	7.2±2.4	(3)	1.7±1.0	(3)	ND	151±16	(3)
Colon submucosa	43±9	(3)	33±7	(3)	ND	67±6	(3)
Colon sm muscle	111±37	(3)	4.9±1.3	(3)	ND	31±4	(3)
Ileum	26±4	(3)	4.7±1.5	(3)	ND	132±22	(3)
Ileum mucosa	1.5±0.4	(3)	1.5±0.4	(3)	ND	50±17	(3)
Ileum submucosa	1.8±0.3	(3)	2.2±0.6	(3)	ND	33±1	(3)
Ileum sm muscle	134±15	(3)	5.7±2.7	(3)	ND	67±10	(3)
Soleus	1.8±0.9	(3)	20±8	(3)	ND	164±23	(3)
Gastrocnemius	0.7	(2)	12	(2)	0.31(2)	170	(2)
Plantaris	0.7±0.5	(3)	15±8	(3)	ND	165±19	(3)
Lung	0.23±0.18	(3)	9.5±2.6	(3)	ND	108±15	(3)
Liver	0.23±0.17	(3)	4.8±1.6	(3)	ND	101±32	(3)
Left atrium	ND		1.7±0.5	(6)	ND	112±13	(6)
Right atrium+SA	ND		3.1±1.3	(3)	4.4±3.7(3)	77±14	(3)
Right atrium–SA	ND		3.1±0.6	(3)	ND	88±26	(3)
Left ventricle	0.33±0.15	(6)	1.7±0.4	(5)	0.09(2)	139±13	(5)
Myocardium	ND		1.6±0.4	(6)	ND	137±8	(6)

ND: not detectable.
Values from each PCR experiment were expressed as a percentage of the BAT3 positive control from the same experiment. These percentages were then used to calculate the mean ± s.e.mean. Numbers in parentheses are the number of tissues from separate animals analysed using each set of PCR primers.
Figures for BAT are not 100% because the level of each mRNA present in additional samples of BAT varied relative to that present in BAT3 (set at 100%).

expression of β_3-AR mRNA, however these tissues also had moderate amounts of adipsin mRNA. Thus the small amount of β_3-AR mRNA found in rat skeletal muscle may be associated with intrinsic fat cells which are dispersed amongst the muscle fibres (Dubowitz, 1981). Lung and liver had very low expression of β_3-AR mRNA, which again can probably be accounted for by the presence of fat. In the heart, β_3-AR mRNA was not detectable in atria or in right ventricular myocardium. There was a low level of β_3-AR mRNA in samples of left ventricle which contained major blood vessels. Although these samples also contained adipsin mRNA, the level was low compared to other tissues. In this case it is not clear whether the β_3-AR mRNA is associated with the blood vessels *per se* or with a small amount of adipose tissue present in the samples. As stated earlier, UCP mRNA was readily detectable in BAT. We also found some UCP expression in one sample of right atrium containing the SA node region. Two other samples of the same tissue gave detectable but much lower levels of UCP mRNA. However although one atrial dissection contained UCP, the amount of β_3-AR mRNA in this preparation was below the detectable limit. Weak signals for UCP mRNA were also found in single preparations of pylorus, gastrocnemius muscle and left ventricle.

Discussion

β_3-AR mRNA was detected in regions of the rat gastrointestinal tract corresponding to sites where atypical β-AR responses have been observed, namely the stomach, ileum and colon. The high level of β_3-AR mRNA expression in the longitudinal/circular smooth muscle of both ileum and colon is consistent with the proposal that these receptors play an important role in the regulation of gut motility via their relaxant effects (Croci *et al.*, 1988; Bianchetti & Manara; 1990; McLaughlin & MacDonald, 1990; van der Vliet *et al.*, 1990; Growcott *et al.*, 1993; Roberts *et al.*, 1995). In the ileum it is predominantly the smooth muscle which contains β_3-AR mRNA, whereas in colon the mucosa contains readily detectable mRNA without significant levels of adipsin. This finding may indicate that the β_3-AR participates in secretory processes in the colon as observed for rat caecum (Canfield & Abdul-Ghaffar, 1992). A dual role may also exist for the β_3-AR in rat stomach, since atypical β-ARs have been shown to mediate both acid secretion (Canfield & Paraskeva, 1992) and relaxation of the fundus (McLaughlin & MacDonald, 1991; Cohen *et al.*, 1995). High levels of β_3-AR mRNA expression were observed in stomach fundus and also moderate levels in the pylorus. Further correlation between the functional effects mediated by atypical β-ARs in the stomach and other regions of the gastrointestinal tract and the localization of β_3-AR mRNA in appropriate cell types will need to be investigated using techniques such as *in situ* hybridization histochemistry.

