Basal adrenergic tone is required for maximal stimulation of rat brown adipose tissue UCP1 expression by chronic PPAR-γ activation

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Festuccia WT, Blanchard PG, Richard D, Deshaies Y. Basal adrenergic tone is required for maximal stimulation of rat brown adipose tissue UCP1 expression by chronic PPAR-γ activation. Am J Physiol Regul Integr Comp Physiol 299: R159–R167, 2010. First published April 14, 2010; doi:10.1152/ajpregu.00821.2009.—We investigated the involvement of basal sympathetic tone in brown adipose tissue (BAT) recruitment and gene expression profile induced by peroxisome proliferator-activated receptor-γ (PPAR-γ) activation. Innervated and surgically denervated BAT pads of rats treated or not with rosiglitazone (15 mg·kg⁻¹·day⁻¹, 7 days) were evaluated for weight, triacylglycerol (TAG) and DNA content, mitochondrial mass, and gene expression. Rosiglitazone induced BAT recruitment (increased mass, TAG and DNA content) and mRNA levels of lipolytic (adipose tissue triglyceride lipase and CPG-48) and lipogenic (lipoprotein lipase, phosphoenolpyruvate carboxykinase, fatty acid binding protein 4, and diacylglycerol acyltransferase 1) proteins independently of tissue innervation status. Mitochondrial mass and mRNA levels of its regulators peroxisome proliferator-activated receptor coactivator-α and CCAAT/enhancer binding protein-β were not affected by rosiglitazone, while being significantly reduced by denervation. By contrast, maximal stimulation of uncoupling protein 1 (UCP1) (thermogenesis), cell death-inducing DNA fragmentation factor-45-like effector A (an inhibitor of UCP1 activity), monoacylglycerol lipase (lipolysis), small heterodimer partner (transcription), and glycogen synthase (TAG synthesis) by rosiglitazone depended on the presence of intact BAT innervation. Cold exposure (5°C, 24 h) significantly increased UCP1 mRNA levels in innervated BAT pads of untreated rats, without affecting the already high BAT UCP1 levels of rosiglitazone-treated animals. A similar pattern of response was found in denervated pads, but with markedly lower UCP1 expression than that in innervated BAT. In conclusion, whereas the mass (hyperplasia and hypertrophy), lipogenic, and lipolytic components of BAT recruitment induced by rosiglitazone occur independently of tissue sympathetic innervation, maximal UCP1 expression induced by PPAR-γ in vivo depends on the presence of basal BAT adrenergic tone. The residual sympathetic tone found under rosiglitazone treatment is, therefore, involved in the modulation of a subset of major components of PPAR-γ-mediated BAT recruitment.

thermogenesis; sympathetic innervation; mitochondria; peroxisome proliferator-activated receptor coactivator-α; rosiglitazone

THE RECENT CONFIRMATION THAT a substantial proportion of adult humans possesses active brown adipose tissue (BAT) has renewed the interest in the metabolic significance of BAT nonshivering thermogenesis as a possible alternative to treat obesity (6, 24, 38, 39, 43). The sympathetic nervous system (SNS), through direct tissue innervation, is the major activator of BAT lipolysis and thermogenesis (5). On activation, BAT sympathetic nerves release norepinephrine (NE) that activates mainly β-adrenergic receptors, lipase activity, and triacylglycerol (TAG) hydrolysis. Whereas lipolysis-derived glycerol is recycled back to TAG by the action of glycerokinase (GyK) (11), lipolysis-derived fatty acids are directed to the mitochondria, where they either are oxidized or allosterically activate uncoupling protein 1 (UCP1) and thus thermogenesis.

