Differential regulation of the expression of α_1 -adrenergic receptor subtype genes in brown adipose tissue

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The physiological control of the expression of the genes for the α_1 -adrenoceptor subtypes was examined in rat brown adipose tissue by analysing Northern blots of poly(A)-enriched RNA with oligonucleotide probes. In control rats, α_{1B} -receptor gene expression was much lower in brown adipose tissue than in liver, but the expression of both α_{1A} and α_{1D} was higher than in the heart, making brown adipose tissue one of the mammalian tissues with the highest expression of these subtypes. During acute exposure to cold, α_{1B} -receptor gene expression was essentially unchanged, α_{1A} -receptor gene expression was increased and α_{1D} -receptor gene expression was transiently decreased. Noradrenaline injection could mimic these effects of acute cold exposure, indicating that the physiologically induced up- and

down-regulations were due to the interaction of noradrenaline with cells within the tissue. In chronically cold-acclimated animals, α_{1B} -receptor gene expression was decreased but that of the α_{1A} -receptor gene remained at a level twice that of controls. α_{1D} -Receptor gene expression was also somewhat decreased. It is suggested that the enhanced expression of the α_{1A} -receptor gene explains the increased α_1 -receptor density in recruited brown adipose tissue reported previously. The intricate and differential regulation of α_1 -receptor gene expression and the markedly enhanced expression of the α_{1A} -receptor may imply that α_1 -receptors are important for the recruitment process or for maintenance of the recruited state in this tissue.

INTRODUCTION

On the basis of both pharmacological studies and analysis of expressed genes [1,2], at least three subtypes of α_1 -adrenergic receptors have been identified during recent years. The mere existence of these different subtypes poses questions about possible different functions and different mechanisms of regulation of expression, in different tissues and developmental states of cells and under different physiological conditions. In order to investigate whether physiologically induced chronic adrenergic stimulation influences the expression of the different α_1 -receptor subtype genes in a differential way, we have here examined the level of gene expression of all the known α_1 -receptor subtypes in brown adipose tissue, a tissue in which adrenergic stimulation has unique universal effects, both on acute cell function and on cell proliferation and differentiation [3–6].

Brown adipose tissue is the major site of non-shivering thermogenesis in mammals. The acute activity of the tissue is regulated by the sympathetic nervous system, with noradrenaline as the transmitter. When the tissue is physiologically activated, e.g. when the animal is in the cold or is exposed to certain diets, there is enhanced sympathetic activity and increased release of noradrenaline [7,8]. Noradrenaline then interacts with different adrenergic receptors in the tissue.

The thermogenic processes in the tissue are mainly stimulated via β -adrenergic pathways, with the β_3 -receptor being the β -receptor involved [9]. Thermogenesis results from activation of pathways that are similar to those well characterized in other tissues, namely an increase in cAMP and activation of hormone-sensitive lipase, leading to the release of fatty acids which are the substrates for thermogenesis and may also be involved in the

activation of the unique brown-adipose-tissue-specific uncoupling protein, thermogenin.

Despite the general notion that the main adrenergic receptor type involved in the acute thermogenic process is the β -receptor, the presence of α_1 -adrenergic receptors in membrane preparations from brown adipose tissue and in isolated brown adipocytes has been demonstrated by ligand-binding studies with [³H]prazosin [10,11]. That these receptors are coupled to intracellular processes is known from demonstrations of α_1 -stimulated IP₃ production [12], release of Ca²⁺ from intracellular stores [13], increase in cytosolic Ca²⁺ levels [14–16] and activation of Ca²⁺-dependent K⁺ channels [17,18] and Cl[−] channels [19,20]. However, only a small thermogenic effect of selective α_1 -adrenergic stimulation can be observed in isolated brown adipocytes from hamsters [21], and in rat brown adipocytes the α_1 -adrenergic component may be even smaller [22]. There are also only small positive effects of α_1 -adrenergic stimulation on the level of expression of the gene for the uncoupling protein in murine brown adipocytes in culture [3,23] and on the expression of the lipoprotein lipase gene in cultured rat brown adipocytes [24].

