Reduction of phosphodiesterase 3B gene expression in peroxisome proliferator-activated receptor γ (+/−) mice independent of adipocyte size

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Received 6 February 2003; revised 1 April 2003; accepted 1 April 2003

First published online 9 April 2003

Edited by Ned Mantei

Abstract Phosphodiesterase 3B (PDE3B) gene expression is generally reduced in large adipocytes of obese, insulin-resistant mice. This reduced gene expression is restored by peroxisome proliferator-activated receptor (PPAR) γ ligands accompanied by a reduced fat cell size. To determine whether PDE3B gene expression is regulated by PPARγ itself, we analyzed lean PPARγ (+/−) mice with adipocyte size comparable to control PPARγ (+/+) mice. In adipocytes of PPARγ (+/−) mice, PDE3B mRNA and protein were both reduced to 63% of wild-type levels. Basal PDE activity tended to be decreased to 70% of wild-type levels, and, similarly, insulin-induced PDE activity was significantly decreased to 70%. Thus, PPARγ is required for PDE3B gene expression independent of adipocyte size.

Key words: Phosphodiesterase 3B; Peroxisome proliferator-activated receptor γ; Adipocyte; Insulin; Type 2 diabetes; Insulin resistance

1. Introduction

Phosphodiesterase (PDE) 3B is the major isoform of PDE in adipocytes [1]. Insulin mediates antilipolytic action via the phosphorylation and activation of PDE3B. The activation of PDE3B results in decreased intracellular cAMP levels, which leads to a reduction in cAMP-dependent protein kinase A activity. This subsequently leads to the inactivation of hormone-sensitive lipase, resulting in a decreased hydrolysis of stored triglycerides, and a reduced release of free fatty acids (FFA) from adipocytes [2,3].

Type 2 diabetes mellitus is characterized by insulin resistance in insulin target tissues such as adipose tissue, skeletal muscle, and liver [4]. Reduced insulin sensitivity in adipose tissues might represent an event prior to overt insulin resistance in skeletal muscle and liver [5,6]. When the antilipolytic action of insulin is impaired, the release of FFA from adipocytes is increased. It is a well-known fact that the elevation of serum FFA causes insulin resistance in skeletal muscle and liver, as well as adipose tissue [7–9].

The reduced PDE3B gene expression in adipocytes could result in an increased FFA output leading to whole body insulin resistance. In fact, PDE3B (−/−) mice show insulin resistance with an elevated lipolytic capacity [10]. We previously showed that PDE3B gene expression is reduced in the large adipocytes of obese insulin-resistant KKAy mice and db/db mice [11–13]. PDE3B mRNA is also reduced in obese insulin-resistant *cp/cp* rats [14]. Therefore, a reduced PDE3B gene expression in large adipocytes appears to be a general phenomenon associated with the obese insulin-resistant state.

We previously reported that peroxisome proliferator-activated receptor (PPAR) γ ligands, thiazolidinediones, increase PDE3B gene expression in adipose tissues of obese insulin-resistant KKAy or db/db mice [11,13]. It has been reported that thiazolidinediones cause an increase in the number of small adipocytes, probably by inducing adipocyte differentiation in obese insulin-resistant Zucker rats [15,16]. We also reported that PDE3B mRNA and its promoter activity are induced on adipocyte differentiation in 3T3-L1 cells [17]. Therefore, PPARγ might enhance PDE3B gene expression directly by activating its gene transcription or indirectly by reducing the fat cell size.

PPARγ is known to be a master regulator of adipocyte differentiation [18]. In fact, the ability of embryonic fibroblasts from PPARγ (+/−) mice to differentiate into adipocytes is approximately 50% lower than that of PPARγ (+/+) mice [19]. Since the numbers of epididymal fat cells do not change irrespective of fat mass in these mice under a standard, high carbohydrate, or high fat diet [19], fat mass should correlate with fat cell size.

