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Selective Up-Regulation of α_{1a}-Adrenergic Receptor Protein and mRNA in Brown Adipose Tissue by Neural and β_3-Adrenergic Stimulation

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SUMMARY

Previous studies have shown that neural stimulation of brown adipose tissue (BAT) reorganizes the expression and activity of signaling proteins in the β-adrenergic adenylyl cyclase pathway. Cold stress increases neural stimulation of BAT and increases α_1-adrenergic receptor number; however, the α_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 α_{1a} and α_{1d}, but not α_{1b}, receptors. Parallel pharmacologic studies of BAT membranes and recombinant α_{1a} and α_{1d} receptors expressed in COS-7 cells demonstrated that α_{1a} receptors predominate in BAT. Exposure of rats to 4° for 4 days increased α_{1a} receptors and mRNA in BAT but did not alter expression of α_{1d} receptors or mRNA. The induction of α_{1a} receptor and mRNA level by cold stress was prevented by selective surgical denervation of BAT. Furthermore, α_{1a} receptor and mRNA expression could be induced in warm-adapted rats by infusions of the selective β_3-adrenergic receptor agonist CL 316,243. These data indicate that neural activation of β_3-adrenergic receptors is an important determinant of α_{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 β-adrenergic receptor subtypes, G protein α subunits, and adenylyl cyclase isoforms (4–7).

Considerable evidence indicates that α_1-adrenergic receptors play an important modulatory role in BAT. Interestingly, cold stress increases the expression of α_1 receptors, and this accompanies recruitment of BAT to the thermoactive state (8). However, the molecular heterogeneity of α_1 receptor subtypes[1] was unknown when cold stress was first shown to increase α_1 receptor levels in BAT. Thus, it is not known which α_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 adenylyl cyclase subtypes, type III is selectively up-regulated by cold stress (6, 7). Moreover, it has not been determined whether the elevation of α_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 α_1 receptors with molecular biological analysis of BAT RNA and parallel pharmacological analysis of BAT membranes and membranes of COS-7 cells expressing recombinant α_1 recep-

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[1] The nomenclature for α_1 receptors used throughout this paper corresponds to that adopted by the International Union of Pharmacology [*Pharmacol. Rev.* **47:**267–270 (1995)]. Specifically, α_{1a} receptor correspond to the pharmacologic α_{1A} and the cloned receptor previously named "α_{1c}." α_{1d} receptors were known previously as the cloned "α_{1a}" and "$\alpha_{1a/d}$" receptors.

ABBREVIATIONS: BAT, brown adipose tissue; IBAT, interscapular brown adipose tissue; HEAT, (±)-β-([^{125}I]iodo-4-hydroxyphenyl)-ethyl-aminomethyl-tetralone; HSP, heat shock protein; NE, norepinephrine; RT, reverse transcription; PCR, polymerase chain reaction; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; AC-III, type III adenylyl cyclase.

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tor subtypes. Additional experiments evaluated the effects of cold exposure and selective surgical denervation of BAT on the expression of α_1 receptor subtypes. These data demonstrated that although BAT contained mRNAs encoding α_{1a} and α_{1d} receptors, only α_{1a} receptors were present in the tissue. Cold exposure increased α_{1a} receptors and its mRNA but did not change levels of α_{1d} receptor mRNA. Finally, both α_{1a} receptor mRNA and binding were induced strongly by infusion of the selective β_3 receptor agonist CL 316,243.

Materials and Methods

Animals and surgery. Male Sprague-Dawley rats (Hilltop, Scottdale, 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 α_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 inosine substitutions for certain degenerate codons. The sense and antisense primers were: TCCGAATTCGTGATCCT(CT)TC(AG)GTGGCCTG and GCTCTAGAGTAIACICGGCAGTACATGACC, respectively. The primer set contained engineered *Eco*RI and *Xba*I restriction sites (underlined) to facilitate directional cloning of the PCR products. RT/PCR analysis was performed as described previously (6). Briefly, RNA from BAT of cold-exposed rats and cerebral cortex was reverse transcribed with random primers. Thirty cycles of PCR were performed on the cDNA with the primer set above as follows: 94° for 2 min, 63° for 1.5 min, and 72° for 2 min. Restriction analysis of the PCR products demonstrated the presence of α_{1a}, α_{1b}, and α_{1d} receptor cDNAs in cortex, whereas only α_{1a} and α_{1d} receptor cDNAs were amplified in BAT. Subsequently, the PCR products encoding the three α_1 receptor subtypes were cloned into pGEM 7z (Promega, Madison, WI) and sequenced by the dideoxy chain termination technique with an automated DNA sequencer (Applied Biosystems, Norwalk, CT).