Despite these apparently modest effects of α_1 -adrenergic stimulation on the ultimate processes, α_1 -adrenergic pathways in the tissue may be physiologically important. Clear synergistic interaction between α_1 - and β -adrenergic stimulation has been reported for the thyroxine 5'-deiodinase enzyme [25], and synergistic α_1 - β interaction appears to be virtually obligatory for adrenergic induction of the expression of the *c-fos* proto-oncogene [16], which is presumed to be important for regulation of cell proliferation and differentiation. Further, there is a strong positive correlation between the density of α_1 -adrenoceptors and the degree of recruitment of the tissue [11]. In obese animals,

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where the tissue is in an atrophied state, there is a reduced density of α_1 -adrenoceptors, whereas in newborn, cafeteria-fed and cold-acclimated animals, where the tissue is in a recruited state, high densities of α_1 -adrenoceptors are found [11]. Thus the higher the degree of recruitment, the greater the increase in α_1 -adrenoceptor density. As recruitment is also associated with chronic adrenergic stimulation of the tissue, this increase in α_1 -receptors may be considered paradoxical, because chronic stimulation is generally expected to lead to desensitization and down-regulation of receptors. The functional significance of the changes in α_1 -adrenoceptor density in brown adipose tissue is not known, but the clear correlation with tissue recruitment state has motivated an investigation of the physiological control of the expression of α_1 -receptor subtype genes in this tissue.

The nomenclature of the α_1 -receptor subtypes is still somewhat complex. Two subtypes can be distinguished by pharmacological studies (often referred to as α_{1A} and α_{1B} subtypes), while three cDNAs have been cloned, originally referred to as α_{1B} , α_{1C} and α_{1D} [1]. There has been some controversy as to the relationship between the three cloned subtypes and the two subtypes defined by pharmacological studies of native receptors [1,26]. The basis for the nomenclature used here is the following. It has been demonstrated that the cloned α_{1B} -receptor corresponds to the native α_{1B} -receptor [1]; this receptor is also here referred to as the α_{1B} -receptor. The cloned α_{1C} -receptor was originally thought to be a distinct new subtype of α_1 -adrenoceptor [27] but it has been shown that it is equivalent to the pharmacologically defined α_{1A} -receptor [1,26,28,29]. We shall here refer to this subtype as the α_{1A} -receptor. The cloned α_{1D} -adrenoceptor was thought originally to correspond to the native α_{1A} -adrenoceptor [30] but it does appear to be a distinct subtype and does not correspond to the native α_{1A} -receptor [31]. We shall here refer to this subtype as the α_{1D} -receptor. Thus, with the nomenclature used here, no α_{1C} -receptor exists. This nomenclature is in agreement with that recommended by the Nomenclature Supplement of Trends in Pharmacological Sciences [32].

In the present study, we demonstrate for the first time that the genes for all three subtypes of α_1 -adrenoceptors are expressed in rat brown adipose tissue (the α_{1A} - and the α_{1D} -receptor genes are very highly expressed), and that the mRNA levels change dramatically in both acute and chronically cold-exposed rats, presumably regulated directly via adrenergic pathways.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing about 150 g, were obtained from a local supplier (Eklunds, Stockholm, Sweden). They were kept at 28 °C in single cages for 4–5 days, and had free access to food (a standard rat pellet diet, Ewos R3) and water. For the cold-exposure experiments, the animals were either kept at 4 °C (cold-exposed) or remained at 28 °C (controls). For the noradrenaline injection experiments, the animals were injected intraperitoneally with either noradrenaline [1 mg of (–)arterenol (Sigma) per kg body weight; dissolved in saline] or with saline only (control). The animals were kept at 28 °C throughout. After the treatments, the animals were killed with CO₂ at the time point indicated in the Figures, and interscapular brown adipose tissue was excised for preparation of total RNA and poly(A)-enriched RNA.