In view of this, we initiated a study of PDE3B gene expression in epididymal fat tissues of lean PPARγ (+/−) mice under standard diet conditions. The body weight as well as epididymal fat pad weight of PPARγ (+/−) mice were comparable to those of PPARγ (+/+) mice, which enabled us to investigate the direct effect of PPARγ independent of the effect of fat cell size. Steady-state mRNA and protein of PDE3B were reduced in adipocytes of PPARγ (+/−) mice. Basal PDE activity tended to be decreased to 70% of wild-type levels, and, similarly, insulin-induced PDE activity was significantly decreased to 70%. The fold induction by insulin was not affected.

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0014-5793/03/$22.00 © 2003 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00339-9

2. Materials and methods

2.1. Animals and tissue preparation

Male PPARγ (+/−) mice (C57BL/6J, CBA, and ICR hybrid background) were generated as described previously [19]. These mice had been backcrossed with C57BL/6J mice more than four times. PPARγ (+/+) wild-type littermates were used as controls. These mice were caged in groups of five and were provided with food and water ad libitum, and were used for experiments at an age of 10–14 weeks. The mice were killed by rapid decapitation under light diethyl ether inhalation, and epididymal fat tissues were rapidly removed. For the RNase protection assay, the tissues were homogenized and stored, as described previously [11]. For an assay for membrane-bound PDE activity and Western blotting, isolated adipocytes were prepared from fresh fat tissues by the collagenase method, as described previously [20]. Blood samples were also collected at the time of death. Serum glucose, FFA, triglycerides, and insulin were determined as described previously [11]. All experimental procedures were approved by the Animal Experimentation Committee of Ehime University School of Medicine.

2.2. RNase protection assay (RPA)

Total RNA was isolated from adipose tissue homogenates, and the RPA was performed as described previously [11]. Since the protected PDE3B and β-actin RNAs are of different sizes (395 and 250 nt, respectively), both RNA species could be quantitated in a single RNA sample. We confirmed that β-actin mRNA could be used to correct the PDE3B mRNA in each sample, as has been shown previously [11].

2.3. Western blotting

PDE3B protein levels in isolated adipocytes were assessed by Western blotting as described previously using an anti-rat PDE3B polyclonal antibody raised in rabbits against the NH_2-terminal peptide (amino acid residues 1–17) [11].

2.4. Membrane-bound PDE catalytic activity

Membrane-bound PDE activity was assayed as described earlier [21,22]. Briefly, isolated adipocytes, which were incubated with or without 4 nmol/l insulin, were homogenized with buffer A (10 mM TES (*N*-tris-[hydroxymethyl]-methyl-2-aminoethanesulfonic acid), pH 7.0 containing 0.25 M sucrose). The membrane-bound fraction was then suspended in buffer A, pH 7.5, and PDE activity was then determined.

2.5. Statistical analysis

An unpaired Student's *t*-test was used for all the experiments, and results are expressed as the mean ± S.E.M. from at least four mice.

3. Results

3.1. Body weight, epididymal fat pad weight, and serum insulin, glucose, FFA and triglycerides levels in PPARγ (+/−) mice

We first compared the body weight, epididymal fat pad weight, serum insulin, glucose, FFA and triglyceride concentrations of the PPARγ (+/−) mice to those of control PPARγ (+/+) mice under standard diet conditions (Table 1). The body weight and epididymal fat pad weight of the PPARγ (+/−) mice were comparable to those of PPARγ (+/+) mice. Since the numbers of epididymal fat cells were not different between PPARγ (+/−) and PPARγ (+/+) mice [19], the size of the fat cells of PPARγ (+/−) mice was considered to be comparable to that of PPARγ (+/+) mice. No significant differences in the other parameters were detected between the PPARγ (+/−) and PPARγ (+/+) mice.

