Selective Up-Regulation of $\alpha_{1a}$-Adrenergic Receptor Protein and mRNA in Brown Adipose Tissue by Neural and $\beta_3$-Adrenergic Stimulation

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ABSTRACT

Previous studies have shown that neural stimulation of brown adipose tissue (BAT) reorganizes the expression and activity of signaling proteins in the $\beta$-adrenergic adenyl cyclase pathway. Cold stress increases neural stimulation of BAT and increases $\alpha_1$-adrenergic receptor number; however, the $\alpha_1$ receptor subtype involved and the mechanism of up-regulation by cold stress have not been determined. Using reverse transcription/polymerase chain reaction analysis and nuclease protection assay, BAT was demonstrated to express mRNAs encoding $\alpha_{1a}$ and $\alpha_{1d}$, but not $\alpha_{1b}$, receptors. Parallel pharmacologic studies of BAT membranes and recombinant $\alpha_{1a}$ and $\alpha_{1d}$ receptors expressed in COS-7 cells demonstrated that $\alpha_{1a}$ receptors predominate in BAT. Exposure of rats to 4°C for 4 days increased $\alpha_{1a}$ receptors and mRNA in BAT but did not alter expression of $\alpha_{1d}$ receptors or mRNA. The induction of $\alpha_{1a}$ receptor and mRNA level by cold stress was prevented by selective surgical denervation of BAT. Furthermore, $\alpha_{1a}$ receptor and mRNA expression could be induced in warm-adapted rats by infusions of the selective $\beta_3$-adrenergic receptor agonist CL 316,243. These data indicate that neural activation of $\beta_3$-adrenergic receptors is an important determinant of $\alpha_{1a}$ adrenergic receptor expression in BAT.

The main function of BAT is to produce heat in response to sympathetic nerve stimulation (1). It is well known that activation of BAT thermogenesis is important in the maintenance of body temperature during cold stress, and recent evidence showing that genetic ablation of BAT results in obesity (2) indicates that this tissue plays an important role in overall body energy homeostasis as well. In the absence of adrenergic stimulation, BAT exists in an involuted, inactivated state. Sustained adrenergic stimulation, as produced by cold stress, not only triggers an acute thermogenic response but also induces expression of key genes that serve to increase the thermogenic capacity of the tissue (1, 3). We have reported previously that adrenergic stimulation dramatically alters the expression and activity of several proteins in the adrenergic signaling cascade, including $\beta$-adrenergic receptor subtypes, G protein $\alpha$ subunits, and adenyl cyclase isoforms (4–7).

Considerable evidence indicates that $\alpha_1$-adrenergic receptors play an important modulatory role in BAT. Interestingly, cold stress increases the expression of $\alpha_1$ receptors, and this accompanies recruitment of BAT to the thermogenic state (8). However, the molecular heterogeneity of $\alpha_1$ receptor subtypes was unknown when cold stress was first shown to increase $\alpha_1$ receptor levels in BAT. Thus, it is not known which $\alpha_1$ receptor subtypes are expressed in BAT or whether cold stress selectively increases the expression of one subtype. In this regard, we have recently shown that although BAT expresses five adenyl cyclase subtypes, type III is selectively up-regulated by cold stress (6, 7). Moreover, it has not been determined whether the elevation of $\alpha_1$ receptor number by cold stress results from direct neural activation of the tissue or is mediated by other factors induced by the cold. In the following study, we have characterized BAT $\alpha_1$ receptors with molecular biological analysis of BAT RNA and parallel pharmacological analysis of BAT membranes and membranes of COS-7 cells expressing recombinant $\alpha_1$ recep-

1 The nomenclature for $\alpha_1$ receptors used throughout this paper corresponds to that adopted by the International Union of Pharmacology [Pharmacol. Rev. 47:267–270 (1995)]. Specifically, $\alpha_{1A}$ receptor correspond to the pharmacologic $\alpha_{1A}$ and the cloned receptor previously named $\alpha_{1c}$; $\alpha_{1A}$ receptors were known previously as the cloned $\alpha_{1a}$ and $\alpha_{1d}$ receptors.