In addition to thermogenesis, sympathetic innervation is a major regulator of brown adipocyte proliferation, differentiation, and apoptosis, among other processes implicated in BAT maintenance and function (5). Chronic sympathetic activation increases BAT mass by enhancing brown adipocyte proliferation and differentiation and by reducing apoptosis (4, 5, 21). Sympathetic activation also stimulates BAT mitochondrial biogenesis and the expression of thermogenic proteins, such as UCP1 and peroxisome proliferator-activated receptor coactivator-α (PGC-α) (7, 26, 33). The functional result of sympathetically mediated BAT recruitment is a marked amplification of the tissue capacity to produce heat (5).

Chronic sympathetic activation was the only recognized inducer of BAT recruitment until the discovery that activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) with synthetic ligands also increases BAT mass and UCP1 levels in rodents (22, 27, 36). PPAR-γ is a nuclear receptor highly expressed in BAT that acts as a master transcriptional regulator of brown adipocyte differentiation required for tissue development, function, and survival (1, 9, 14–16, 25). Remarkably, however, PPAR-γ-mediated BAT recruitment is associated with a reduction in BAT sympathetic activity (∼50%) and thyroid status, which seem to prevent the translation of a high thermogenic capacity (BAT mass and UCP1 content) into increased functional thermogenic activity (12).

In brown adipocytes in vitro, PPAR-γ activation competently induces thermogenic features, such as UCP1 expression and mitochondrial biogenesis, independently of adrenergic activation (25, 35). Although basal respiration is unchanged, acute noradrenaline elicits a robust response in PPAR-γ-exposed brown adipocytes commensurate with their higher mitochondrial and UCP1 content (25, 35). A major factor to consider when comparing in vitro and in vivo BAT recruitment is that, in the latter, BAT is continuously under the influence of an adrenergic tone, the intensity of which varies, according to ambient temperature and feeding status (5). Considering that the sympathetic tone, by modulating the levels of PPAR-γ (20) and its coactivator PGC-α (26), is liable to modulate the transcriptional activity of this nuclear receptor, and that the adrenergic and PPAR-γ pathways share common targets (e.g., UCP1), we investigated the involvement of BAT residual sympathetic activity in the in vivo effects of PPAR-γ ligand treatment on tissue recruitment (increase in tissue mass/cellu-
larity) and gene expression profile of several proteins implicated in BAT maintenance and function. This was achieved in rats subjected to surgical sympathetic hemidenervation of interscapular BAT and treated or not with the PPAR-γ ligand rosiglitazone for 7 days. Additional animals were also challenged with a short period of cold exposure, a situation characterized by a marked increase in BAT sympathetic activity (13), to evaluate whether simultaneous PPAR-γ and sympathetic activation have additive effects on BAT UCP1 expression.

MATERIALS AND METHODS

Animals. BAT denervation, and treatment. Animal care and handling were performed in accordance with the Canadian Guide for the Care and Use of Laboratory Animals. All experimental procedures received prior approval of the Laval University animal care committee. Male Sprague-Dawley rats (125–150 g) were individually housed in stainless steel cages in a room kept at 23 ± 1°C with a light-dark cycle of 12:12 h (lights on at 0800). After a 4-day adaptation period, rats were anesthetized with isoflurane and an incision was made in the midline interscapular region. Unilateral BAT denervation was performed by removing a 5-mm section of five branches of the right intercostal nerve bundles, as previously described (11). The contralateral pad was left intact and used as a within-animal control. Before and 2 days following surgery, rats were given an analgesic (ketoprophen, 5 mg/kg, twice a day). After a 7-day recovery period, rats were matched by weight and divided into control and rosiglitazone-treated groups. Following surgery, rats were given an analgesic (ketoprophen, 5 mg/kg, twice a day). After a 7-day recovery period, rats were matched by weight and divided into control and rosiglitazone-treated groups. Additional animals were also chal-

