The Guanosine Monophosphate Reductase Gene Is Conserved in Rats and Its Expression Increases Rapidly in Brown Adipose Tissue during Cold Exposure*

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Non-shivering thermogenesis is required for survival of rodents during cold stress. Un coupling protein-1 acts in brown adipose tissue (BAT) to transport protons, thus dissipating the proton gradient across the inner mitochondrial membrane. This permits respiration uncoupled from ATP synthesis. UCP-1 function is inhibited by the binding of purine nucleotides, with GTP/GDP being more potent than ATP/ADP. We used a cDNA subtraction analysis to identify cDNAs rapidly induced by cold exposure. One of these encodes rat guanosine monophosphate reductase (GMP-r). This was surprising in that previous data had suggested that this enzyme was absent in rodents. Rat GMP-r is 96% identical to human GMP-r, and its mRNA is increased 30-fold in BAT within 6 h of cold exposure. The gene is also expressed (but not cold-responsive) in muscle and kidney, but not in white fat. We speculate that the physiological function of the marked increase in BAT GMP-r during cold stress may be to deplete the brown adipocyte of guanine nucleotides, converting them to IMP, thus permitting enhanced UCP-1 function. This is a previously unrecognized regulatory aspect of thermogenesis, an essential physiological response of rodents to cold.

Adaptation to cold in rodents requires a physiological process called non-shivering or facultative thermogenesis. Brown adipose tissue (BAT) plays the dominant role in this response through its capacity to produce heat by uncoupling oxidative phosphorylation. Thermogenesis in BAT is initiated in the hypothalamus and is effected via the intense sympathetic nervous innervation of this tissue. Many genes are, either directly or indirectly, transcriptionally up-regulated by the adrenergic innervation of this tissue. Many genes are, either directly or indirectly, transcriptionally up-regulated by the adrenergic innervation of this tissue. In fact, several previous reports concluded that GMP reductase, present in Escherichia coli and humans, was lost during the evolution of rodents and that an unrelated enzyme catalyzes this reaction in those species (11, 12). The present studies show that this is not the case and demonstrate, furthermore, a marked and rapid increase in GMP reductase in BAT after cold exposure suggesting a previously unrecognized critical role for the reaction catalyzed by this enzyme in non-shivering thermogenesis. As mentioned, guanine nucleotides are more potent inhibitors than are adenine nucleotides of fatty acid transport by UCP-1 (8, 9, 13). We speculate that GMP reductase may enhance UCP-1 function by reducing its inhibition by endogenous guanine nucleotides.

EXPERIMENTAL PROCEDURES

Cold Exposure and RNA Isolation—Male Sprague-Dawley rats (100–125 g) were obtained from Zivic-Miller Laboratories and used under a protocol approved by the Animal Use Committee at Harvard Medical School. Rats were divided into two groups: one (control group) was kept at 21 °C (cDNA(−)), and the other was maintained at 4 °C (cDNA(+) ). Rats were killed, and interscapular BAT was dissected and immediately frozen in liquid N2. Total RNA was isolated by the guanidinium/phenol method (14). Poly(A)+ RNA was selected by passage through an oligo(dT) column (Amersham Pharmacia Biotech, Type T7).

Subtraction Library and cDNA Cloning—A PCR-based subtractive hybridization strategy was followed to isolate cDNAs that were rapidly increased during cold exposure (10). The two mRNA populations were converted to double-stranded cDNAs, fragmented by digestion with 

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† The abbreviations used are: BAT, brown adipose tissue; GMP-r, GMP reductase; UCP, uncoupler protein; PCR, polymerase chain reaction; bp, base pair(s); MOPS, 4-morpholinepropanesulfonic acid.

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**RESULTS**

**Cloning and Sequence Analysis of Rat GMP Reductase cDNA**—To identify genes involved in cold adaptation in brown adipose tissue, a PCR-based subtractive cDNA library was prepared using interscapular BAT poly(A)+ mRNA from control and cold-stimulated rats. One of the cDNA fragments (clone 4) derived from the cDNA(+) that met our selection criteria (expressed exclusively in BAT and markedly increased during acute cold exposure) was subcloned and sequenced. The sequence obtained was highly similar to the human GMP reductase, and the partial cDNA was used to screen a cDNA library prepared from BAT from cold-exposed rats (see “Experimental Procedures”). A 1452-bp clone was identified and sequenced (Fig. 1). It contained an open reading frame of 1035 nucleotides, encoding a deduced protein of 345 amino acids that is 96% identical to the human GMP reductase (Fig. 2). The two cDNAs share a 79% identity at the nucleotide level. There is a polyadenylation site (AATAA) in the cDNA located at nucleotide 1492, 14 nucleotides upstream of the poly(A) tail. On the basis of this high homology, this cDNA encodes the rat GMP reductase.