ABBREVIATIONS: BAT, brown adipose tissue; IBAT, interscapular brown adipose tissue; HEAT, $\pm\beta(\pm [\text{I}^{25}])$ido-4-hydroxyphenyl)-ethyl-amino-methyl-tetraolone; HSP, heat shock protein; NE, norepinephrine; RT, reverse transcription; PCR, polymerase chain reaction; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(\(\beta\)-aminoethyl ether)-N,N',N'-tetraacetic acid; AC-III, type III adenyl cyclase.
tor subtypes. Additional experiments evaluated the effects of cold exposure and selective surgical denervation of BAT on the expression of $\alpha_1$ receptor subtypes. These data demonstrated that although BAT contained mRNAs encoding $\alpha_{1a}$ and $\alpha_{1d}$ receptors, only $\alpha_{1a}$ receptors were present in the tissue. Cold exposure increased $\alpha_{1a}$ receptors and its mRNA, but did not change levels of $\alpha_{1d}$ receptor mRNA. Finally, both $\alpha_{1a}$ receptor mRNA and binding were induced strongly by infusion of the selective $\beta_3$ receptor agonist CL 316,243.

Materials and Methods

**Animals and surgery.** Male Sprague-Dawley rats (Hilltop, Scottsdale, PA) weighing 175–250 g were used. All rats had continuous access to water and Purina laboratory chow (Ralston Purina, St. Louis, MO). To increase physiological levels of sympathetic nerve stimulation, rats were exposed at 4°, whereas control rats remained at room temperature (22°). IBAT consists of bilateral pads that receive independent sympathetic innervation (9, 10). To eliminate sympathetic neural stimulation, one IBAT pad was denervated surgically as described previously (9), and the contralateral pad was used as the intact control. After 4 days, animals were killed and harvested tissues were frozen at −80°. For drug infusions, neurally intact rats were implanted with an osmotic minipump (model 2001; Alza, Palo Alto, CA) containing vehicle, NE (100 nmol/hr), or CL 316,243 (15 nmol/hr). CL 316,243 was provided by American Cyanamid (Pearl River, NY). Animals were maintained at 22° for 4 days, and then tissue was harvested as described above.

**RT/PCR analysis.** The amino acid sequences of the three cloned rat $\alpha_1$ receptors (11–14) were aligned to identify short sequences that are completely conserved among the subtypes. PCR primers were synthesized based on common cDNA sequences, with mixed base or are completely conserved among the subtypes. PCR primers were designed on the genomic DNA sequences (underlined) to facilitate directional cloning of the PCR products.

**Radioligand binding.** $\alpha_1$ receptors were characterized with $[125\text{I}]$HEAT (DuPont-New England Nuclear) as described previously (15). Briefly, membrane pellets were resuspended in binding buffer containing 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 10 mM MgCl$_2$, 1 mM ascorbic acid, and 0.01 mg/ml leupeptin. The homogenate was filtered through glass wool and then centrifuged at 1,100 × g for 15 min. The supernatant was removed and centrifuged at 48,000 × g for 15 min. The resulting pellet was resuspended in buffer and centrifuged at 48,000 × g.

**Statistical analysis.** Except where noted, data are reported as mean ± standard error. Differences between treatments were evaluated by analysis of variance or Student’s t-test; $p < 0.05$ (two-tailed) was judged as significant.
tained all three subtypes. Probes of \( \alpha_{1a} \) and \( \alpha_{1d} \) receptor mRNA were of equal specific activity and were assayed in the same reaction sample. Therefore, BAT expressed comparable levels of \( \alpha_{1a} \) and \( \alpha_{1d} \) receptor mRNA.

The above experiment established the presence of \( \alpha_{1a} \) and \( \alpha_{1d} \) receptor mRNAs in BAT. To determine the relative expression of the protein(s) encoded by these mRNAs, pharmacological analysis of \(^{\text{[125]}}\)HEAT binding sites was performed in BAT membranes and in membranes of COS-7 cells transfected with \( \alpha_{1a} \) and \( \alpha_{1d} \) receptor cDNAs. Membranes from BAT and transfected COS-7 cells displayed saturable (\( K_d \) values between 55 and 75 pm) \(^{\text{[125]}}\)HEAT binding sites that were displaced specifically by 1 \( \mu \text{M} \) prazosin. Competition analysis was performed with various compounds in the presence of 50 pm \(^{\text{[125]}}\)HEAT. Fig. 2 illustrates a typical experiment. Oxymetazoline, 5-methylurapidil, and BMY 7378 had dramatically different affinities for recombinant \( \alpha_{1a} \) and \( \alpha_{1d} \) receptors expressed in COS-7 cells, as expected from previous studies of the recombinant receptors (11–13). Competition curves for \(^{\text{[125]}}\)HEAT binding sites in BAT membranes were identical to COS-7 cells expressing \( \alpha_{1a} \) receptors. Table 1 summarizes the \( K_i \) values of various subtype-selective and nonselective compounds in displacing \(^{\text{[125]}}\)HEAT binding sites. The pharmacological characteristics of the BAT \( \alpha_1 \) receptor were virtually identical to those of COS-7 cells expressing the recombinant \( \alpha_{1a} \) receptor and clearly different from the \( \alpha_{1d} \) receptor. These data indicate that the dominant, if not exclusive, subtype in BAT is the \( \alpha_{1a} \) receptor.