Quantification of α_1 receptor mRNAs. α_1 receptor mRNAs were determined by probe-excess nuclease protection assay as described previously (4, 6). The cloned PCR products were used as templates for the generation of riboprobes. Briefly, radioactive cRNA probes were transcribed *in vitro* with [^{32}P]CTP [DuPont-New England Nuclear (Boston, MA) or Andotek Life Sciences (Irvine, CA)] using the T7 promoter. Tissue RNA (15–30 μg) was coprecipitated with 3×10^4 cpm of [^{32}P]-labeled probe. For cases in which the protected probe fragments could be adequately resolved, two subtypes were determined simultaneously in the same sample. Samples were resuspended in 30 μl of hybridization buffer containing 75% formamide, 400 mM NaCl, 1 mM EDTA, and 40 mM piperazine-*N*,*N*′-bis(2-ethane-sulfonic acid), pH 6.4, and hybridized at 55° for 12–18 hr. Samples were diluted in 10 volumes of 300 mM NaCl, 5 mM EDTA, and 10 mM Tris, pH 7.5, and 300 units of T-1 ribonuclease was added to each sample. Digestions were stopped after a 60-min incubation at 37° and samples were precipitated in ethanol. The [^{32}P]RNA probes that were protected from RNase digestion were resolved electrophoretically on a denaturing polyacrylamide gel containing 8 M urea. The gels were dried and exposed to Kodak XAR-5 film for 18–72 hr. The resulting autoradiograms were scanned with an E-C System densitometer coupled to a Shimadzu Chromatograph integrator (Shimadzu, Kyoto, Japan).

COS-7 cell transfections and membrane preparation. COS-7 cells were grown in 90% Dulbecco's modified Eagle's medium containing 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (0.1 mg/ml), and 2 mM glutamine. Cells were plated onto 10-cm dishes and incubated at 37° in a humidified incubator with 5% CO_2. After reaching 50–80% confluency, cells were transfected with rat α_{1a} and rat α_{1d} cDNAs with lipofectamine reagent (GIBCO BRL, Gaithersburg, MD). Full-length cDNAs encoding rat α_{1a} and rat α_{1d} receptors in the expression vector pMT2 were generously provided by Dr. Dianne Perez (Cleveland Clinic, Cleveland, OH). For each transfection, 5 μg of DNA and 30 μl of lipofectamine reagent were diluted into 2 ml serum-free medium and incubated at room temperature for 45 min. Cells were incubated with the DNA-liposome complexes for 6 hr at 37°, and then the medium was replaced with complete medium and incubations were continued for 48 hr.

COS-7 cell membranes were prepared by scraping cells into 20 mM HEPES buffer containing 2 mM $MgCl_2$, 1 mM EGTA, and 0.01 mg/ml leupeptin, pH 8.0, and centrifuging at 48,000 × *g* for 15 min. IBAT membranes were prepared by homogenizing pads in 3 ml of cold 20 mM Tris·HCl buffer, pH 7.4, containing 250 mM sucrose, 2 mM $MgCl_2$, 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*.

Radioligand binding. α_1 receptors were characterized with [^{125}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. For saturation curve studies, [^{125}I]HEAT concentrations ranged from 25 to 800 pM. For competition analysis, [^{125}I]HEAT concentration was 50 pM. Membrane concentrations were adjusted to yield similar levels of total binding per tube. Nonspecific binding was defined as [^{125}I]HEAT binding that was displaced by 1 μM prazosin (Pfizer Central Research, Groton, CT). Nonspecific binding at 50 nM [^{125}I]HEAT was 7%, 31%, and 36% for α_{1a}, α_{1d}, and BAT membranes, respectively. Phentolamine was obtained from Ciba Geigy (Summit, NJ), and all other compounds used in the competition analyses were from Research Biochemicals, Inc. (Natick, MA). The reactions were incubated at room temperature for 1 hr and stopped by rapid filtration onto Whatman GF/C glass fiber filters. Data were analyzed by computer using nonlinear regression analysis (Enzfitter, Elsevier-Biosoft, Cambridge, UK).

