

Improvement in Insulin Resistance and the Restoration of Reduced Phosphodiesterase 3B Gene Expression by Pioglitazone in Adipose Tissue of Obese Diabetic KK_{Ay} Mice

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Phosphodiesterase (PDE) 3B is a key enzyme in the mediation of the antilipolytic action of insulin in adipocytes, and activation of this molecule results in a reduced output of free fatty acids (FFAs). An elevation of serum FFAs is known to cause insulin resistance in skeletal muscle and liver, which could be the primary cause of type 2 diabetes. To elucidate whether PDE3B is involved in this disease, we examined the PDE3B gene expression in epididymal fat tissues of obese insulin-resistant diabetic KK_{Ay} mice. We also examined the effect of an insulin-sensitizing drug, pioglitazone, on this gene expression. In adipose tissue of KK_{Ay} mice, PDE3B mRNA and its corresponding protein were reduced to 48 and 43% of those in C57BL/6J control mice. Basal and insulin-stimulated membrane-bound PDE activities were also decreased to 50 and 36% of those in the controls, respectively. Pioglitazone increased both PDE3B mRNA and protein levels by 1.8-fold of those in untreated KK_{Ay} mice. Basal and insulin-induced membrane-bound PDE activities were also increased by 1.6- and 2.0-fold, respectively. Pioglitazone reduced the elevated levels of serum insulin, glucose, FFAs, and triglyceride in KK_{Ay} mice. Thus, the reduced PDE3B gene expression in adipose tissues could be the primary event in the development of insulin resistance in KK_{Ay} mice, which was improved by pioglitazone possibly because of the restoration of the reduced PDE3B gene expression. *Diabetes* 48:1830–1835, 1999

Seven different gene families involving mammalian cyclic nucleotide phosphodiesterase (PDE1–7) are described in the review by Beavo et al. (1), and these family members have increased since that report (1–7). PDE3 is an isoform of these families, which is characterized by an insertion with the catalytic domain. PDE3B is activated by insulin and is a major isoform in adipocytes, whereas PDE3A is mainly expressed in platelet,

heart, and vascular smooth muscle. Insulin exerts an antilipolytic action via the phosphorylation and activation of PDE3B. The activation of this enzyme results in a decrease in intracellular cAMP, which in turn, leads to a reduction in cAMP-dependent protein kinase A activity. This subsequently leads to the dephosphorylation and inactivation of hormone-sensitive lipase (HSL), leading to the decreased hydrolysis of stored triglycerides and a reduction in the release of free fatty acids (FFAs) from adipocytes (8–11).

Type 2 diabetes is clinically characterized by insulin resistance in target tissues such as adipose tissue, skeletal muscle, and liver. Skeletal muscle and liver represent major sites of insulin resistance in the case of type 2 diabetes (12). Reduced insulin sensitivity in adipocytes could represent an event before overt insulin resistance in such tissues (13–15). When the antilipolytic action of insulin is impaired, the rate of release of FFAs from fat is increased. It is a well-known fact that the elevation of serum FFAs causes insulin resistance in skeletal muscle and liver as well as adipose tissues (16–18).

In a prior study, we have demonstrated that basal and insulin-induced membrane-bound PDE (mainly PDE3B) activities are impaired in epididymal adipocytes of insulin-resistant diabetic KK mice, spontaneously obese rats, and dexamethasone-treated rats (19–21). Other studies have also concluded that PDE activity is reduced in adipocytes of diabetic patients or rats (22–24). This reduced PDE activity can be restored by appropriate treatment. These findings suggest that PDE3B gene expression might well be correlated with insulin resistance or diabetic state.

In view of this, we have begun to analyze PDE3B gene expression in epididymal fat tissues of obese insulin-resistant diabetic KK_{Ay} mice. Our findings show that basal and insulin-induced membrane-bound PDE activities and either steady-state protein or mRNA are all reduced in adipose tissues of these mice. Treatment with insulin-sensitizing thiazolidinedione (pioglitazone [PIO]) restores all these parameters in parallel with an improvement in hyperglycemia, elevated FFAs, and insulin resistance.