Table 1. Pairs of primers used for quantification of real-time PCR

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ATGL, adipose tissue triglyceride lipase; β3-ADR, β3-adrenergic receptor; C/EBP, CCAAT enhancer binding protein; CIDEA, cell death-inducing DNA fragmentation factor-κB-like effector A; COX4, cytochrome oxidase 4; mCPT1, muscle type carnitine palmitoyltransferase 1; DGAT1, diacylglycerol acyltransferase 1; FABP4, fatty acid binding protein 4; FATP1, fatty acid transporter protein 1; FJF1, fatty acid transporter protein 1; GyK, glycerokinase; LPL, lipoprotein lipase; MGL, monacylglycerol lipase; ND2, NADH dehydrogenase subunit 2; PECCK, phosphoenolpyruvate carboxykinase; PGC, peroxisome proliferator-activated receptor coactivator; PPAR, peroxisome proliferator-activated receptor; PRDM16, positive regulatory domain containing 16; SHP, small heterodimer partner (NROB2); SIRT, sirtuin; UCPI, uncoupling protein 1.

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the PCR products was achieved with SYBR Green I (Molecular Probes, Willamette Valley, OR). At the end of each run, melt curve analyses were performed, and a few samples representative of each experimental group were run on agarose gel to ensure the specificity of the amplification. Results are expressed as the ratio of the expression of the target gene to that of the housekeeping gene 36B4 (NM_022402), which was selected because no significant variation in its expression was observed after sympathetic denervation or rosiglitazone treatment.

Adipocyte morphology by light microscopy. Innervated and denervated BAT were harvested, and portions of the tissues were fixed in 0.1 mM PBS, pH 7.4, containing 4% paraformaldehyde and embedded in paraffin. Thin sections were mounted on glass slides and dyed with hematoxylin/eosin. Digital images of tissue slices were captured and analyzed as previously described (12).

BAT TAG and DNA contents. BAT TAG content was measured after lipid extraction with chloroform-methanol (2:1) with an enzymatic kit (Roche Diagnostics, Montreal, Canada). Tissue DNA content was determined using the DNeasy Tissue Kit (QIAGEN), following manufacturer’s instructions.

Statistical analysis. Results are expressed as means ± SE. Multifactorial ANOVA, followed by the Newman-Keuls’ multiple-range test, were used to analyze the effects of denervation, rosiglitazone treatment, cold exposure, and their interaction. P < 0.05 was chosen as the threshold of significance. The large number of comparisons increases the probability of type I errors; however, the calculated level of significance of individual comparisons (not shown) was, in many cases, well below the 0.05 threshold, thus reducing the likelihood of false positives.

RESULTS

Confirming previous studies, rosiglitazone treatment increased body weight gain (20%) and food intake (9%) and reduced plasma insulin (~37%), nonesterified fatty acid (~56%), and TAG (~42%) levels (Table 2). Thus BAT hemi-denervation did not interfere with the positive effects of rosiglitazone on energy balance and metabolic indexes of insulin sensitivity and lipemia.

The effects of rosiglitazone-mediated PPAR-γ activation on NE content (an index of sympathetic innervation density), morphology, cellularity, and receptor gene expression in innervated and denervated BAT pads are depicted in Fig. 1. Rosiglitazone did not affect NE content, regardless of innervation status, whereas denervation, as expected, markedly reduced NE (~90%) in both untreated and ligand-treated rats (Fig. 1A). Regarding tissue recruitment, rosiglitazone induced similar increases in BAT mass (~2-fold), DNA (~2.5-fold), and TAG (~1.6-fold) content, and the percentage of unilocular cells (~1.6-fold) in both intact and denervated BAT pads (Fig. 1, B–E, respectively). Rosiglitazone significantly reduced PPAR-γ2 mRNA levels in innervated (~50%), but not denervated, BAT pads (Fig. 1F). Levels of PPAR-γ1 and β3-adrenergic receptor mRNA were not affected by rosiglitazone or denervation (Fig. 1F).