**Tissue Expression of the GMP Reductase Gene in the Rat**—The identification of the GMP reductase cDNA prompted us to investigate factors regulating its expression in greater detail. To explore its expression during cold exposure, we analyzed nine rat tissues by Northern blot analysis comparing the GMP reductase expression of control and cold-stimulated rats. As shown in Fig. 3, the GMP reductase mRNA is highly expressed in BAT as compared with other tissues. It is also expressed in skeletal and cardiac muscle, but in those tissues its expression is 5-fold lower than in unstimulated BAT, and it does not increase during cold stress. GMP reductase is not expressed in testis, lung, liver, or spleen, but it is weakly expressed in kidney from cold-stimulated animals. In contrast to the high levels of GMP reductase mRNA in brown fat, no specific signal could be detected in white fat (Fig. 3).

**Effect of Cold Exposure on GMP Reductase mRNA and Protein**—Exposure to 4°C for 6 h caused a marked increase in GMP reductase mRNA only in BAT. To analyze this issue further we compared GMP reductase mRNA and protein expression after increasing the time of cold exposure. After only 3 h at 4°C, GMP-reductase mRNA increased 10-fold reaching the maximum levels of 30-fold after 6 h (Fig. 4A). The mRNA remained elevated during 4 days at 4°C. The increases in GMP reductase mRNA are similar in timing to those of D2 mRNA (Fig. 4B), the enzyme that generates 3,5,3′-triiodothyronine in BAT. Actin mRNA did not change significantly in the same samples (Fig. 4C).

Western blotting with an antisera directed against residues 2 to 16 of the human GMP reductase (100% identical in the rat protein (Fig. 2)) showed a 37-kDa protein in BAT lysates that is not visible in spleen (Fig. 5A). After 6 h of cold exposure the 37-kDa band is increased 4-fold over control and reaches its maximum level at 24 h (7-fold). It is still 3-fold higher than control after 72 h of cold exposure (Fig. 5B).

**Effect of Thyroid Status on GMP Reductase in BAT**—Thyroid hormone levels influence adenrenergic status and lipogenesis in rat BAT with increased noradrenaline turnover in hypothryroid animals and reduced sympathetic tone during hypothyroidism (reviewed in Refs. 1 and 17). Interestingly, thyroid status strongly affected GMP reductase expression at both the mRNA and protein levels. As shown in Fig. 3, GMP reductase mRNA is 4-fold higher in BAT from hypothyroid rats maintained at 21 °C than in controls. On the other hand, BAT GMP reductase mRNA is reduced 3-fold in hypothyroid rats. In thyroidectomized animals, GMP reductase protein is also in...
creased 3-fold compared with euthyroid rats, and conversely, it is suppressed to 30% of control in BAT from hyperthyroid animals (Fig. 5B).

**DISCUSSION**

In rodents, cold adaptation is essential for survival. During the first hours of cold exposure there is a marked stimulation of cold adaptation genes.

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**Fig. 1.** Nucleotide sequence of the cDNA encoding the rat GMP reductase gene.

**Fig. 2.** Comparison between rat and human GMP reductase (GMP-r) genes at the amino acid level.
BAT through the sympathetic nervous system culminating in thermogenesis in this organ (2). We have used a PCR subtraction strategy to identify a previously unrecognized gene involved in these early events. After only 3 h of cold exposure, we detect a marked increase in the expression of GMP reductase mRNA and protein. This enzyme reduces GMP, producing IMP, thus representing the only step by which guanine nucleotides can be converted to IMP and subsequently to adenine nucleotides.

The human GMP reductase enzyme has an unusual history. It was initially cloned as one portion of a cDNA cloning artifact and thought to represent part of a post-transcriptionally generated chimeric protein (11, 18). This error was recognized subsequently (16, 19, 20), but since then, no further studies

**FIG. 3.** Northern blot analysis showing the tissue distribution of GMP reductase in rat tissues, the effects of cold exposure changes, and the thyroid status level on GMP reductase mRNA expression in BAT and other tissue. Twenty μg of total RNA were electrophoresed and blotted as described under “Experimental Procedures.”