RESEARCH DESIGN AND METHODS

Animals and tissue preparation. Male nondiabetic C57BL/6J mice and insulin-resistant KK_{Ay} mice were purchased from Clea Japan (Osaka, Japan) and were used for the experiments at an age of 10–12 weeks. KK_{Ay} mice result from a cross between glucose-intolerant black KK female mice and male yellow obese Ay mice and are known to serve as excellent models of obese insulin-resistant type 2 diabetes (25). C57BL/6J mice are generally used as these controls, including the PIO experiments (26). The mice were individually housed and were provided with food

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FFA, free fatty acid; HSL, hormone-sensitive lipase; PDE, phosphodiesterase; PIO, pioglitazone; PPAR, peroxisome proliferator-activated receptor; RPA, RNase protection assay.

and water ad libitum. The treatment group of KKAY and C57BL/6J mice received PIO in their food at a level of 0.01% (from Takeda Chemical Industries, Osaka, Japan), which resulted in the ingestion of 20 mg · kg⁻¹ · day⁻¹ over a 4-day period. The mice were killed by rapid decapitation under light diethyl ether inhalation, and the epididymal fat tissues were rapidly removed. For the RNase protection assay, the adipose tissues were homogenized with Tri Reagent LS (Molecular Research Center, Cincinnati, OH), frozen in liquid nitrogen, and stored at -80°C. For Western blotting and a PDE 3B activity assay, free adipocytes were prepared from fresh fat tissues by the collagenase method (27). Blood samples were also collected at the time of death. Serum glucose was measured with Glucorder-MkII, an automated glucose analyzer (A&T, Tokyo). Serum FFAs and triglyceride were determined by Cobas Mira Plus (Roche, Tokyo). Serum insulin was detected by an ELISA kit (MIA Institute of Biological Science, Yokohama, Japan). All these procedures were approved by the Committee of Animal Experimentation at the Ehime University School of Medicine.

RNase protection assay. Total RNA was isolated from the homogenates of mouse fat tissues in Tri Reagent LS according to the manufacturer's instructions (Molecular Research Center). The integrity of each RNA sample was confirmed by intact 18S and 28S rRNA that was stained with ethidium bromide on an agarose gel. The concentration and purity was assessed by absorbance at 260 nm and by the 260/280 nm ratio, respectively. A mouse PDE3B cDNA fragment corresponding to nt 201–595 in the partial mouse PDE3B cDNA (Genebank Accession no. X95521) was amplified via polymerase chain reaction using the 5' primer: 5'CCCGATCCAAGCTTAGTCATTTCTCTGCGG and the 3' primer: 5'CCGGAATTCGGTACCCTTCTCAGACCTGCATGGTGCG as described (28), with addition of *Bam*HI and *Hind*III restriction sites, respectively. This nt 201–595 PDE3B cDNA with generated restriction sites was cloned into pGEM7 (Promega, Madison, WI) at the unique *Bam*HI and *Hind*III sites in reverse orientation when transcribed from the T7 promoter. The plasmids were linearized by *Hind*III digestion, and a 449-nt biotinylated cRNA probe containing a 395-nt antisense RNA was transcribed using the MAXIscript In Vitro Transcription T7 Kit (Ambion, Austin, TX) and Biotin-16-UTP (Boehringer Mannheim, Indianapolis, IN). A 300-nt biotinylated RNA probe containing a 250-nt mouse β -actin antisense RNA was also produced using the pTRI- β -actin mouse control template DNA (Ambion). The full-length transcripts were isolated from acrylamide gel using the usual protocol. The biotinylated RNA molecular weight markers (100-, 200-, 300-, 400-, and 500-nt RNA) were transcribed from the RNA Century Marker Template Set (Ambion).