BAT enlargement induced by PPAR-γ activation was associated with a significant increase, independently of tissue innervation status, in the mRNA levels of several lipogenic proteins, including lipoprotein lipase (LPL), phosphoenolpyruvate carboxykinase (PEPCK), fatty acid binding protein 4 (FABP4, also known as aP2), and diacylglycerol acyltransferase 1 (DGAT1) (Fig. 2, A, D, E, and F). No effect of denervation was seen on the mRNA levels of those genes in untreated rats, except PEPCK, whose mRNA levels were significantly upregulated by denervation. Rosiglitazone marked reduced fatty acid transporter protein 1 (FATP1) mRNA levels in innervated BAT to an extent identical to that achieved by denervation (Fig. 2B). Rosiglitazone significantly increased GyK mRNA levels in both innervated and denervated BAT pads, an effect that was significantly larger in the former than in the latter (Fig. 2C). In addition to attenuating rosiglitazone-mediated induction of GyK, denervation also significantly reduced GyK mRNA levels (~70%) in untreated rats.

In innervated BAT, rosiglitazone did not affect mitochondrial mass, as indicated by the lack of change in the mRNA levels of the mitochondrial proteins cytochrome oxidase 4 (COX4), NADH dehydrogenase subunit 2 (ND2) and F0F1 ATPase (F0F1) (Fig. 3, A–C). Denervation, however, induced a significant reduction (~35 to ~50%) in COX4, ND2, and F0F1 mRNA levels in both untreated and ligand-treated rats (Fig. 3, A–C). By contrast to markers of mitochondrial mass, rosiglitazone markedly increased BAT mRNA levels of UCP1 and cell death-inducing DNA fragmentation factor-45-like effector A (CIDEA; an attenuator of UCP1 activity) in innervated and, to a lesser extent, in the denervated BAT pads (Fig. 3, D and E). In addition to attenuating rosiglitazone-mediated induction of UCP1 and CIDEA, denervation significantly reduced UCP1 (~45%), but not CIDEA mRNA levels in untreated rats. No effect of rosiglitazone or denervation was seen on the mRNA levels of muscle type carnitine palmitoyltransferase 1, the rate-limiting step in mitochondrial fatty acid entry (Fig. 3F). Regarding proteins involved in lipolysis, rosiglitazone significantly increased mRNA levels of adipose triglyceride lipase (1.6-fold) and CGI58 (2-fold) independently of tissue innervation status (Fig. 3, G and H). Denervation did not affect mRNA levels of these proteins. Rosiglitazone (~2.5-fold increase) and denervation (~50% reduction) exerted opposite, independent effects on monacylglycerol lipase mRNA levels, whereas no treatment effect was seen on mRNA levels of hormone-sensitive lipase (data not shown).

In association with UCP1 and CIDEA, mRNA levels of key coregulators of nuclear receptor activity implicated in the control of BAT maintenance and function were also affected by the treatments. Rosiglitazone administration did not affect mRNA levels of PGC-α and β and CCAAT/enhancer binding protein (C/EBP)-α, -β, and -δ (Fig. 4, A, B, G, H, and I). By contrast, sympathetic denervation induced a significant reduction in the mRNA levels of PGC-α and C/EBP-β (~70 and ~55%, respectively), but not PGC-β and C/EBP-α and -δ.

Table 2. Final body weight, cumulative food intake, and serum concentrations of insulin and metabolites in hemi-denervated rats treated or not with RSG for 7 days

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<tr>
<td>Initial body weight, g</td>
<td>193 ± 3.8</td>
<td>194 ± 4.1</td>
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<tr>
<td>Final body weight, g</td>
<td>263 ± 3.6</td>
<td>279 ± 4.1*</td>
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<td>Body weight gain, g</td>
<td>71.2 ± 1.3</td>
<td>85.6 ± 3.7*</td>
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<tr>
<td>Food intake, g</td>
<td>210 ± 4.3</td>
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<tr>
<td>Insulin, pmol/l</td>
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<td>124 ± 21*</td>
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<td>Glucose, mmol/l</td>
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<tr>
<td>NEFA, mmol/l</td>
<td>0.51 ± 0.05</td>
<td>0.22 ± 0.06*</td>
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<tr>
<td>TAG, mmol/l</td>
<td>2.1 ± 0.3</td>
<td>1.2 ± 0.2*</td>
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Values are means ± SE of 12 rats. RSG, rosiglitazone; NEFA, nonesterified fatty acid; TAG, triacylglycerol. *P < 0.05 vs. control.