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.

Results

Preliminary analysis of BAT and cerebral cortical RNA by RT/PCR demonstrated the presence of α_{1a}, α_{1b}, and α_{1d} receptor cDNAs in cortex, whereas only α_{1a}, and α_{1d} receptor cDNAs were amplified in BAT. The PCR products were cloned, and sequence analysis confirmed them as α_{1a}, α_{1b}, and α_{1d} receptor cDNAs. Probe-excess solution hybridization analysis was then performed to quantify the relative abundance of α_1 receptor mRNAs. As shown in Fig. 1, BAT and white adipose tissue contained α_{1a} and α_{1d} but no α_{1b} receptor mRNA. Liver, which was used as a positive control, contained only α_{1b} receptor mRNA, whereas cerebral cortex con-

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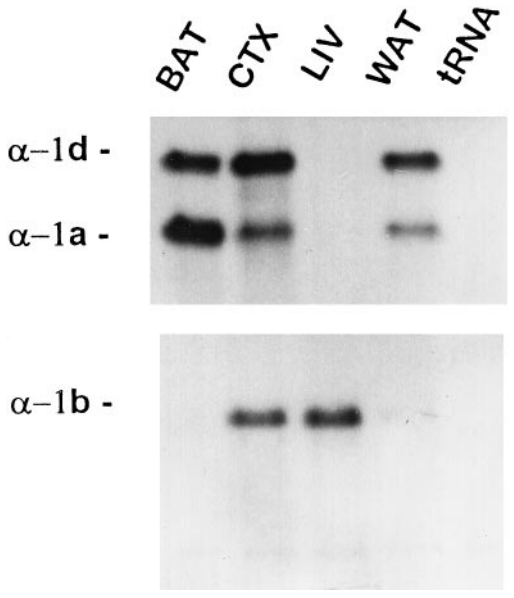

Fig. 1. Detection of α_1 receptor subtype mRNAs by nuclease protection assay. Autoradiograms identifying α_1 subtype mRNA in BAT, white adipose tissue (*WAT*), cerebral cortex (*CTX*), liver (*LIV*), and tRNA are shown.

tained all three subtypes. Probes of α_{1a} and α_{1d} receptor mRNA were of equal specific activity and were assayed in the same reaction sample. Therefore, BAT expressed comparable levels of α_{1a} and α_{1d} receptor mRNA.

The above experiment established the presence of α_{1a} and α_{1d} receptor mRNAs in BAT. To determine the relative expression of the protein(s) encoded by these mRNAs, pharmacological analysis of [^{125}I]HEAT binding sites was performed in BAT membranes and in membranes of COS-7 cells transfected with α_{1a} and α_{1d} receptor cDNAs. Membranes from BAT and transfected COS-7 cells displayed saturable (K_d values between 55 and 75 pM) [^{125}I]HEAT binding sites that were displaced specifically by 1 μM prazosin. Competition analysis was performed with various compounds in the presence of 50 pM [^{125}I]HEAT. Fig. 2 illustrates a typical experiment. Oxymetazoline, 5-methylurapidil, and BMY 7378 had dramatically different affinities for recombinant α_{1a} and α_{1d} receptors expressed in COS-7 cells, as expected from previous studies of the recombinant receptors (11–13). Competition curves for [^{125}I]HEAT binding sites in BAT membranes were identical to COS-7 cells expressing α_{1a} receptors. Table 1 summarizes the K_i values of various subtype-selective and nonselective compounds in displacing [^{125}I]HEAT binding sites. The pharmacological characteristics of the BAT α_1 receptor were virtually identical to those of COS-7 cells expressing the recombinant α_{1a} receptor and clearly different from the α_{1d} receptor. These data indicate that the dominant, if not exclusive, subtype in BAT is the α_{1a} receptor.