Purified cRNA probes of PDE3B and β -actin were hybridized with 10 μ g each of the total RNA extracted from mouse epididymal fat tissues using the HybSpeed RNase protection assay (RPA) (Ambion). The protected RNAs were separated by 5% acrylamide per 8 mol/l urea gel electrophoresis at 250 V for 1.25 h. Size-fractionated RNAs were then electrophoretically transferred to a Brightstar-Plus Positively Charged Nylon Membrane (Ambion). The transferred biotinylated cRNAs were then detected by the Brightstar BioDetect Kit (Ambion). The chemiluminescently detected cRNAs were exposed to film (Kodak, Rochester, NY) and quantitated using The 1D Image Analysis Software (Kodak Digital Science, Kodak). Because the protected PDE3B and β -actin RNAs are of a different size (395 and 250 nt, respectively), both RNA species could be quantitated in a single RNA sample. Because thiazolidinedione does not affect β -actin mRNA in vitro, we chose this as an internal control for our experiments (29,30). When the same amount of each intact RNA sample was examined, no significant difference in the level of β -actin mRNA in adipose tissues between PIO-treated and untreated KKAY or C57BL/6J mice was detected (C57BL 1.00 \pm 0.08, PIO-C57BL 0.95 \pm 0.10, KKAY 1.03 \pm 0.11, PIO-KKAY 1.07 \pm 0.12; data are means \pm SE when the mean value of untreated C57BL is defined as 1; n = 8). Thus, the β -actin mRNA level could be used to correct the PDE3B mRNA in each sample for meaningful comparisons between experiments. The sequence of our subcloned PDE3B cDNA was consistent with the cDNA sequence from 201 to 595 of mouse PDE3B (registered as cGIPDE1 gene in DBGET, GenBank-today database, Accession x95521) (31), except for one

nucleotide at nt 405, which contained C instead of T. The sequence of the cDNA from different mouse adipocytes or 3T3-L1 adipocytes RNA were identical.

Western blotting. Adipocytes were isolated from fresh epididymal fat tissues and then homogenized in STS (250 mmol/l sucrose, 10 mmol/l N-tris[hydroxy-methyl]methyl-2-amino) ethanesulfonic acid [TES], pH 7.0 solution) buffer (15 μ g/ml pepstatin, 15 μ g/ml leupeptin, 3 mmol/l benzamide, 0.5 mmol/l phenylmethylsulfonyl fluoride). The membrane-bound fraction was obtained from an isolated adipocyte homogenate as described previously (32,33). To examine the difference between KKAY mice with and without PIO treatment, fresh fat tissues were directly homogenized with STS buffer. Each 20- μ g sample of protein was electrophoresed on 7.5% polyacrylamide gels, and the size-fractionated proteins were electrophoretically transferred to Immobilon membranes (Millipore, Bedford, MA). These membranes were incubated with anti-rat PDE3B polyclonal antibody raised in rabbits against the NH₂-terminal peptide (amino-acid residues 1–17). Visualization of PDE3B protein was carried out with the enhanced chemiluminescence detection system as described (33). The autographs were quantitatively analyzed by 1D Image Analysis Software (Kodak Digital Science). To confirm the specificity of our anti-rat PDE3B polyclonal antibody, the absorption test was carried out by preincubating the antibody with the NH₂-terminal peptide (1–17 aa) of rat PDE3B as a competitor.

Membrane-bound PDE (mainly PDE3B) catalytic activity. Membrane-bound PDE activity was assayed exactly as described earlier (32,33). Briefly, isolated adipocytes were incubated with or without 1 nmol/l insulin for 10 min at 37°C in Krebs-Ringer HEPPES albumin buffer (pH 7.4, 20 mg/ml bovine serum albumin). Adipocytes were washed and homogenized with STS buffer; the membrane-bound fraction was then suspended in 250 mmol/l sucrose, 10 mmol/l TES, pH 7.5 buffer; and the PDE activity was determined.

Statistic analysis. An unpaired Student's *t* test was used for all the experiments, and results are expressed as means \pm SE from four to eight mice.

RESULTS

Effect of PIO on serum insulin, glucose, triglyceride and FFA levels in KKAY mice. We first compared body weight, epididymal fat pad weight, serum glucose, FFAs, triglyceride and insulin concentrations of KKAY mice to those of control C57BL/6J mice (Table 1). KKAY mice were obese, hyperinsulinemic, hyperglycemic, and hyperlipidemic compared to the controls. A 4-day treatment with PIO resulted in a reduction in FFA, triglycerides, glucose and insulin levels in KKAY mice but did not affect these levels in the C57BL/6J mice. PIO had no effect on body weight and epididymal fat pad weight in the KKAY mice and C57BL/6J mice.

Effect of PIO on PDE3B mRNA in adipose tissues of KKAY mice. To estimate PDE3B mRNA, we used a non-isotopic RPA method. We first confirmed that the mouse PDE3B and β -actin cRNA undigested probes were of the correct sizes. After hybridization with mouse fat total RNA and digestion with RNase, the sizes of protected probes were exactly the same as expected (Fig. 1A). Thus, the PDE3B mRNA could be successfully determined using this sensitive non-isotopic RPA.