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Rosiglitazone markedly reduced mRNA levels of positive regulatory domain containing 16 (PRDM16), with such effect being similar to that induced by sympathetic denervation (Fig. 4C). Rosiglitazone also significantly increased mRNA levels of the small heterodimer partner (SHP, also known as NROB2) in intact BAT pads (2-fold), an effect that was completely abolished by denervation (Fig. 4D). Denervation alone also induced a 50% reduction in SHP mRNA levels. Both rosiglitazone and denervation equally reduced mRNA levels of the sirtuins (SIRT) 3 and 5 (Fig. 4E and F). An additive reduction in SIRT3, but not SIRT5, mRNA levels was induced by the combination of rosiglitazone and denervation.

We have previously shown that the rosiglitazone-induced increase in BAT thermogenic capacity, which does not translate into increased thermogenic activity in a warm environment, fully does so when the sympathetic drive to BAT is increased by administration of a β3-adrenergic agonist (30). Here we wished to examine whether the response of BAT UCP1 expression to an acute cold exposure, a physiological situation characterized by increased BAT sympathetic activity (13), is additive to rosiglitazone, and whether it is maintained in denervated BAT. As expected, 24-h exposure to cold increased UCP1 expression almost threefold in innervated BAT pads of untreated rats, without inducing any further increase in the already high UCP1 levels of rosiglitazone-treated rats (Fig. 5). A similar pattern of response was found in denervated pads, but with markedly lower UCP1 expression than in innervated BAT. This increase is probably related to the activation by cold of the sympathetic nerves that were not destroyed by surgical denervation, as well as to cold-induced release of catecholamines by the adrenals.

DISCUSSION

In the present study, by combining BAT sympathetic denervation with rosiglitazone treatment, we were able to elucidate the involvement of basal adrenergic tone in the induction of several components of BAT recruitment by PPAR-γ activation in vivo. Our main findings (Table 3) indicate that the rosiglitazone-induced increase in BAT mass, cellularity (DNA content), and mRNA levels of lipogenic (LPL, PEPCK, FABP4, and DGAT1) and lipolytic (adipose triglyceride lipase and CGI58) proteins occur independently of tissue basal adrenergic tone. By contrast, maximal stimulation of the thermogenic
(UCP1) and anti-thermogenic (CIDEA) mRNA levels by rosiglitazone depended on the presence of intact tissue sympathetic nerves. Further sympathetic activation by cold exposure did not amplify the rosiglitazone-induced induction of UCP1. Mitochondrial mass (COX4, ND2, and F0F1) and its regulators PGC-α and C/EBP-β were unaffected by rosiglitazone, but strongly reduced by denervation. These data strongly support the notion that, even under activation by a specific, potent ligand, PPAR-γ activity toward the UCP1 thermogenic component of BAT recruitment requires a minimum of adrenergic input to be maximally efficient.

![Fig. 2. Relative mRNA levels of proteins involved in lipogenesis in hemi-denervated brown adipose tissue of rats treated or not with RSG for 7 days. A: lipoprotein lipase (LPL); B: fatty acid transporter protein 1 (FATP1); C: glycerokinase (GyK); D: phosphoenolpyruvate carboxykinase (PEPCK); E: fatty acid binding protein 4 (FABP4); F: diacylglycerol acyltransferase 1 (DGAT1). Each bar represents the mean ± SE of 12 rats. a,b,c,d Means not sharing a common superscript are significantly different from each other, P < 0.05.](image1)