No pharmacological evidence could be found for the presence of α_{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 α_1 receptor mRNA and binding in COS-7 cells transiently transfected with the respective cDNAs. Transfection resulted in nearly equivalent levels of α_{1a} and α_{1d} receptor mRNA, but levels of α_{1d} receptor binding were less than 2% of α_{1a} binding (Fig. 3).

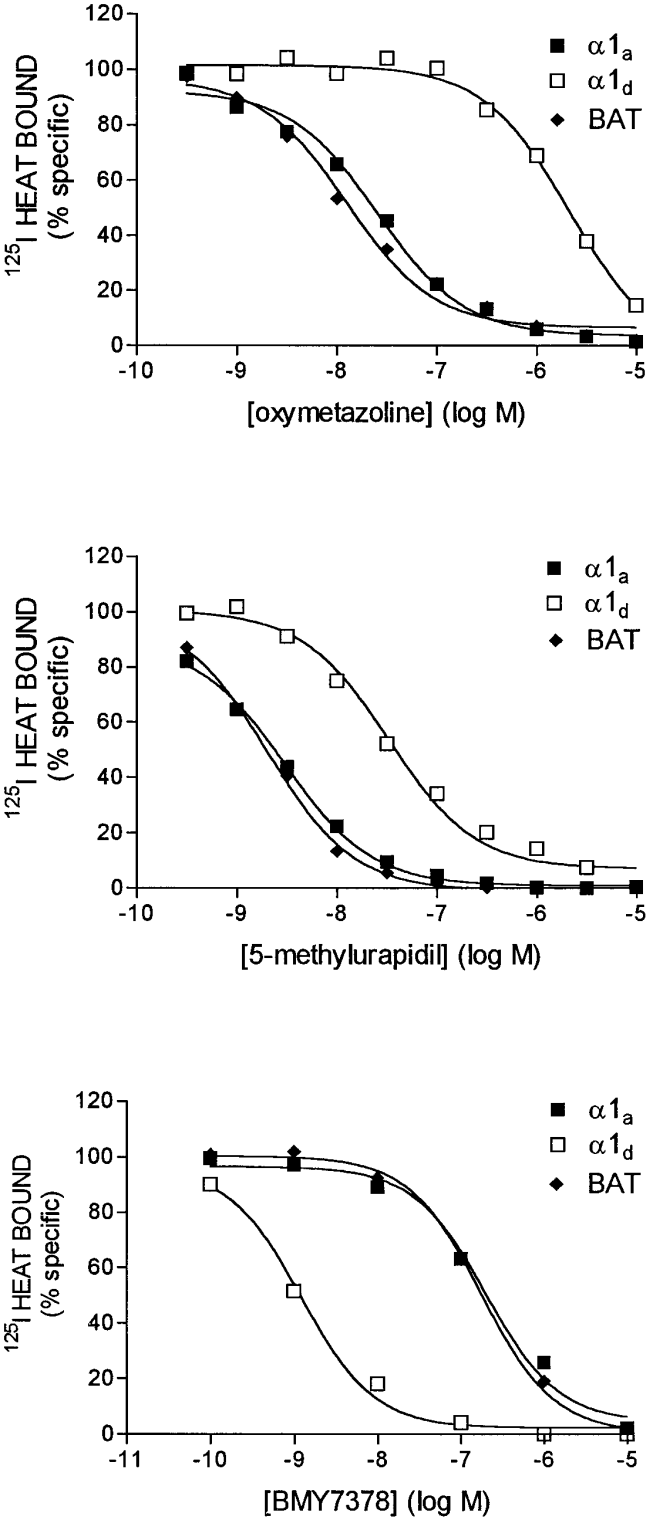

Fig. 2. Pharmacological characterization of α_1 receptors in BAT. Competition analysis of [^{125}I]HEAT binding sites was performed in membranes from BAT and COS-7 cells expressing recombinant rat α_{1a} and rat α_{1d} receptors. Representative experiments performed in duplicate are shown. Each experiment was performed independently at least three times, and data are summarized in Table 1.