Oligonucleotide probes

The sequences of the 48- and 75-mer oligonucleotides synthesized for identification of α_{1B} - and α_{1D} -adrenoceptor mRNAs were

those described by Meister et al. [33]. The sequences were 5'-GCCGCTGTCATCCAGAGAGTCCTTCCGCGACTGTGA-TCTCTCCAGCGA-3' (α_{1B}) and 5'-CACGGAAGAGAAGAT-TGCATAGCCCACCTCCTCGGTGATGCCGCAGAAAC-GCTCATCCGGGGGCACTGGCTCCTT-3' (α_{1D}). The sequence of the 48-mer oligonucleotide synthesized for identification of α_{1A} -adrenoceptor mRNA was obtained by using the GCG software to analyse the sequence of the rat α_{1A} -adrenergic receptor reported by Stewart et al. [34]. The sequence decided upon was 5'-CACTGCTGACTCCGCCCTTCTGC-AGGGACATTTTTACGGTGGATGC-3'. This sequence was compared with all nucleotide sequences in the GenEMBL data bank; similarity scores for the closest non- α_{1A} -adrenoceptor sequences (which were prokaryote sequences) were less than half those against the reported α_{1A} -receptor sequences.

The oligonucleotide probes and oligo(dT)₁₈ were synthesized commercially (Innovagen). All probes were labelled at the 5' end with T4 polynucleotide kinase (Pharmacia) and [γ -³²P]ATP (5000 Ci/mmol; Amersham) and subsequently purified on Sephadex G-50 columns (Pharmacia).

Northern-blot analysis

Total and poly(A)-enriched RNA was obtained from heart, brain, liver and brown adipose tissue by using a total RNA/mRNA isolation kit (Biotex Laboratories Inc.). Routinely, only one cycle of poly(A) enrichment was run. The yield was normally approx. 10 µg of poly(A)-enriched RNA from 300 µg of total RNA.

For Northern-blot analysis, poly(A)-enriched RNA was denatured with formamide/formaldehyde/20 × SSC (20:7:2, by vol.) (where 1 × SSC is 0.15 M NaCl/0.015 M sodium citrate) and separated by electrophoresis on 1.25 % agarose containing 6.2 % formaldehyde (8–13 µg per lane). After electrophoresis, the RNA was transferred to a nylon membrane (Hybond-N; Amersham) by capillary blotting. After blotting, the membrane was fixed by UV cross-linking. Prehybridization was performed at 45 °C for 1–2 h in a solution containing 50 % formamide, 5 × SSC, 0.5 % SDS, 5 × Denhardt's and 50 mM sodium phosphate (where 1 × Denhardt's is 0.02 % Ficoll 400/0.02 % polyvinylpyrrolidone/0.02 % BSA). Hybridization was performed at 45 °C for approx. 16 h in the prehybridization solution with a ³²P-labelled oligonucleotide probe. The membranes were washed with 2 × SSC/0.1 % SDS (2 × 20 min at 25 °C for α_{1B} and α_{1A} and at 45 °C for α_{1D}) and 0.2 × SSC/0.1 % SDS (1 × 20 min at 48 °C). The membranes were then exposed to a PhosphorImager screen which was scanned on a Molecular Dynamics PhosphorImager 1–2 days later for quantitative analysis. The same membranes were probed consecutively for the α_{1B} -, the α_{1A} - and the α_{1D} -adrenoceptor mRNAs, after being stripped in between by washing with 0.1 % (w/v) SDS.

Standardization of the amount of RNA

To standardize the amount of total and poly(A)-enriched RNA, a small denatured aliquot from every sample was blotted on to a nylon membrane in a slot-blot apparatus (Minifold 2; Schleicher & Schuell) and washed with 20 × SSC. The membranes were then hybridized with labelled oligo(dT)₁₈ as probe, principally as described by Harley [35]. Prehybridization was performed for 30 min at room temperature with 5 × SSC/0.5 % SDS/5 × Denhardt's, and hybridization for 2 h under the same conditions. The membranes were washed four times with 2 × SSC/0.5 % SDS and exposed and analysed as described for the Northern blots. All α_1 -receptor data were expressed relative to the poly(A) content as estimated in this way, but the poly(A)-

enriched RNA samples were found to contain very similar levels of poly(A), and this correction had therefore only marginal effects on the data shown.

RESULTS

Presence of α_1 -adrenoceptor subtype mRNAs in brown adipose tissue

In the present investigation, we used oligonucleotide probes to investigate the physiological control of the expression of the different α_1 -adrenoceptor subtypes in brown adipose tissue. In order to confirm that the labelled oligonucleotide probes detected the relevant subtype of α_1 -adrenoceptor mRNA, we compared the tissue distribution and the transcript lengths obtained here with those reported previously from different rat tissues. We used heart, brain and liver, as did Stewart et al. [34]. The Northern blots included samples from brown adipose tissue of control rats, which thus allowed a comparison of the expression level in brown adipose tissue with that in the other, previously characterized tissues.