**FIG. 4.** Effect of cold exposure on GMP reductase (A), type 2 deiodinase (B), and β-actin (C) mRNA levels in rat BAT. The filter was first hybridized with an 0.8-kilobase pair probe from the rat GMP reductase cDNA. Thereafter, a second hybridization was performed on the same filter with a type 2 deiodinase-specific probe (B). C shows results of probing with rat actin, and D shows the ethidium bromide-stained gel prior to transfer.

**FIG. 5.** Western blot analysis of GMP reductase protein expression in BAT and spleen from rats exposed to 4 °C for 5 h (A) and various times (B). Tissue homogenate protein (50 μg) from control BAT and from cold-stimulated rats were examined by Western blot analysis using antibody directed against residues 2–16 of GMP reductase (B) as described previously. Equal protein loading and transfer were verified by Ponceau staining of the membrane. In the last two lanes of panel B, BAT protein from rats 10 days after thyroidectomy (Tx) or after treatment with 3,5,3′-triiodothyronine (T3) for 7 days (see “Experimental Procedures”) was similarly analyzed.
have been performed on this gene besides the characterization of its structure and genetic polymorphism and its assignment to human chromosome 6 (12, 21, 22). Previous evidence suggested that this enzyme, present in *E. coli* and humans, was somehow lost during evolution in rodents. The failure to detect a similar cDNA in the mouse and hamster genomic DNA (21), together with earlier data showing that rat tissue can convert guanine to hypoxanthine (23), had previously suggested that an unrelated or distantly related enzyme could catalyze this reaction in these species (20). Here we show that this is not the case since not only is GMP reductase expressed in rat BAT, but its expression is highly regulated in this tissue. Rat GMP reductase is highly homologous to human GMP reductase, sharing over 95% identity at the amino acid level.

The tissue distribution of GMP reductase has not been explored previously. Rat GMP reductase mRNA is highly expressed in BAT, even under basal conditions, and is also present in skeletal and cardiac muscle and kidney, but is absent in testis, liver, lung, spleen, and interestingly, white fat. Furthermore, in BAT, its expression is responsive to conditions that regulate BAT function such as cold stimulation or thyroid status (Figs. 3 and 4). The presence of GMP reductase mRNA in other tissues implies more general functions of this enzyme outside those of thermogenesis. However, after only 3 h of cold exposure, BAT GMP reductase RNA increases 10 times over control, reaching the maximum expression level of 30-fold after 6 h (Fig. 4). Previous work clearly demonstrated that optimal control, reaching the maximum expression level of 30-fold after exposure, BAT GMP reductase RNA increases 10 times over other tissues implies more general functions of this enzyme status (Figs. 3 and 4). The presence of GMP reductase mRNA in more, in BAT, its expression is responsive to conditions that function in skeletal and cardiac muscle and kidney, but is absent in expressed in BAT, even under basal conditions, and is also pres-

GMP reductase is highly homologous to human GMP reductase, and GMP reductase may also play a role in the brown fat response. The reductive deamination of GMP to IMP could potentially somehow lost during evolution in rodents. The failure to detect a similar cDNA in the mouse and hamster genomic DNA (21), together with earlier data showing that rat tissue can convert guanine to hypoxanthine (23), had previously suggested that an unrelated or distantly related enzyme could catalyze this reaction in these species (20). Here we show that this is not the case since not only is GMP reductase expressed in rat BAT, but its expression is highly regulated in this tissue. Rat GMP reductase is highly homologous to human GMP reductase, sharing over 95% identity at the amino acid level.

How can a requirement for GMP reductase in the acute response of brown adipocyte be explained? It can be hypothe-
sized that the nucleotide binding site of UCP-1 is normally blocked primarily by guanine, rather than adenine nucleotides, due both to a higher affinity of the guanine nucleotides and as a high ratio of guanine to adenine nucleotides in the mitochondri-

The parallel effects of cold exposure to increase UCP-1, D2, and GMP reductase mRNAs (Figs. 3–5) point to overlapping and synergistic mechanisms that have evolved for stimulation of heat production in rodents. Presumably, these genes will be found to contain common transcriptional control elements, such as cyclic AMP response elements, to explain their similar response. The fact that GMP reductase, like D2, is increased in BAT of hypothyroid rats and decreased in hyperthyroidism (Figs. 5) shows that compensatory changes occur in the expression of both genes to modulate facultative thermogenesis in a physiologically appropriate fashion.

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