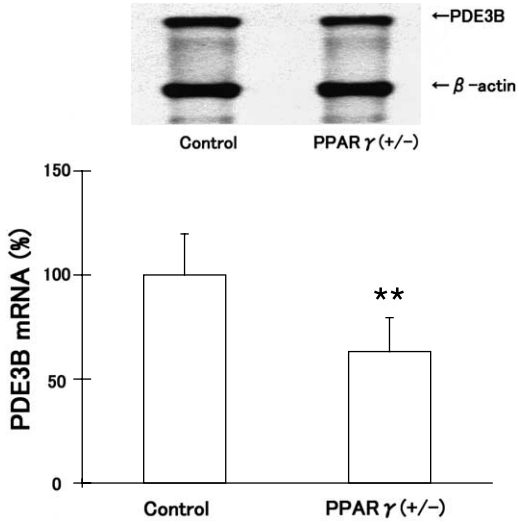

Fig. 1. PDE3B mRNA in adipose tissues of PPARγ (+/−) mice. PDE3B mRNA was measured by RPA using epididymal fat tissues from 10–14 week old PPARγ (+/−) and control PPARγ (+/+) mice as described in Section 2. PDE3B mRNA is expressed as the intensity of the protected PDE3B band corrected by that of the protected β-actin band in each lane. The mean value in the control mice is then defined as 100% and the relative value is shown as the means ± S.E.M. (%). $^{**}P < 0.01$, significant difference compared to control PPARγ (+/+) mice ($n = 5$).

3.2. PDE3B mRNA in adipose tissues of PPARγ (+/−) mice

To determine whether PDE3B gene expression is directly regulated by PPARγ, the PDE3B mRNA in adipose tissues of PPARγ (+/−) mice was first compared to that of control PPARγ (+/+) mice using RPA (Fig. 1). The PDE3B mRNA in the PPARγ (+/−) mice was decreased to 63% of PPARγ (+/+) mice (PPARγ (+/+) 100 ± 20 vs. PPARγ (+/−) 63 ± 16%, $n = 5$, $P < 0.01$). Since we have consistently observed that PDE3B mRNA in adipose tissues correlates with PDE3B protein in adipocytes [11–13,24], we assume that changes in PDE3B mRNA levels in adipose tissue reflect alterations in adipocytes.

3.3. PDE3B protein in adipocytes of PPARγ (+/−) mice

To examine whether the decreased PDE3B mRNA in the adipose tissues of PPARγ (+/−) mice is related to PDE3B protein in their isolated adipocytes, we next assessed the steady-state PDE3B protein level by Western blotting (Fig. 2). The specificity of our antibody was verified by an absorption test using a peptide to which the antibody had been

Table 1
Total body and fat pad weight, and serum insulin, glucose, FFA, and triglyceride levels in PPARγ (+/−) and control PPARγ (+/+) mice

Experimental group	*n*	Body weight (g)	Epididymal fat pad weight (g)	Insulin (ng/ml)	Glucose (mg/dl)	FFA (mEq/l)	Triglycerides (mg/dl)
Control PPARγ (+/+) mice	4	40.5 ± 1.2	0.76 ± 0.16	3.04 ± 0.44	245.8 ± 1.9	1.68 ± 0.26	194 ± 41
PPARγ (+/−) mice	10	41.4 ± 1.1	0.73 ± 0.09	3.41 ± 0.62	243.5 ± 4.9	1.84 ± 0.13	199 ± 19

Data are mean ± S.E.M. No significant difference ($P < 0.05$) between control PPARγ (+/+) and PPARγ (+/−) mice was detected.

raised, as described previously [11]. PDE3B protein in adipocytes of the PPARγ (+/−) mice was decreased to 63% of PPARγ (+/+) mice (PPARγ (+/+) 100 ± 11 vs. PPARγ (+/−) 63 ± 4%, $n = 4$, $P < 0.05$) (Fig. 2). Thus, PDE3B protein levels were decreased in adipocytes of PPARγ (+/−) mice, which correlates with the levels of PDE3B mRNA in adipose tissues.

