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SPECIAL REPORT

Expression of β_3-adrenoceptor mRNA in rat brain

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The reverse transcription/polymerase chain reaction was used to demonstrate β_3-adrenoceptor mRNA in rat brain regions. Levels were highest in hippocampus, cerebral cortex and striatum and lower in hypothalamus, brainstem and cerebellum.

Keywords: β_3-Adrenoceptor; rat brain; polymerase chain reaction

Introduction In rat brain, β_1-adrenoceptors are present in cerebral cortex, striatum and hippocampus whereas β_2-adrenoceptors are found mainly in cerebellum and hippocampus. *In situ* hybridisation localisation of β_1- and β_2-adrenoceptor expression showed β_1-mRNA in cerebral cortex, dentate gyrus, pineal gland, and a variety of nuclei in the brain and spinal cord, and of β_2-mRNA in olfactory bulb, piriform cortex, dentate gyrus, and thalamic nuclei (Nicholas *et al.*, 1993). Neither probe detected β-adrenoceptor mRNA in striatum. There is sparse evidence for β_3-adrenoceptors in rat brain but the β_3-adrenoceptor agonist pro-drug SR 58611A is active in animal models of depression (Simiand *et al.*, 1992). This study utilises the reverse transcription/polymerase chain reaction to demonstrate β_3-adrenoceptor mRNA in regions of rat brain.

Methods *Tissue dissection and RNA preparation* Male Sprague-Dawley rats (200-250 g) were anaesthetized, brains were removed, regions dissected (Glowinski & Iversen, 1966) and pulverised in a stainless-steel anvil cooled in liquid N_2 and RNA extracted (Chomczynski & Sacchi, 1987). RNA yield and quality were assessed by absorbance measurements and by gel electrophoresis, and samples were DNase treated to remove contaminating genomic DNA.

Reverse transcription/PCR RNA (1 μg) was reverse transcribed using oligo $(dT)_{15}$ and AMV reverse transcriptase (Promega). PCR amplification utilised cDNA (from 100 ng RNA) and oligonucleotide primers based on Genbank entries for either β_3-adrenoceptors (forward 514-538; reverse 1006-1030 or intron spanning 1213-1237), adipsin (forward 167-185; reverse 736-755), uncoupling protein (UCP) (forward 180-199; reverse 1083-1102) and transferrin receptor (forward 204-233; reverse 569-598). PCR mixes contained 2.5 pmol of primers, Taq polymerase (Promega), the buffer supplied, 3.5 mM $MgCl_2$, 200 μM dNTP's, and cDNA in 10 μl. Each experiment also contained a negative control (RT reaction containing no added RNA) and a positive control (cDNA from brown adipose tissue-BAT). Following heating at 95°C for 2 min, amplification cycles were 30 s at 95°C, 30 s anneal, 30 s extension at 72°C. Annealing temperatures/cycle numbers were 65°C/30 for β_3-AR, 56°C/24 for adipsin, 53°C/30 for UCP and 64°C/32 for transferrin receptor. PCR products were separated by gel electrophoresis on 1.3% agarose and transferred onto Hybond N^+ (Amersham).

Detection and measurement of PCR products PCR products were fixed to nylon membranes (u.v. light 2 min) and hybridised at 42°C to β_3-adrenoceptors (840-860), adipsin (400-419) or UCP (270-296) probes end-labelled with [γ-^{33}P]-ATP (2000 Ci mmol^{-1}; Bresatec) and T4 polynucleotide kinase (Pharmacia). Filters were washed in 2 × SSC/0.1% SDS at 42°C. In some experiments, reverse primers were end labelled as above and after transfer of labelled PCR products, Hybond N^+ membranes were dried and radioactivity detected using a Molecular Dynamics SI phosphorimager.

Results *Verification of PCR products* Bands of an appropriate size were produced for mRNA coding for β_3-adrenoceptors, adipsin and UCP in the BAT control and in brain regions that expressed these genes (Figure 1). Parallel experiments were also conducted using the β_3-adrenoceptor forward primer and a reverse primer corresponding to the coding region 3′ to intron 1 of the rat β_3-adrenoceptor gene. The PCR