The expression levels of the three α_1 -receptor subtypes were very different in the different tissues. α_{1B} -Adrenoceptor transcripts were readily detected in heart, brain and liver (Figure 1), in agreement with previous reports [29,34]. Liver had the highest levels, and, in this tissue, we observed two mRNA species (approx. 2.4 and 3.0 kb). In a similar preparation, Stewart et al. [34] observed only the smaller transcript (approx. 2.2 kb), whereas Lomasney et al. [30] also observed two transcripts. In comparison with the high expression of the α_{1B} -receptor subtype in these tissues, the expression in brown adipose tissue was very low, and only the larger transcript was visible. The low expression was unexpected, as the only α_1 -receptor detected previously in white adipose tissue by both pharmacological means and mRNA identification was the α_{1B} -receptor [36].

α_{1A} -Adrenoceptor transcripts were not detected in liver but were observed in brain and at even higher levels in heart (Figure 1), again in agreement with previous reports [29,34]. Also the observation of several transcript sizes was in agreement with

previous reports [34]; the cause or significance of the different transcript sizes has not been elucidated [34]. The α_{1A} -adrenoceptor transcripts in brown adipose tissue were also of two to three different sizes, and the level in brown adipose tissue was even higher than that in the heart. As the level of expression in heart was the highest in the series of tissues investigated by Rokosh et al. [29], it would seem that brown adipose tissue is the mammalian tissue exhibiting the highest expression of this gene.

α_{1D} -Adrenoceptor transcripts (of which only one size was observed) were not found in liver but the level was high in both heart and brain, again in agreement with previous reports [29,34]. The level of expression in brown adipose tissue was also very high, even higher than that found in the heart. Only the vas deferens has previously been reported to have a higher level of expression than the heart [29]. Thus it would seem that the level of expression of this subtype in brown adipose tissue is at least as high as is in this classical tissue for α_1 -receptor studies.

Thus the tissue distribution (and the molecular masses, not discussed in detail here) of the three different α_1 -receptor subtype transcripts identified with the oligonucleotides used here were very similar to those reported previously. Therefore we concluded that the oligonucleotides were correctly identifying the relevant transcripts.

Acute effects of cold exposure on α_1 -adrenoceptor subtype gene expression

When animals are acutely exposed to cold, the sympathetic activity of the tissue is increased, and the brown adipocytes are acutely exposed to increased levels of noradrenaline [7]. We investigated whether this would lead to physiologically induced up- or down-regulation of the expression of the α_1 -receptor subtype genes.

Rats were exposed to a temperature of 4 °C for the times indicated in Figure 2, and poly(A)-enriched RNA was extracted. As the physiological stimulus may lead to alterations in the composition of the RNA and as this may lead to an altered recovery of total poly(A) RNA in the poly(A)-enriched fractions,

Figure 1 Northern-blot analysis of α_1 -adrenoceptor subtype mRNAs in different rat tissues

For each tissue indicated, 20 μ g of poly(A)-enriched RNA obtained from control rats were subjected to Northern-blot analysis with 32 P-labelled oligonucleotide probes for the indicated α_1 -adrenoceptor mRNAs. RNA size markers are indicated on the left and the position of ribosomal RNAs on the right. BAT, brown adipose tissue.

Figure 2 Effect of acute cold exposure on α_1 -receptor subtype gene expression in rat brown adipose tissue

Rats were exposed to cold for the indicated times and poly(A)-enriched RNA fractions prepared as described in the Materials and methods section. (A) Northern blots from one experimental series. Poly(A)-enriched RNA was subjected to Northern-blot hybridization with the indicated ^{32}P -labelled oligonucleotide probes (lowest row is slot blot). (B)–(D) Compilation of results from three independent series of experiments. For each sample, the ratio (α_1 -adrenoceptor mRNA subtype amount)/[poly(A) amount] was calculated. In each series, the value at each time point was expressed as a percentage of the control value at 0 h for that series. Results are thus means $\pm$ S.E.M. for the three independent experiments. For the quantitative presentation, we analysed only the small transcript of α_{1A} -receptor mRNA (approx. 3 kb); the other appeared to change in almost the same way. (Stewart et al. [34] have suggested that the larger transcripts might reflect incomplete mRNA processing.) (E) The amount of poly(A) RNA in the total RNA fractions, as estimated by hybridization with oligo(dT). The amount was found to be positively correlated with time in the cold ($P < 0.05$; regression analysis with the independent variable being logarithmically transformed time and the dependent variable being the mean values shown).