![Fig. 3. Relative mRNA levels of proteins involved in mitochondrial function, thermogenesis, and lipolysis in hemi-denervated brown adipose tissue of rats treated or not with RSG for 7 days. A: cytochrome oxidase 4 (COX4); B: NADH dehydrogenase subunit 2 (ND2); C: F0F1 ATPase (F0F1); D: uncoupling protein 1 (UCP1); E: cell death-inducing DNA fragmentation factor-45-like effector A (CIDEA); F: muscle type carnitine palmitoyltransferase 1 (mCPT1); G: adipose tissue triglyceride lipase (ATGL); H: CGI58; I: monoacylglycerol lipase (MGL). Each bar represents the mean ± SE of 12 rats. a,b,c,d Means not sharing a common superscript are significantly different from each other, P < 0.05.](image2)
We have previously shown that rosiglitazone significantly reduces BAT sympathetic activity (~50%), as estimated by NE turnover rate, without affecting BAT NE content (12), a marker of the amount of sympathetic nerves that reach a given tissue rather than activity (3, 34). Although PPAR-γ activation is capable of recruiting BAT in vitro independently of adrenergic input, given the importance of the sympathetic drive in the function of BAT in vivo, here we used surgical denervation to further reduce BAT sympathetic drive in rosiglitazone-treated rats, with the goal of delineating the contribution of basal adrenergic tone to BAT recruitment induced by the ligand. As estimated by tissue NE content, the surgery was effective in reducing BAT innervation to ~10% of that of intact pads, resulting in near-complete abolition of the sympathetic drive. It should be noted that surgical denervation also destroys sensory nerves that run along with the sympathetic fibers. BAT is innervated by various populations of sensory nerves (8) whose physiological functions are unknown. Thus it is difficult to predict their involvement in the paradigm under study here. An additional limitation of the study is that, although rosiglitazone has a high specificity for PPAR-γ, the possibility that some of its actions may be receptor-independent cannot be excluded.

Confirming previous studies, in vivo PPAR-γ ligand treatment was associated with BAT recruitment characterized by a marked increase in tissue mass (10, 12, 30, 36). Here we extend this notion by showing that BAT mass and its determinants in the context of PPAR-γ activation, namely hyperplasia (increase in cell number) and hypertrophy (increase in cell diameter) (2, 10, 12), were amplified independently of the presence of sympathetic innervation. The rosiglitazone-induced decrease in PPAR-γ mRNA in intact BAT did not affect the magnitude of recruitment. Rosiglitazone increases BAT TAG synthesis (lipogenesis) by enhancing LPL-mediated fatty acid...
provision and tissue uptake, glycerol 3-phosphate generation, and glycerol phosphate acyltransferase and DGAT activities (10, 19). In accordance with the sympathetically independent brown adipocyte hypertrophy, rosiglitazone increased mRNA levels of lipogenic proteins (LPL, PEPCCK, FABP4, and DGAT) also independently of BAT basal adrenergic tone, which demonstrates that sympathetic innervation is not required for PPAR-γ-induced BAT mass expansion. Among the lipogenic enzymes investigated, only GyK and FATP1 responded to denervation, with their reduction being expected because of their well-established modulation by the SNS in BAT (11, 41). Maximal induction of GyK mRNA levels by rosiglitazone depended on the presence of intact BAT innervation, although a smaller, but significant increase in GyK levels was induced by the ligand in denervated BAT pad, suggesting some independence of ligand action from adrenergic signaling. By contrast to GyK, FATP1 mRNA levels were reduced equally by rosiglitazone and denervation without additivity. This finding suggests that both denervation and ligand act through the same mechanism, probably the reduction in BAT adrenergic tone, and that the 50% reduction achieved by PPAR-γ activation (2, 10, 12) is sufficient for maximal effect on FATP1.