No pharmacological evidence could be found for the presence of \( \alpha_{1d} \) receptors in BAT despite the substantial levels of mRNA encoding this protein. To evaluate whether the discrepancy between mRNA and protein is a general phenomenon, we measured \( \alpha_1 \) receptor mRNA and binding in COS-7 cells transiently transfected with the respective cDNAs. Transfection resulted in nearly equivalent levels of \( \alpha_{1a} \) and \( \alpha_{1d} \) receptor mRNA, but levels of \( \alpha_{1d} \) receptor binding were less than 2% of \( \alpha_{1a} \) binding (Fig. 3).

We next turned our attention to the regulation of \( \alpha_1 \) receptor expression in BAT by cold exposure. As mentioned above, cold exposure was shown to increase \(^{\text{[3H]}}\)prazosin binding
sites in BAT, although the mechanism of the increase and the subtype involved were not addressed previously (8). Saturaton analysis (Fig. 4, top) demonstrated that cold exposure doubled total $^{125}$I-HEAT binding sites in neurally intact BAT ($p < 0.01$). Competition analysis with oxymetazoline demonstrated that these sites corresponded exclusively to $\alpha_{1a}$ receptors in all treatment conditions (Fig. 4, bottom). Surgical denervation had no effect on $\alpha_{1a}$ receptor mRNA levels in denervated BAT to levels of seen in intact controls, suggesting involvement of a cold-induced humoral factor, possibly epinephrine.

The above experiment indicates that sympathetic nerve activity is a major determinant of $\alpha_{1a}$ receptor mRNA and protein levels in BAT. NE is the major neurotransmitter of the sympathetic innervation of BAT and is released in response to cold stress. Furthermore, $\beta_3$-adrenergic receptors are highly expressed in this tissue and are believed to play a central role in recruitment of BAT during cold stress (16). We next examined whether infusion of NE or the selective $\beta_3$ receptor agonist CL 316,243 could reproduce the effects of cold stress in warm-adapted rats. Drugs were delivered by minipump for 4 days at rates that have been shown to induce BAT recruitment (6, 17). Infusion of CL 316,243 strongly and selectively induced $\alpha_{1a}$ binding and mRNA (both $p < 0.01$) in warm-adapted rats (Fig. 6). There was a trend for NE infusion to increase $\alpha_{1a}$ binding and mRNA; however, this effect was not statistically significant. It should be noted that NE, unlike CL 316,243, activates $\beta_3$-adrenergic receptors and stimulates BAT hyperplasia (17). In the present experiment, NE infusions increased total $\alpha_{1a}$ binding and mRNA per pad by approximately 40%, an increase that kept pace with the growth of the tissue.
Discussion

The present study characterized the $\alpha_1$ receptor subtypes expressed in BAT and examined the regulation of these receptors by adrenergic stimulation. Using RT/PCR and direct hybridization techniques, BAT was shown to express mRNAs encoding $\alpha_{1a}$ and $\alpha_{1d}$ but not $\alpha_{1b}$ receptors. Parallel radioligand binding studies comparing the pharmacologic properties of BAT $\alpha_1$ receptors with those of the recombinant $\alpha_{1a}$ and $\alpha_{1d}$ expressed in COS-7 cells demonstrated that the receptors present in BAT are predominantly, if not exclusively, the $\alpha_{1a}$ subtype. Although $\alpha_{1a}$ and $\alpha_{1d}$ receptor mRNAs were expressed at similar levels in BAT, no $\alpha_{1d}$ binding sites could be detected. A similar discrepancy between functional responses mediated by $\alpha_{1d}$ receptors and its mRNA was reported recently in rat mesenteric and renal arteries, although receptor protein levels were not determined directly (18). The present work demonstrated a clear discrepancy between mRNA and protein in COS-7 cells transfected with the recombinant receptors. These observations point to strong intrinsic differences in the post-transcriptional processing of the $\alpha_1$ receptor subtypes and indicate that the presence of abundant $\alpha_{1d}$ receptor mRNA levels does not necessarily indicate abundant expression of the protein.