To determine whether PDE3B mRNA is altered in KKAY mice, we compared PDE3B mRNA of these mice to those of

TABLE 1

Effect of PIO on body and total epididymal fat pad weight, serum glucose, FFAs, triglyceride, and insulin concentrations in KKAY and C57BL/6J mice

Experimental groups	<i>n</i>	Body weight (g)	Epididymal fat pad weight (g)	Glucose (mg/dl)	FFAs (mEq/l)	Triglyceride (mg/dl)	Insulin (ng/ml)
Untreated C57BL mice	8	26.5 \pm 0.4	0.44 \pm 0.02	203.1 \pm 11.8	0.94 \pm 0.07	83 \pm 7	0.5 \pm 0.0
PIO-treated C57BL mice	6	26.8 \pm 0.5	0.45 \pm 0.03	201.1 \pm 9.3	0.94 \pm 0.04	84 \pm 10	0.4 \pm 0.1
Untreated KKAY mice	8	43.2 \pm 1.0*	1.74 \pm 0.09*	736.9 \pm 23.1*	3.97 \pm 0.17*	440 \pm 65*	43.9 \pm 7.7*
PIO-treated KKAY mice	8	44.4 \pm 0.5	1.81 \pm 0.07	398.1 \pm 42.9†	2.49 \pm 0.18†	195 \pm 22†	20.4 \pm 5.3†

Data are means \pm SE. *Significant differences compared with C57BL/6J mice, P < 0.01. †Significant differences compared with untreated KKAY mice, P < 0.01.

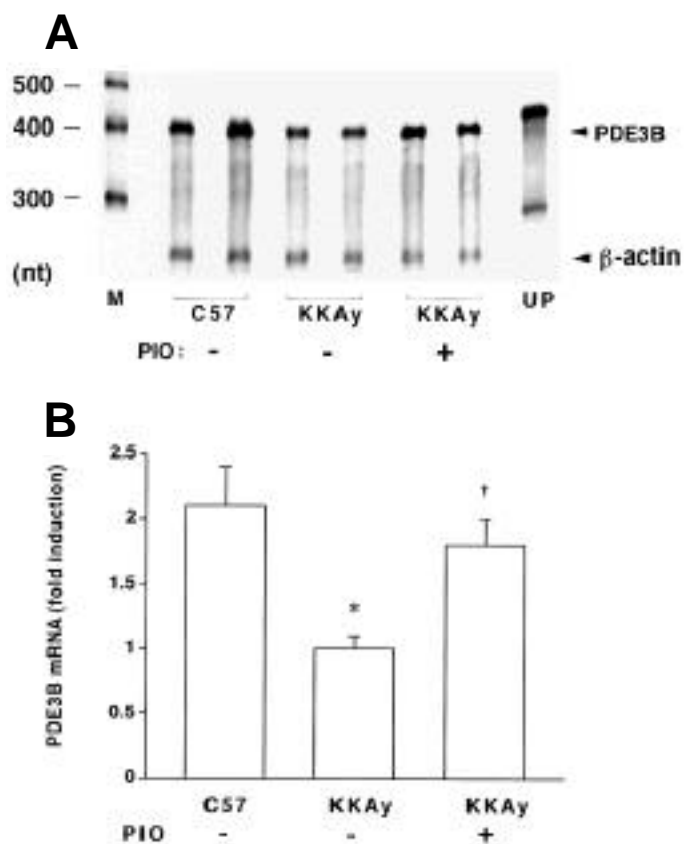


FIG. 1. Effect of PIO on PDE3B mRNA in adipose tissues of KKAY mice. PDE3B mRNA was measured by RPA in epididymal fat tissues from C57BL/6J, KKAY, and PIO-treated KKAY mice as described in METHODS. All mice ($n = 8$ in each experimental group) were 10–12 weeks old. PIO ($20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) was administered for 4 days in the case of the treated KKAY mice. **A:** The position of the full-length and protected PDE3B and β -actin (internal control) cRNA probes. Arrowheads indicate the 395 nt PDE3B protected probes and the 250 nt protected β -actin probes. Numbers indicate nucleotides of RNA size markers. M, RNA molecular marker; UP, undigested probes. **B:** Quantitative analysis of PDE3B mRNA in adipose tissues of C57BL/6J, KKAY, and PIO-treated KKAY mice. 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 untreated KKAY mice is then defined as 1.0, and the fold induction is shown as means \pm SE (fold). *Significant difference compared with C57BL mice ($P < 0.01$). †Significant difference compared with untreated KKAY mice ($P < 0.01$).