3.4. Basal and insulin-induced membrane-bound PDE activities in adipocytes of PPARγ (+/−) mice

To examine whether the heterozygous deficiency of PPARγ affects the insulin signaling pathway leading to PDE3B activation, basal and insulin-induced membrane-bound PDE (mainly PDE3B) activities were measured (Fig. 3). In epididymal adipocytes of PPARγ (+/−) mice, basal membrane-bound PDE activity was slightly reduced to 74% of PPARγ (+/+) mice, although the difference did not quite reach significance (PPARγ (+/+) 40.0 ± 3.0 pmol/mg/min vs. PPARγ (+/−) 29.6 ± 4.3 pmol/mg/min, $n = 5$, $P = 0.08$). This tendency correlates with PDE3B mRNA and protein levels. Insulin-induced membrane-bound PDE activity was reduced to 70% of PPARγ (+/+) mice (PPARγ (+/+) 83.0 ± 7.1 pmol/mg/min vs. PPARγ (+/−) 57.9 ± 6.0 pmol/mg/min, $n = 4$, $P < 0.05$). The fold induction by insulin (insulin-induced/basal activity) was not affected in the PPARγ (+/−) mice (PPARγ (+/+) 2.2 ± 0.1-fold vs. PPARγ (+/−) 2.1 ± 0.3-fold, $n = 4$, $P = 0.71$). Therefore, in adipocytes of PPARγ (+/−) mice, PDE3B gene expression was reduced, whereas the insulin signaling pathway leading to PDE3B activation was not probably affected.

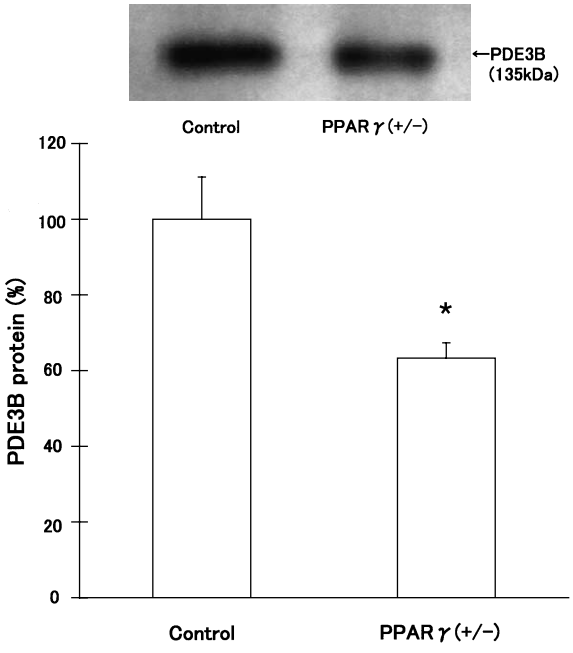

Fig. 2. PDE3B protein in adipocytes of PPARγ (+/−) mice. Western blotting was carried out using an anti-PDE3B antibody raised against the N-terminal peptide (residues 1–17) of rat PDE3B as described in Section 2. All mice were 10–14 weeks of age. The mean value of the PDE3B protein intensity of the PPARγ (+/−) mice is defined as 100% and the relative value is shown as the mean ± S.E.M. (%). *$P < 0.05$, significant difference compared to control PPARγ (+/+) mice ($n = 4$).

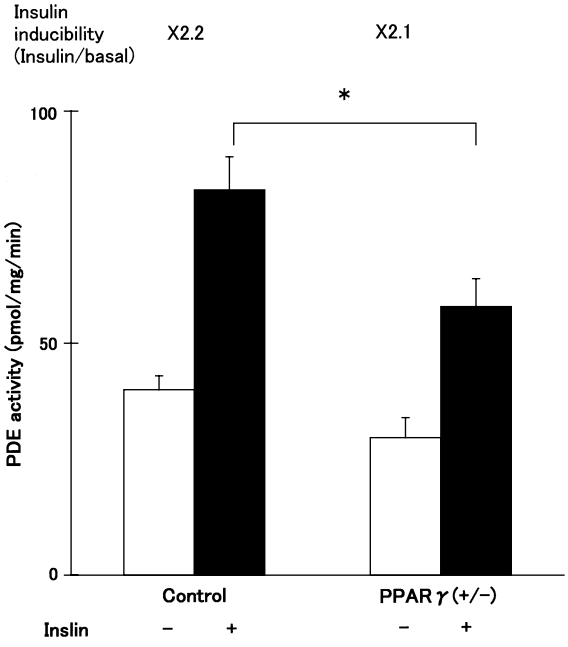

Fig. 3. Basal and insulin-induced membrane-bound PDE activities in adipocytes of PPARγ (+/−) mice. The basal and insulin-induced activities of the membrane-bound PDE were determined in epididymal adipocytes from 10–14 week old PPARγ (+/−) and control PPARγ (+/+) mice as described in Section 2. Columns represent the mean ± S.E.M. of PDE catalytic activity (pmol/mg/min). Numbers indicate the insulin inducibility of PDE activity (insulin-treated/basal activities, fold induction). *$P < 0.05$, significant difference compared to basal activity of PPARγ (+/−) mice ($n = 4$).