we elected to express all subtype mRNA levels as a fraction of the total mRNA [poly(A) RNA]. Thus all samples were also analysed for the content of poly(A) RNA and all values were expressed relative to this. However, we found no statistically significant effect of cold exposure on the poly(A) RNA content of the poly(A) RNA-enriched fractions (not shown), and results expressed directly per μg of poly(A)-enriched RNA would only marginally differ from those shown here.

In Figure 2(A), Northern blots of one series of experiments are shown, and in Figure 2(B)–2(E) the results from three independent series are compiled. The expression of the α_{1B} -receptor

gene, which was only weakly expressed in the tissue in control animals (Figure 1), was not significantly affected by acute cold exposure (Figure 2B). The effect of cold exposure on the expression of the α_{1A} -receptor gene was much more dramatic (Figure 2A). The levels of all transcript sizes were affected by this physiological stimulus, almost in parallel; for the quantitative analysis, only the data for the approx. 3 kb transcript are shown (Figure 2C). Three phases of change in α_{1A} -receptor mRNA levels seemed to occur. Within 2–4 h of cold exposure, the levels had increased to about 160 % of the control value. They then consistently but transiently returned to the control value at 8 h

Figure 3 Effect of noradrenaline injection on α_1 -receptor gene expression in rat brown adipose tissue

Rats were injected with noradrenaline (filled symbols) or with saline (open symbols) at 28 °C and killed after the time indicated. The initial control was uninjected. Poly(A)-enriched fractions were prepared as described in the Materials and methods section. (A) Northern blots (and slot-blot) from one experimental series. (B)–(E) Compilation of results from three independent series of experiments. For further details, see the legend to Figure 2. Statistics for (B)–(D) are discussed in the text. In (E) there was a significant effect of noradrenaline compared with saline injection ($P < 0.05$; paired t test on the mean values shown for the 1, 2 and 4 h time points). NA, noradrenaline.

before increasing gradually again, the levels being approximately doubled after 48 h. Two phases of change in α_{1D} -receptor mRNA levels were observed as an effect of acute cold exposure (Figure 2D). After as little as 1 h, the amount had decreased to 50 % of the control value; it then reached the lowest value (25 % of the control value) by 2–4 h. Despite the physiological stimulation remaining the same, the expression gradually recovered, returning to the control value after 24 h and then apparently remaining stable.

As all the above data were expressed as changes in levels of transcripts in the poly(A)-enriched RNA, it could be envisaged that the changes were secondary to changes in the relative content of mRNA [poly(A) RNA] in the total RNA (i.e. there could, for example, be an apparent decrease in gene expression as a result of dilution of the transcripts under study by increased amounts of mRNA transcribed from other genes). We therefore followed the amount of poly(A) RNA ($\approx$ mRNA) in the total RNA preparations from brown adipose tissue during the same time. As seen in Figure 2(E), the fraction of total RNA that was mRNA changed only to a limited extent, but there was a significant increase of about 20 % during the first few hours of cold exposure. This increase in the relative amount of mRNA cannot, however, explain the changes in the levels of the transcripts of the α_1 -receptor subtypes reported above (Figures

2B–2D), except perhaps for the small initial tendency to a decrease in α_{1B} -receptor mRNA level which is mirrored by the change in poly(A) RNA. The changes in α_{1A} -receptor mRNA levels would be even greater if expressed per total RNA. The decrease in α_{1D} -receptor mRNA was much greater than could be explained by dilution.

Ability of noradrenaline to mimic acute effects of cold exposure

In order to study whether the acute effects of cold exposure on α_1 -adrenoceptor gene expression in brown adipose tissue were due to the increased level of endogenous noradrenaline that would result from the enhanced sympathetic nervous activity during cold exposure [7], we examined whether acute noradrenaline treatment could mimic the effect of acute cold.