control C57BL/6J mice using RPA (Fig. 1B). PDE3B mRNA in the KKAY mice was decreased to 48% of those in the control C57BL/6J mice (C57BL 2.1 ± 0.3 -fold vs. KKAY 1.0 ± 0.1 -fold, $n = 8$, $P < 0.01$). With PIO treatment, PDE3B mRNA was partially restored to 89% of that in the control mice (C57BL 2.1 ± 0.3 -fold vs. PIO-treated KKAY 1.8 ± 0.2 -fold, $n = 8$, $P < 0.01$). This increase was significant when compared with that of untreated KKAY mice ($n = 8$, $P < 0.01$). PIO had no effect on PDE3B mRNA in adipose tissue of C57BL/6J mice (C57BL 1.00 ± 0.09 vs. PIO-C57BL 1.05 ± 0.17 , $n = 6$). Thus, PDE3B mRNA in adipocytes was reduced in obese insulin-resistant diabetic KKAY mice, and this reduction could be restored by treatment with PIO.

Effect of PIO on PDE3B protein in adipose tissues of KKAY mice. To examine whether reduced PDE3B mRNA of KKAY mice and the restoration of the mRNA with PIO are in parallel to PDE3B protein, we next assessed the steady-state

PDE3B protein by Western blotting (Fig. 2). The specificity of our antibody was verified by the absorption test using the peptide (the NH_2 -terminal sequence of 1–17 amino acid fragment of rat PDE3B protein) to which the antibody had been raised (Fig. 2A). The 135-kDa band representative of PDE3B disappeared completely after peptide absorption (compare lane 1 with lane 2 in Fig. 2A). Thus, the PDE3B protein detected by this method is specific.

PDE3B protein was then quantitated by Western blotting using this PDE3B antibody. PDE3B protein from epididymal adipocytes of KKAY mice was reduced to 43% of the controls (C57BL 2.3 ± 0.1 -fold vs. KKAY 1.0 ± 0.1 -fold, $n = 6$, $P < 0.01$) (Fig. 2B). PIO treatment increased the PDE3B protein to 1.8 ± 0.1 -fold that of the untreated KKAY mice ($n = 4$, $P < 0.01$) (Fig. 2C). Thus, PDE3B protein in adipocytes was reduced in diabetic KKAY mice, and this altered PDE3B protein was restored by PIO.

Effect of PIO on basal and insulin-induced membrane-bound PDE activities in adipose tissues of KKAY mice.

As a final output of PDE3B gene expression, the basal and insulin-induced membrane-bound PDE (mainly PDE3B) activities were measured (Fig. 3). In the case of epididymal fat tissues of untreated KKAY mice, basal and insulin-induced membrane-bound PDE activities were both reduced compared with those of nondiabetic C57BL/6J mice (53.9 ± 8.0 vs. $107.2 \pm 2.2 \text{ pmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ in basal activity and 65.6 ± 9.6 vs. $180.3 \pm 3.0 \text{ pmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ in insulin-induced activity, $n = 6$, $P < 0.01$). PIO resulted in an increase in both the basal and insulin-induced PDE activities of KKAY mice (83.6 ± 7.0 and $130.5 \pm 14.1 \text{ pmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$, respectively, $P < 0.05$, $n = 6$). The insulin-induced increase (insulin-induced/basal activity) was also decreased in KKAY mice (KKAY vs. C57BL mice 1.2 ± 0.1 vs. 1.7 ± 0.1 , $P < 0.01$, $n = 6$). This reduction was restored with PIO (1.6 ± 0.1 -fold, $P < 0.01$, $n = 6$). Thus, basal and insulin-induced PDE activities in adipocytes were reduced in diabetic KKAY mice, and these altered activities were restored by PIO.

DISCUSSION

The data herein show that PDE3B gene expression in adipocytes is reduced in obese insulin-resistant diabetic KKAY mice and that this altered PDE3B gene expression is restored by PIO. The changes of this gene expression as evidenced by steady-state mRNA, protein levels, and PDE catalytic activities are correlated with serum FFA concentrations and insulin resistance.