We next turned our attention to the regulation of α_1 receptor expression in BAT by cold exposure. As mentioned above, cold exposure was shown to increase [^{3}H]prazosin binding

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TABLE 1

Pharmacologic analysis of ^{125}I-HEAT binding sites in membranes from BAT and COS-7 cells expressing recombinant rat α_{1a} and rat α_{1d} receptors

Values are means ± standard error; n = 3–4.

Compounds	K_i		
	BAT	α_{1a}	α_{1d}
		nM	
Oxymetazoline	5.0 ± 0.4	11.6 ± 0.37	1210.0 ± 45.0
5-Methylurapidil	1.27 ± 0.1	2.17 ± 1.2	16.0 ± 4.0
(*S*)-(+)-Niguldipine	15.9 ± 10.3	9.0 ± 3.8	137.0 ± 19.9
Prazosin	0.11 ± 0.01	0.21 ± 0.07	0.08 ± 0.01
Phentolamine	4.9 ± 0.2	7.1 ± 0.4	20.5 ± 2.1
BMY 7378	119.1 ± 11.9	132.7 ± 9.6	0.9 ± 0.2

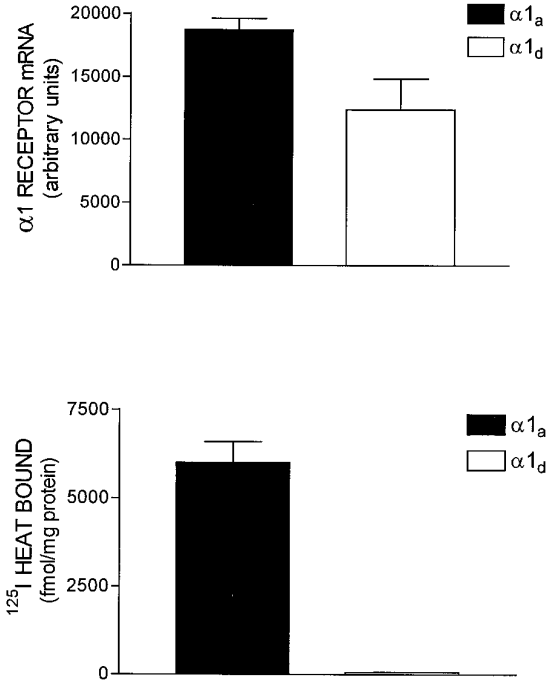

Fig. 3. Relationship between [^{125}I]HEAT binding sites and α_1 receptor mRNA expression in transfected COS-7 cells. *Top*, α_{1a} and α_{1d} receptor mRNA in transfected COS-7 cells. *Bottom*, number of α_1 receptors specifically labeled by [^{125}I]HEAT in membranes from transfected cells.

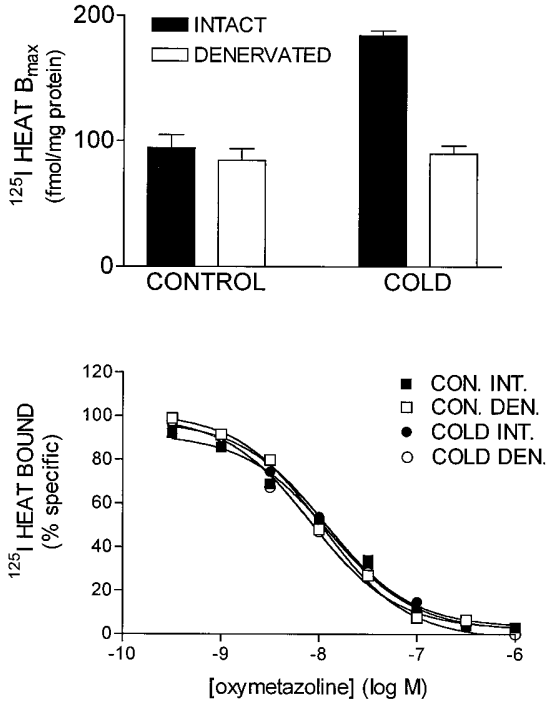

Fig. 4. Effect of denervation and cold exposure on α_1 receptor expression in BAT. *Top*, total α_1 receptor number (B_{max}) estimated by [^{125}I]HEAT saturation binding and nonlinear regression analysis. Values are mean ± standard error (three experiments). *Bottom*, competition analysis of [^{125}I]HEAT binding sites with α_{1a}-selective agonist oxymetazoline. A representative experiment is shown. In two independent replications, K_i values for oxymetazoline ranged from 3.4 to 5.6 nM in the various treatment groups. *CON*, control; *INT*, intact; *DEN*, denervated.