By contrast to tissue enlargement, mitochondrial mass, a functionally important component of BAT recruitment, was not affected by rosiglitazone, as evidenced by the lack of change in mRNA levels of the mitochondrial markers COX4, ND2, and FO1. These findings differ from those obtained in brown adipocytes in vitro, in which rosiglitazone upregulated mitochondrial mass independently of adrenergic stimulation (25). The reasons for this discrepancy between in vivo and in vitro findings are unknown and clearly deserve further investigation; possible mechanisms are addressed below. While insensitive to PPAR-γ activation, BAT mitochondrial mass was significantly affected by denervation, confirming the SNS as an important regulator of mitochondrial biogenesis. Taking into consideration that the sympathetic drive to BAT is reduced by PPAR-γ ligand treatment (12), one may have expected a reduction in mitochondrial mass in innervated BAT pads of rats treated with rosiglitazone. That this was not the case suggests that only severe changes in BAT sympathetic drive, such as those achieved by surgical denervation, can affect mitochondrial mass.

A likely candidate to mediate the interaction between BAT adrenergic tone and mitochondrial mass is PGC-α, a coactivator of PPAR-γ transcriptional activity. Expression of PGC-α and its transcriptional regulator C/EBP-β followed a pattern of modulation similar to that of the mitochondrial markers (no effect of rosiglitazone and reduction by denervation). These findings are in accordance with in vitro and in vivo studies showing that BAT PGC-α expression is under adrenergic control through cAMP response-element binding protein and C/EBP-β activation and correlates with mitochondrial mass (18, 26, 37). Interestingly, rosiglitazone increased mRNA levels of the orphan nuclear receptor SHP (NROB2), a negative regulator of PGC-α expression and energy expenditure in brown adipocytes (40), and reduced those of both SIRT3, a protein involved in the control of BAT PGC-α expression and mitochondrial biogenesis (31), and PRDM16, a zinc-finger protein that exerts its action in BAT by interacting with and modulating PPAR-γ transcriptional activity (17, 28, 29). SIRT3 is highly expressed in BAT, localizes at the mitochondrial inner membrane, and is upregulated by cold exposure (31). Forced expression of SIRT3 is associated with increased PGC-α and UCP1 levels and mitochondrial biogenesis (31). Similar to SIRT3, PRDM16 has been implicated in the determination of several BAT characteristics, including mitochondrial number, PGC-α, and UCP1 levels (29). Whether the upregulation of SHP and reduction in SIRT3 and PRDM16 levels are involved in the lack of effect of rosiglitazone on PGC-α expression and mitochondrial mass in vivo remains to be investigated. In addition to SIRT3, mRNA levels of SIRT5, which is expressed in BAT at significant levels (31), were also downregulated by rosiglitazone and denervation. The physiological role of SIRT5 in BAT, however, has not yet been elucidated.

Although BAT mitochondrial mass was not affected by rosiglitazone, UCP1 mRNA levels were significantly upregulated by the ligand, confirming earlier findings (12). Interestingly, denervation partially but robustly reduced UCP1 induction by rosiglitazone, demonstrating that maximal activation of UCP1 expression by PPAR-γ requires the presence of an intact BAT sympathetic innervation and basal adrenergic tone. These results are also in contrast with those found in vitro, where rosiglitazone maximally stimulated brown adipocyte UCP1 expression in the absence of an adrenergic stimulus (25). In fact, although much more modestly than in innervated BAT, rosiglitazone increased UCP1 mRNA levels, even in denervated BAT pads, suggesting some degree of independence of PPAR-γ action from adrenergic signaling. Here again, PGC-α is likely causal in the failure of rosiglitazone to maximally stimulate UCP1 expression in denervated BAT pad, as invalidation of PGC-α severely blunts adrenergic stimulation of UCP1 in brown adipocytes (37). Thus, by abolishing the residual basal sympathetic tone found under PPAR-γ activation, thereby reducing PGC-α, sympathetic denervation im-

| Table 3. Regulation of BAT recruitment by basal sympathetic tone and RSG-induced PPAR-γ activation in rats |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Components of BAT Recruitment | Denervation | RSG | Dependent on Basal Sympathetic Tone |
| Mass | – | – | No |
| Cellularity | – | – | No |
| Lipogenic genes (LPL, PEPCCK, FABP4, and DGAT1) | – | – | No |
| Lipolytic genes (ATGL and CGI58) | – | – | No |
| Mitochondrial mass (COX4, ND2, and FO1) | ↓ | – | Yes |
| Thermogenesis related (UCP1 and CIDEA) | ↓ | – | Yes |
| Nuclear factors (PGC-α and C/EBP-β) | ↓ | – | Yes |

BAT, brown adipose tissue; –, unaltered; ↓, reduced; ↑, increased.
pedes the ability of rosiglitazone to optimally increase UCP1 expression.