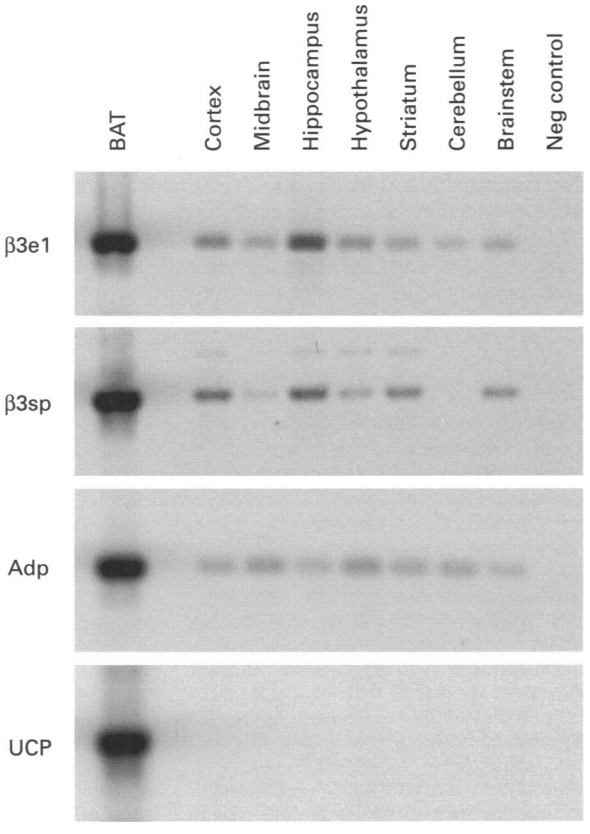

Figure 1 Expression of β_3-adrenoceptors, adipsin, and uncoupling protein (UCP) in rat brain regions. RT/PCR utilised the unlabelled primers: β3e1 (β_3-adrenoceptor exon 1), β3sp (β_3-adrenoceptor intron-spanning), Adp (adipsin), UCP (uncoupling protein). Following electrophoresis and transfer to nylon membranes, the PCR products were identified by hybridisation to independent probes. Product sizes were determined by comparison with a 100 bp DNA ladder (Pharmacia).

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Table 1 Levels of mRNA in rat brain relative to brown adipose tissue (BAT)

	β_3-*Adrenoceptor*	*Adipsin*	*TR*
BAT	100	100	100
Cortex	3.1±0.7 (5)	1.7±0.5 (5)	109±24 (5)
Midbrain	1.5±0.6 (3)	1.4±0.7 (3)	110±17 (3)
Hippocampus	5.6±1.5 (4)	1.5±0.6 (4)	123±8 (4)
Hypothalamus	0.83±0.25 (4)	2.2±0.5 (4)	99±7 (4)
Striatum	2.3±1.0 (4)	1.5±0.5 (4)	120±10 (4)
Cerebellum	0.13±0.07 (5)	1.3±0.3 (5)	139±10 (5)
Brain stem	0.18±0.11 (3)	0.76±0.28 (3)	112±19 (3)

Values are expressed as %BAT control from the same experiment. These percentages were then used to calculate the mean±s.e.mean (*n*). TR: gene transferrin receptor.

products were of the expected size (724 bp) for mRNA (Figure 1) and not from contaminating genomic DNA.

Measurement of mRNA in regions of rat brain mRNA coding for β_3-adrenoceptors, adipsin, UCP and transferrin receptor was measured using labelled reverse primers. All values are expressed as % BAT cDNA in the same experiment but no other corrections were made. The levels of β_3-adrenoceptor mRNA were highest in hippocampus > cortex > striatum > midbrain > hypothalamus > brainstem > cerebellum (Table 1). The ratio of levels in hippocampus:cerebellum was 42 fold. Low levels of adipsin mRNA were found in all areas, probably associated with the myelin sheath of nerves (Cook *et al.*, 1987), and did not correlate with the levels of β_3-adrenoceptor mRNA. The ratio of levels in hypothalamus:brainstem was 2.9 fold. The BAT marker UCP was not measurable in any region but clearly visible in the positive control. Levels of the housekeeping gene transferrin receptor (TR) were highly reproducible within regions and showed only small differences between regions (range 99 – 139%).

Discussion β_3-Adrenoceptor mRNA was detected in hippocampus, cortex and striatum using a RT/PCR method. The levels are low compared to those in BAT but did not result from genomic DNA since the signal was unaffected by DNase treatment and the product size using intron-spanning primers indicated that it was derived from RNA. Low signals from the fat marker adipsin and different distribution indicated that the β_3-adrenoceptor mRNA signal was not derived from fat contamination. Although early studies in man failed to detect β_3-adrenoceptor mRNA in brain (Berkowitz *et al.*, 1995), a recent report indicates low levels of β_3-adrenoceptor mRNA in several brain regions and markedly (100 fold) higher levels in infants (Rodriguez *et al.*, 1995). It remains to be established whether expression of β_3-adrenoceptors in rat hippocampus, cortex and striatum reflects a generalised low level of expression or as is the case for β_1- and β_2-adrenoceptor mRNA, expression that is highly localised to particular structures (Nicholas *et al.*, 1993). Functional evidence for β_3- or atypical β-adrenoceptors in the CNS has been demonstrated with the β_3-adrenoceptor agonist pro-drug SR58611A which is active in several animal models of depression including antagonism of apomorphine or reserpine induced hypothermia, potentiation of yohimbine toxicity and reversal of learned helplessness (Simiand *et al.*, 1992). The effects were not antagonized by subtype-selective β-adrenoceptor antagonists and only by high doses of the non-selective antagonists alprenolol and propranolol. These characteristics are those of an atypical or β_3-adrenoceptor (Arch & Kaumann, 1993). The finding that β_3-adrenoceptor mRNA is present in rat cortex and hippocampus lends support to this functional evidence. The gross distribution of β_3-adrenoceptor mRNA in rat brain closely resembles that of β_1-adrenoceptor mRNA with high levels in cortex, hippocampus and low levels in cerebellum. The major difference is in the striatum which has β_3- but no β_1-adrenoceptor mRNA (Nicholas *et al.*, 1993).

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