sites in BAT, although the mechanism of the increase and the subtype involved were not addressed previously (8). Saturation 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 α_{1a} receptors in all treatment conditions (Fig. 4, *bottom*). Surgical denervation had no effect on α_{1a} receptors in warm-adapted animals but completely prevented the cold-induced elevation ($p < 0.01$).

Levels of α_1 receptor mRNA also were measured in tissue samples from these same animals (Fig. 5). Cold stress did not alter α_{1d} receptor mRNA in neurally intact BAT (101 ± 9% versus 100 ± 7% in controls). Because α_{1d} receptor mRNA was unaffected by cold stress, we routinely monitored its level as an internal control for RNA recovery. In contrast to α_{1d} receptors, cold exposure significantly increased levels of α_{1a} receptor mRNA ($p < 0.001$). Surgical denervation reduced levels of α_{1a} receptor mRNA in control animals ($p <$ 0.05), indicating a role for tonic sympathetic stimulation in the regulation of α_{1a} receptor mRNA. Denervation also prevented full induction of α_{1a} receptor mRNA by cold ($p < 0.01$ versus intact cold-exposed). Cold exposure, however, increased α_{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 α_{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, β_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 β_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 α_{1a} binding and mRNA (both $p < 0.01$) in warm-adapted rats (Fig. 6). There was a trend for NE infusion to increase α_{1a} binding and mRNA; however, this effect was not statistically significant. It should be noted that NE, unlike CL 316,243, activates β_1-adrenergic receptors and stimulates BAT hyperplasia (17). In the present experiment, NE infusions increased total α_{1a} binding and mRNA per pad by approximately 40%, an increase that kept pace with the growth of the tissue.

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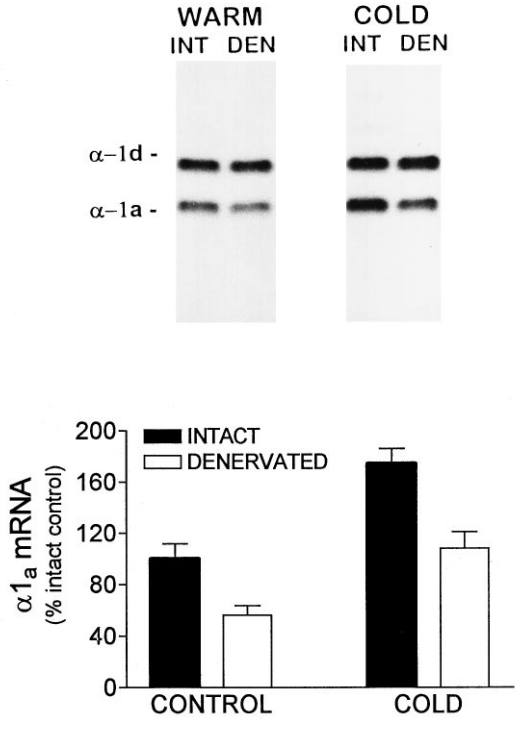

Fig. 5. Effect of denervation and cold exposure on α_1 receptor mRNA expression in BAT. *Top*, α_{1a} and α_{1d} receptor mRNA were determined in the same sample by nuclease protection assay. An autoradiogram of probes protected by tissue RNA is shown. *INT*, intact; *DEN*, denervated. *Bottom*, summary of densitometric analysis of autoradiograms (seven or eight independent samples).