Another germane finding of the present study is the previously unrecognized upregulation of CIDEA by rosiglitazone. CIDEA is a mitochondrial protein highly expressed in BAT that interacts with and inhibits UCP1 uncoupling activity (42). CIDEA levels are downregulated by cold exposure, such effect being mediated by the sympathetic drive to BAT (32). The present findings suggest that, in addition to a reduced BAT sympathetic activity and thyroid status (12), CIDEA upregulation may be implicated in the absence of translation of the increased BAT mass and UCP1 content into higher functional thermogenic activity and energy expenditure on PPAR-γ ligand treatment (22, 27, 36).

Remarkably, 24 h of cold exposure, a situation characterized by a marked increase in BAT sympathetic activity (13), did not synergize with rosiglitazone to further increase UCP1 expression in intact BAT pads. Such lack of additivity may conceivably be due to either an already maximal induction of UCP1 expression by rosiglitazone or a prevention of cold-induced sympathetic activation by the ligand. These possibilities remain to be tested; however, arguing against the latter hypothesis is the fact that chronic administration of a β3-adrenergic agonist to PPAR-γ ligand-treated mice does not further increase BAT UCP1 mRNA levels (30).

In conclusion, whereas the rosiglitazone-induced expansion of BAT mass and its determinants (hypertrophy and hyperplasia) occurs independently of tissue sympathetic innervation, we identified major differences in BAT recruitment in vivo compared with that reported to occur in cultured brown adipocytes. Indeed, rosiglitazone in vivo does not affect BAT mitochondrial mass and PGC-α levels and requires the presence of a basal sympathetic tone to optimally induce UCP1 expression. Thus the residual sympathetic tone found under PPAR-γ ligand treatment is involved in the modulation of a subset of major components of PPAR-γ-mediated BAT recruitment in vivo.

Perspectives and Significance

Decades ago, BAT nonshivering thermogenesis was suggested as a possible alternative to treat obesity, a concept that has gained support and interest with the recent confirmation that a substantial number of adult humans possesses active BAT (6, 24, 38, 39, 43). At this point, little is known about the impact of BAT on human energy balance, but it is plausible that the efficacy of such therapy will depend on the induction of BAT recruitment in humans. The present study draws attention to three major issues regarding the major modulators of BAT recruitment. First, the SNS and the PPAR-γ pathway exist in a reciprocally inhibitory relationship with each other, that is, although both work toward the same goal (BAT recruitment), the SNS reduces PPAR-γ expression (20), and PPAR-γ activation reduces the SNS drive to BAT (12). Second, despite such mutual antagonism, PPAR-γ activation requires basal SNS activity to achieve maximal stimulation of UCP1 expression, as shown here. Third, the two systems share modulation of some (mass, cellularity, UCP1), but not all (PGC-α, mitochondrial biogenesis), components of BAT recruitment. Such differences widen the mechanistic options for intervention and may lead to the development of strategies that will enhance the induction of specific, rather than all such components, which may, in turn, help optimize the efficacy and innocuity of BAT recruitment. Also to be considered is that simply increasing BAT mass and mitochondrial genesis does not necessarily lead to an increase in thermogenesis without concomitant increase or at least maintenance of the SNS drive to BAT; thus genetic or pharmacological manipulations that promote increases in BAT mass in humans, in and of themselves, will not guarantee increased thermogenesis and thereby possible decreases in body fat.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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