Roberts *et al.* (1993) showed the presence of ICYP binding sites with the characteristics of atypical β-ARs in rat soleus muscle. In addition, two other studies have reported effects of specific β_3-AR agonists (BRL35135A or its active product BRL37344) on glucose uptake in skeletal muscle (Abe *et al.*, 1993; Liu & Stock, 1995). In both cases, the effect on oxidative muscle such as soleus was greater than on the glycolytic parts typified by gastrocnemius or quadriceps muscles. Glucose uptake was also increased by 65% or 2 to 3 fold in WAT, compared to 10 to 12 fold in BAT. Although β_3-AR mRNA was detected in rat skeletal muscles (Summers *et al.*, 1995 and present results), the presence of substantial adipsin mRNA expression coupled with the very low values for β_3-AR mRNA suggest that the β_3-AR may be present only in fat cells. These cells are almost certainly WAT, since UCP mRNA was detected at a very low level in only one of the skeletal muscle samples. It is unlikely that β_3-AR stimulated glucose uptake in WAT can account for the overall effect seen in skeletal muscle, as the increase seen, for example, in soleus muscle is greater than that seen in homogeneous WAT (Liu & Stock, 1995). Thus it is difficult to reconcile the observed functional effects of β_3-AR agonists in rat skeletal muscle with our observations of very low levels of β_3-AR mRNA.

The same conclusions can be drawn about the heart. One line of evidence for atypical β-ARs in the rat heart is the finding that a series of β-AR partial agonists related to pindolol cause positive chronotropic effects at concentrations

greatly in excess of their affinities at β_1 and β_2-ARs (Kaumann *et al.*, 1979; Kaumann, 1989). Mediation of these effects would presumably be via receptors present in the sinoatrial (SA) node. We were unable to detect any β_3-AR mRNA in this region of the heart at the level of PCR amplification used in this study, indicating that expression of β_3-AR mRNA in right atrium/SA node is at least 250 fold lower than in BAT (the lower limit of detection). As in the study of Krief *et al.* (1993), the possibility that the β_3-AR is present in a small number of specialised pacemaker cells cannot be ruled out. Again this could be assessed only by high resolution *in situ* hybridization. Evidence for a role of atypical β-ARs in stimulating glucose uptake in heart is conflicting, with one study showing a 2 fold increase in response to a β_3-AR agonist (Abe *et al.*, 1993), and the other a 44% decrease (Liu & Stock, 1995). Even if agonists such as BRL37344 have a measurable effect on glucose uptake in the heart, this cannot be mediated via existing β_3-ARs as their overall expression is far too low.

It was demonstrated (Figure 2) that amplification of β_3-AR cDNA using primers which span the first intron gave results which closely paralleled those obtained using forward and reverse primers within exon 1. As well as verifying that PCR fragments were not derived from any contaminating genomic DNA, these results indicate that splicing of the β_3-AR transcript follows the same pattern in BAT and all other tissues examined. Our reverse b3.1237 primer (Table 1) essentially acts as a marker for the presence of the final 12 amino acids encoded by exon 2 (Granneman *et al.*, 1992; Bensaid *et al.*, 1993). The results provide evidence that the C-terminal tail of the β_3-AR is invariant between tissues, and hence that under the conditions of this experiment the receptor would lack the phosphorylation sites which are found in the C-terminal tail of the β_1 and β_2-ARs and contribute to receptor desensitization (Bouvier *et al.*, 1988; Hausdorff *et al.*, 1989; Emorine *et al.*, 1991). This conclusion is of interest given that patterns of desensitization of the β_3-AR vary between tissues. For example, in WAT the β_3-AR appears to be resistant to acute desensitization (Granneman, 1992; Carpene *et al.*, 1993), whereas rapid desensitization does occur in stomach and caecum (Canfield & Paraskeva, 1992; Canfield & Abdul-Ghaffar, 1992). Our results are consistent with those of Chaudry & Granneman (1994), who showed that transfection of the authentic β_3-AR cDNA into different cell lines gave rise to varying degrees of acute desensitization, and concluded that resistance to desensitization is not intrinsic to the β_3-AR. Thus regulation of a single β_3-AR appears to occur via differing mechanisms depending on the cellular environment.

In conclusion, the expression of β_3-AR mRNA in rat tissues has been examined together with that of the adipose tissue marker, adipsin and the BAT marker, UCP. β_3-AR mRNA was expressed at high levels in WAT, BAT, stomach fundus, smooth muscle of colon and ileum and colon submucosa, moderate levels in pylorus and colon mucosa, and low but readily detectable levels in ileum mucosa and submucosa. In rat skeletal muscles low levels of β_3-AR mRNA expression were associated with expression of adipsin mRNA and were therefore probably due to intrinsic fat. β_3-AR mRNA was virtually undetectable in heart, lung and liver. In addition to its established roles in lipolysis, thermogenesis and regulation of gut motility the detection of β_3-AR mRNA in the gut mucosa and submucosa suggests that the receptor may subserve other functions in these regions.

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