A single injection of noradrenaline (1 mg/kg body weight) was given to the rats. This dose is known to induce maximal heat production *in vivo* [37]. On the basis of the duration of the thermogenic response, it can be envisaged that the level of noradrenaline remains elevated for 1–2 h after the injection.

In Figure 3(A), Northern blots from one series of experiments are shown, and in Figures 3(B)–3(E) the results from three independent series are compiled. The results may thus be directly compared with the earliest time points obtained during acute

cold exposure (Figure 2). There was no significant effect of noradrenaline injection on the α_{1B} -receptor mRNA level (Figure 3B), in agreement with the absence of effect of acute cold exposure (Figure 2B).

For α_{1A} -receptor mRNA, an increase in the saline-treated control was observed, and there was no consistent noradrenaline effect on top of this. Thus even the saline-injected animals showed the up-regulation observed during cold exposure (Figure 2B). It may nevertheless be implied that the effect of acute cold exposure on α_{1A} -receptor gene expression is mediated by noradrenaline. This is because saline injection in itself leads to some stress effect and thus to some sympathetic stimulation as evidenced for example by a short-lived increase in thermogenesis [37]. If the α_{1A} -receptor gene is very sensitive to noradrenaline, a positive effect of saline treatment on gene expression can thus occur.

The results for the α_{1D} -receptor mRNA were very clear (Figure 3D). The level in the controls was stable, but noradrenaline injection led to a significant decrease ($P < 0.05$). Thus the effect of acute cold exposure (Figure 2D) was mimicked by the noradrenaline injection.

There were only marginal effects of noradrenaline injection on the relative content of mRNA in total RNA (Figure 3E). However, if anything, the level was increased, in accordance with the tendency seen in animals during acute cold exposure (Figure 2E).

Thus for each subtype of α_1 -adrenoceptor, noradrenaline injection induced responses fairly similar to those of acute cold exposure. These results are therefore consistent with the idea that the differential up- and down-regulation of the gene expression of the α_1 -receptor subtypes during acute cold exposure is mediated via the sympathetic nervous system and is due to the increased intensity of noradrenaline stimulation.

Effect of acclimation to cold on α_1 -receptor subtype gene expression

For several parameters, including β_3 -adrenoceptor gene expression in brown adipose tissue [38], there are marked differences between the effects of acute and chronic exposure to cold. The effects observed in acute experiments may be secondary to the transition period as such and the parameters may return to initial levels in the completely cold-acclimated state. We therefore examined the effect of cold acclimation on the expression of the α_1 -receptor subtypes. Complete acclimation to cold is expected to be reached after about 3 weeks in the cold. In the present experiments, we analysed α_1 -receptor gene expression in animals that had been acclimated to the cold for 4 weeks.

In Figure 4, Northern blots from one series of experiments are shown, and in Table 1 the results from three independent series are compiled. The results may be compared with those obtained during acute cold exposure (Figure 2).

The α_{1B} -receptor gene, which was already very weakly expressed in the control rats, showed a reduced expression in the cold-acclimated animals (Table 1). No such tendency was observed during acute cold exposure (Figure 2B). However, the expression level is close to the level of detection and the absolute degree of reduction cannot be accurately determined. In cold-acclimated animals, the α_{1A} -receptor mRNA level was twice that in controls (Table 1). Thus the increase in level already observed after 24 h (Figure 2C) persisted throughout prolonged cold exposure but was not further enhanced. The level of α_{1D} -receptor mRNA was somewhat lower in the cold-acclimated than in the control rats (Table 1). Thus after the initial transient down-regulation and

recovery (Figure 2D), a tendency to a further decrease in α_{1D} -receptor gene expression was seen.

In the fully cold-acclimated state, the fraction of mRNA in the total RNA was $103 \pm 10\%$ of that observed in control animals of the same age (Table 1). Therefore the increase in the fraction seen during acute exposure to cold was apparently transient and may have been associated with an increased demand for protein synthesis during the transition phase.

Thus, in the fully acclimated state, two significant alterations in α_1 -receptor gene expression had occurred. There was a clear decrease in α_{1B} -receptor mRNA level; however, as the absolute level of expression of this subtype was so low, it may be envisaged that this alteration in itself is of minor physiological significance. Most notably, there was a doubling of the α_{1A} -receptor mRNA level; as the expression of this gene was high, even in the control rat, the further enhanced expression may be of physiological importance.