Previous work has shown that $\alpha_1$ receptors are up-regulated by cold stress in adult rats (8). The present work confirmed these observations and has further identified the up-regulated receptor as being the $\alpha_{1a}$ subtype. Cold stress increases norepinephrine release in BAT (1) and selective surgical destruction of the noradrenergic innervation to BAT prevented the cold-induced increase in $\alpha_{1a}$ receptor expression. Taken together, these results indicate that cold-induced up-regulation of $\alpha_{1a}$ receptor expression results from enhanced noradrenergic stimulation of the tissue. Considering these observations, it was somewhat surprising that systemic infusions of NE simply increased expression of $\alpha_{1a}$ receptors in proportion to the growth of the tissue but did not mimic the effects of cold stress on receptor density. In contrast, the selective $\beta_3$ agonist CL 316,243 strongly elevated expression of $\alpha_{1a}$ receptors in BAT. There are numerous differences between the actions of infused NE and that of CL 316,243 and neurally released NE that could account for the observed differences. Like neurally released NE, the direct actions of CL 316,243 are almost exclusively limited to adipose tissue, owing to fat-specific expression of the $\beta_3$ receptor (16, 17, 19). Furthermore, because the $\beta_3$ receptor is resistant to desensitization (20), CL 316,243 remains highly responsive to CL 316,243 during prolonged infusions. By contrast, systemic infusions of NE stimulate adrenergic receptors in numerous tissues, which could indirectly influence regulation of $\alpha_{1a}$ receptors in BAT. In addition, NE has a higher affinity for $\beta_1$ versus $\beta_3$ receptors (20). It is possible that the concentrations of NE used in the present study predominately activate $\beta_1$ receptors, which are desensitized rapidly (20). Unfortunately, higher doses of NE that would be required to strongly activate $\beta_3$ receptors are not well tolerated by rats owing to systemic effects of the agonist.
The increased expression of α1a binding sites was paralleled by selective up-regulation of α1a receptor mRNA levels, suggesting that the increase involves increased receptor synthesis. Recently, NE was found to selectively increase expression of α1a receptors in cardiac myocytes via α1 adrenergoreceptors (21). In contrast, the ability of β2 receptor activation to fully induce α1a receptor mRNA and protein expression in BAT indicates that this effect most likely involves generation of cyclic AMP (16). Up-regulation of α1α-adrenergic receptors by a cyclic AMP-dependent mechanism has been reported in DDT1 MF-2 smooth muscle (22, 23) and FRTL-5 thyroid cells (24). In the latter cells, up-regulation seems to result from increased gene transcription, and a functional cyclic AMP response element has been identified in the proximal promoter of the rat α1a gene (24). Little is known about the structure of the α1a receptor gene; however, the present results suggest that the α1α receptor gene might contain DNA sequences capable of binding cAMP response element-binding protein-related transcription factors that are capable of integrating β- and α1-adrenergic signals.

Neural stimulation of BAT increases the expression of key genes that allow greater sustained thermogenesis during cold stress. This process, termed recruitment, involves the reorganization of several proteins in the adrenergic signaling pathway. Although β-adrenergic receptors play a central role in BAT recruitment, several studies indicate that α1 receptors are also involved. One means by which α1 receptors participate in recruitment is by greatly enhancing β-adrenergic responsiveness (6, 25–27). Although the molecular basis of this phenomenon is not understood, it seems to involve synergistic activation of adenylyl cyclase (25). In this regard, it is interesting to note that AC-III and α1α receptors both are induced by a variety of stimuli that increase neural stimulation of BAT (6, 7). AC-III is believed to be sensitive to the products of phospholipase C activation as well as Gα (28–30), suggesting that AC-III might integrate signals from the α1 and β-adrenergic pathways (6). The coordinate expression of α1α receptors and AC-III suggests that the interaction of α1 and β-adrenergic receptors might be greatest in neurally recruited BAT. Studies are in progress to examine this hypothesis.

In addition to interactions with β receptor activation, α1α-adrenergic stimulation alone potently induces expression of HSPs in BAT without stimulating thermogenesis (31). HSPs have been shown to be important in the import and maturation of mitochondrial proteins (32, 33). Whereas β-adrenergic stimulation is known to trigger expression of key mitochondrial proteins, like uncoupling protein (1, 17), α1 receptor stimulation might facilitate mitochondriogenesis by induction of HSPs, which serve as molecular chaperones for the newly synthesized mitochondrial proteins (31). Of course, the natural adrenergic neurotransmitter in BAT, NE, would activate both α1α and β-adrenergic receptors. It is therefore possible that the up-regulation of α1α receptors reported here facilitates mitochondriogenesis and BAT recruitment during sustained neural stimulation.

References


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