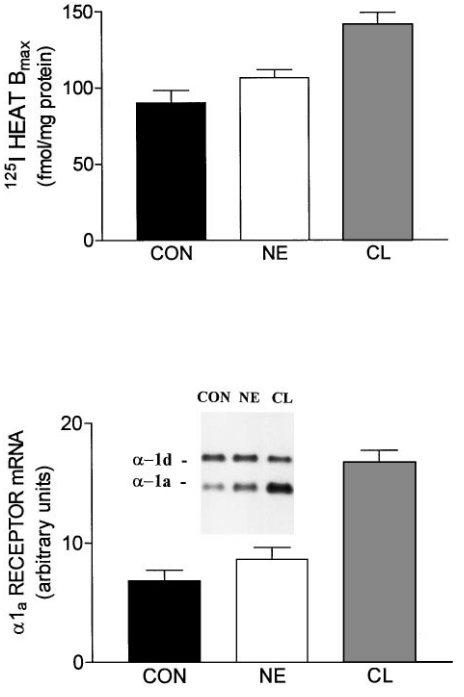

Fig. 6. Effects of agonist infusion on α_{1a} receptor number (*top*) and mRNA expression (*bottom*) in BAT. Rats were infused with vehicle (*CON*), NE (100 nmol/hr), or CL 316,243 (15 nmol/hr) for 4 days. α_{1a} receptor number was determined by [^{125}I]HEAT saturation binding analysis (four to five experiments), and α_{1a} receptor mRNA was determined by nuclease protection assay (seven to eight independent samples). *Inset*, autoradiogram of α_{1a} receptor and α_{1d} receptor mRNAs detected by nuclease protection assay. Levels of α_{1d} mRNA monitored concurrently were: 8.4 ± 1.0, 8.3 ± 0.9, and 8.0 ± 0.5 for control, NE-treated, and CL 316,243-treated rats, respectively.

Discussion

The present study characterized the α_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 α_{1a} and α_{1d} but not α_{1b}, receptors. Parallel radioligand binding studies comparing the pharmacologic properties of BAT α_1 receptors with those of the recombinant α_{1a} and α_{1d} expressed in COS-7 cells demonstrated that the receptors present in BAT are predominantly, if not exclusively, the α_{1a} subtype. Although α_{1a} and α_{1d} receptor mRNAs were expressed at similar levels in BAT, no α_{1d} binding sites could be detected. A similar discrepancy between functional responses mediated by α_{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 α_1 receptor subtypes and indicate that the presence of abundant α_{1d} receptor mRNA levels does not necessarily indicate abundant expression of the protein.

Previous work has shown that α_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 α_{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 α_{1a} receptor expression. Taken together, these results indicate that cold-induced up-regulation of α_{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 α_{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 β_3 agonist CL 316,243 strongly elevated expression of α_{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 β_3 receptor (16, 17, 19). Furthermore, because the β_3 receptor is resistant to desensitization (20), BAT 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 α_{1a} receptors in BAT. In addition, NE has a higher affinity for β_1 versus β_3 receptors (20). It is possible that the concentrations of NE used in the present study predominately activate β_1 receptors, which are desensitized rapidly (20). Unfortunately, higher doses of NE that would be required to strongly activate β_3 receptors are not well tolerated by rats owing to systemic effects of the agonist.

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The increased expression of α_{1a} binding sites was paralleled by selective up-regulation of α_{1a} receptor mRNA levels, suggesting that the increase involves increased receptor synthesis. Recently, NE was found to selectively increase expression of α_{1a} receptors in cardiac myocytes via α_1 adrenoreceptors (21). In contrast, the ability of β_3 receptor activation to fully induce α_{1a} receptor mRNA and protein expression in BAT indicates that this effect most likely involves generation of cyclic AMP (16). Up-regulation of α_{1b}-adrenergic receptors by a cyclic AMP-dependent mechanism has been reported in DDT_1 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 α_{1b} gene (24). Little is known about the structure of the α_{1a} receptor gene; however, the present results suggest that the α_{1a} receptor gene might contain DNA sequences capable of binding cAMP response element-binding protein-related transcription factors that are capable of integrating β- and α_1-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 α_1 receptors are also involved. One means by which α_1 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 α_{1a} 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 G_s (28–30), suggesting that AC-III might integrate signals from the α_1- and β-adrenergic pathways (6). The coordinate expression of α_{1a} receptors and AC-III suggests that the interaction of α_1- 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, α_1-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), α_1 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 α_1- and β-adrenergic receptors. It is therefore possible that the up-regulation of α_{1a} receptors reported here facilitates mitochondriogenesis and BAT recruitment during sustained neural stimulation.

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