Figure 4 Effect of chronic cold exposure on expression of α_1 -receptor subtype gene expression in rat brown adipose tissue

Rats were acclimated to cold (CA) for 4 weeks or remained at 28 °C (cont). Northern blots (and slot-blot) are from one experimental series (a compilation of results from three independent series of experiments is found in Table 1). Poly(A)-enriched fractions were prepared as described in the Materials and methods section. For further details, see the legend to Figure 2.

Table 1 Effect of chronic cold exposure on expression of α_1 -receptor subtype gene expression in rat brown adipose tissue

Results from three independent series of experiments performed as illustrated in Figure 4 are compiled. Rats were acclimated to cold for 4 weeks or remained at 28 °C. Poly(A)-enriched fractions were prepared as described in the Materials and methods section. For further details, see the legend to Figure 2. The data (means $\pm$ S.E.M.) are expressed as a percentage of the values from the age-matched controls. * and ** indicate statistically significant effects of acclimation to cold ($P < 0.05$ and $P < 0.01$ respectively).

	Expression (%)	
	Control	Cold-acclimated
α_{1B}	100	$46 \pm 10^{**}$
α_{1A}	100	$184 \pm 13^{**}$
α_{1D}	100	$68 \pm 7^*$
Poly(A)	100	103 ± 9

DISCUSSION

In the present study, we have examined the physiological control of expression of the different α_1 -adrenergic receptor subtypes in a tissue in which most essential processes are under adrenergic control, namely brown adipose tissue. We have demonstrated that mRNAs for all three subtypes of the α_1 -adrenoceptor are present in rat brown adipose tissue. The α_{1B} -receptor transcripts were only found in trace amounts, orders of magnitude lower than in liver. However, the α_{1A} -receptor transcripts were found to be more abundant in brown adipose tissue than even in the heart, and the α_{1D} -receptor mRNA may be as abundant in brown adipose tissue as it is in vas deferens. Each of the α_1 -receptor subtypes showed dramatic but differential changes during acute cold exposure or chronic cold acclimation. The acute down- and up-regulations were mimicked by noradrenaline treatment of rats, implying that they were direct responses to adrenergic stimulation of the cells in the tissue. To our knowledge, this is the first study to show that physiological stimulation that enhances sympathetic nerve activity leads to dramatic changes in α_1 -adrenoceptor subtype gene expression.

Expression levels of α_1 -adrenoceptor subtype genes in brown adipose tissue

We found that the α_{1B} -receptor gene was only weakly expressed in brown adipose tissue. In contrast, it has previously been observed that this is the only α_1 -receptor gene expressed to a measurable extent in white adipose tissue [36]. If this distinction is valid, the α_1 -receptor subtypes represent one of the few gene families that are qualitatively differentially expressed in brown and white adipose tissue. Except for the nearly definitional expression only in brown adipose tissue of the uncoupling protein, only the α -subunits of the G_i -proteins [39,40], leptin [41] and a carnitine-palmitoyltransferase-1-like protein [42] have hitherto been suggested to show distinct expression patterns in the two adipose tissues.

In contrast with the low expression of the α_{1B} -receptor gene in brown adipose tissue, the α_{1A} - and α_{1D} -receptor subtypes were very well expressed; in fact, brown adipose tissue seems to be one of the mammalian tissues with the highest levels of expression of these genes. Considering the fact that the thermogenic processes in brown adipose tissue are mainly stimulated via β -adrenergic receptors and that there are only modest thermogenic effects of α_1 -adrenergic stimulation (see the Introduction), these results are surprising. They could imply that the α_1 -receptors are more involved in brown adipose tissue function than has generally hitherto been assumed.

Possible divergent functional roles and localization of the different α_1 -receptor subtypes

No differential physiological roles for the different α_1 -receptor subtypes have as yet been suggested. In different systems it has been shown that each of the different α_1 -receptor subtypes (α_{1B} [2], α_{1A} [43] and α_{1D} [44]) may induce the expected cellular responses (IP_3 formation, increases in intracellular Ca^{2+} levels and protein kinase C activation). Thus it is likely that the α_1 -receptor subtypes all utilize identical second messengers. As all these second messengers have been observed in brown adipocytes (see the Introduction), any of the three α_1 -receptor subtypes may be coupled to α_1 -adrenergic stimulation of the brown fat-cells.