4. Discussion

The data herein show that PDE3B mRNA and the corresponding protein levels were both reduced in adipocytes of PPARγ (+/−) mice under standard diet conditions. Although not significant, the basal PDE3B activity showed a consistent decrease. Insulin-induced PDE3B activities were also reduced, whereas the fold induction by insulin (insulin-induced/basal) was not affected.

PDE3B gene expression was decreased in adipocytes of lean PPARγ (+/−) mice. Since the size of fat cells of PPARγ (+/−) mice is comparable to that of PPARγ (+/+) mice, PPARγ may induce PDE3B gene expression, at least in part, independent of fat cell size. PPARγ is known to regulate gene transcription by binding to its DNA elements or by interacting with other factors without directly binding to DNA [18,23].

PDE3B gene expression appears to be regulated by at least two factors, fat cell size and PPARγ. We reported that PDE3B gene expression is reduced in large adipocytes of obese insulin-resistant KKAy or db/db mice [12,13], whereas this gene expression is enhanced in small adipocytes of lean insulin-resistant IRS-1 (−/−) mice [24]. The present data suggest that PPARγ itself is also required for inducing PDE3B gene expression in adipocytes.

No significant differences in serum FFA and triglyceride levels were found between PPARγ (+/−) mice and PPARγ (+/+) mice. In adipocytes of PPARγ (+/−) mice, PDE3B mRNA and protein were both reduced to 63% of PPARγ (+/+) mice. Basal PDE activity tended to be decreased to 70% of PPARγ (+/+) mice, and, similarly, insulin-induced PDE activity was significantly decreased to 70%. This modest

reduction of basal and insulin-induced PDE3B activity may not be sufficient to cause significant changes in serum FFA and triglyceride levels in PPARγ (+/−) mice. Unaffected levels of serum FFA and triglycerides have also been reported by another group [25].

The fold induction of PDE3B activity by insulin was not affected in adipocytes of PPARγ (+/−) mice. The reduction in the basal PDE3B activity appears to be comparable with that in the insulin-induced PDE3B activity. These reduced PDE3B activities could result from reduced PDE3B protein levels whereas molecules involved in the insulin signaling pathway leading to PDE3B activation do not appear to be affected in adipocytes of these mice.

In PPARγ (+/−) mice, PDE3B gene expression and insulin-induced PDE3B activity were reduced, which could result in a reduced capacity for triglyceride storage in these adipocytes. In fact, in the case of a high fat diet, PPARγ (+/−) mice remain lean and insulin-sensitive, whereas control PPARγ (+/+) mice become obese and insulin-resistant [19]. This phenomenon appears to be paradoxical since a PPARγ ligand has an insulin sensitizing effect. It has been proposed that the relation between insulin sensitivity and the amount of PPARγ fits into an inverted U shape [26]. An appropriate amount of PPARγ, probably 50% of the normal amount in these mice, provides better insulin sensitivity under conditions of a high fat diet.

In summary, PDE3B gene expression was decreased in adipocytes of PPARγ (+/−) mice compared to control PPARγ (+/+) mice. The size of fat cells of PPARγ (+/−) mice is comparable to that of PPARγ (+/+) mice, suggesting that PPARγ itself may be required for inducing PDE3B gene expression independent of fat cell size. The issues of how PDE3B gene expression is regulated by PPARγ and how a reduced PDE3B gene expression protects against obesity in PPARγ (+/−) mice under a high fat diet remain unclear. Further experiments will be required to clarify these points.

Acknowledgements: This work was supported mainly by a Grant-in-Aid for Scientific Research on Priority Areas (C) 'Medical Genome Science' (No. 12204007), Grants-in-Aid for Scientific Research (C) (Nos. 14571097, 14571095, and 14571096) from the Ministry of Education, Culture, Science, Sports and Technology of Japan, and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan by the Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists (No. 60206-00). We thank F. Tanabe and M. Murase for technical assistance and suggestions.

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