It may therefore be suggested that instead of differing in intracellular pathways, the α_1 -receptor subtypes may have different affinities or may react differently to chronic stimulation,

exhibiting up- or down-regulation etc., as we indeed have observed here. Further, the α_1 -receptor subtypes may not only be differently expressed in different tissues but they may also be differently expressed in different differentiation states of the cells or on different cell populations in a tissue. Indeed, the data presented here include all types of cells present in the brown adipose tissue, as do estimates of subtype expression in other tissues (see Figure 1 and for example [34]). The absence of certain α_1 -receptor subtypes in certain tissues (Figure 1) makes it, however, unlikely that the expression of a given subtype in a given tissue should primarily be ascribed to non-parenchymal cells such as blood vessels, connective tissue or nerves. Rather, the differential tissue distribution indicates that the specific subtype expression is mainly in the parenchymal cells of each tissue.

Differential adrenergically induced up- and down-regulation of α_1 -receptor gene expression depending on subtype

For two of the receptor subtypes investigated here, we have observed down-regulation of gene expression: α_{1B} (in long-term cold acclimation) and α_{1D} (transiently during cold exposure, noradrenaline induction, and in long-term cold acclimation). It has previously been reported that noradrenaline treatment induces down-regulation of α_{1B} -receptor mRNA in rabbit aortic smooth-muscle cells [45], and such homologous down-regulation is well known in other adrenergic systems (e.g. [46,47]). We have also recently observed a rapid α_1 -adrenergic desensitization process in cultured brown adipocytes (S.-J. Zhang and J. Nedergaard unpublished work). This may be a manifestation of the down-regulation of the α_{1D} -receptor reported here. However, as low α_{1B} -receptor gene expression (versus α_{1A} - and α_{1D} -receptor gene expression) may distinguish brown from white adipose tissue, the late decrease in α_{1B} -receptor gene expression observed in the recruited state may instead be interpreted as a manifestation of an increased degree of differentiation of the tissue, induced by the constant physiological stimulation.

For the third of the α_1 -receptor subtypes investigated here, the α_{1A} -receptor, we instead observed up-regulation of gene expression, with similar tendencies being observed during acute cold exposure, as an acute effect of noradrenaline injection and after cold acclimation. In another system, up-regulation of α_{1A} -receptor gene expression in response to an adrenergic agonist has also been found [48]. In earlier studies in which α_1 -receptor density in brown adipose tissue was determined by radioligand binding of [3H]prazosin, we found that the density of these receptors increased markedly during cold exposure [11]. From the present study, in which also total tissue samples were used for the estimation of expression, it is possible to tentatively identify the α_1 -receptor gene the expression of which may be responsible for this increase in α_1 -receptor density. The α_{1A} -receptor mRNA was the only subtype that increased during chronic cold exposure (Figure 4) and the increase was of similar magnitude to that in receptor density. We suggest therefore that the increased α_1 -adrenoceptor density in brown adipose tissue during cold exposure is due to an increase in α_{1A} -adrenoceptor gene expression. This conclusion is not contradicted by analysis of the pharmacological data known. The α_1 -receptor antagonist WB-4101 is reported to have a pA_2 (affinity) of approx. 10 on this subtype [1] while it is clearly lower on the α_{1B} subtype and somewhat lower on the α_{1D} subtype. In competition experiments against [3H]prazosin binding to brown adipocytes, WB-4101 was found to be more competent than prazosin itself, with a pK_i of about 9.2 [10].

Thus, recruitment of brown adipose tissue is probably positively correlated with an enhanced expression of the α_{1A} -adreno-

ceptor. Although it cannot unequivocally be concluded from the present investigation that this is due to each brown adipocyte expressing this gene to a higher level, it would be in accordance with α_{1A} -receptor gene expression being a differentiation marker of the development of these cells and of a general increase in differentiation state of the cells in the tissue.

It is not known to what extent this physiologically induced up-regulation affects the function of the tissue, nor are the adrenergic receptors mediating the response or the intracellular pathways underlying the up-regulation as yet known.

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