One of the distinct characteristics of obesity is enlargement of adipocytes and fat mass (34). The basal and catecholamine-stimulated rate of lipolysis as well as intracellular cAMP concentration and HSL activity positively correlate with fat cell size (35,36). In fact, lipolysis and circulating FFAs are both increased in obese humans and animals (37,38). PDE3B is thought to be involved in the initial step in the enhanced levels of FFAs released from adipocytes (11). Inactivation of PDE3B can result in increased HSL activity, which leads to an enhanced release of FFAs. Thus, it is possible that reduced PDE3B gene expression in the KKAY mice contributes to an enhanced rate of FFA release. Nagaoka et al. (28) have also recently shown that PDE3B mRNA is reduced in white adipose tissues in obese insulin-resistant diabetic *cp/cp* rats. These collective findings are consistent with our previous report that concluded that the basal mem-

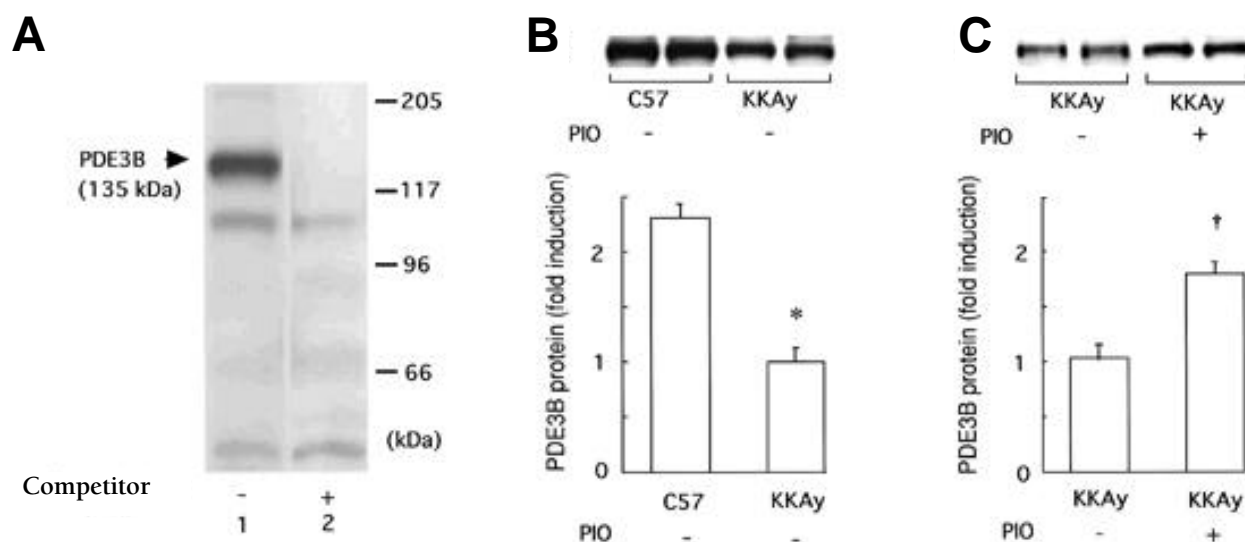


FIG. 2. Effect of PIO on PDE3B protein in adipose tissues of KKAY mice. Western blotting was carried out using the anti-PDE3B antibody raised against the NH₂-terminal peptide (1–17) of rat PDE3B as described in METHODS. All mice were 10–12 weeks old. PIO (20 mg · kg⁻¹ · day⁻¹) was administered for 4 days in the case of the treated KKAY mice. **A:** Specificity of the anti-PDE3B antibody was confirmed by an absorption test. Lane 1 shows the 135-kDa PDE3B band visualized with this antibody. Lane 2 represents the disappearance of this band when the NH₂-terminal peptide (1–17) of rat PDE3B protein was included in the reaction as a competitor. An arrowhead indicates the 135-kDa PDE3B band. Numbers indicate molecular weights of proteins. **B:** Quantitative analysis of PDE3B protein in adipocytes of C57BL/6J mice and KKAY mice. The mean value of PDE3B protein intensity of the untreated KKAY mice is defined as 1.0, and the fold induction is shown as means ± SE (fold). *Significant difference compared with C57BL mice ($P < 0.01$, $n = 6$). **C:** Quantitative analysis of PDE3B protein in adipose tissues of PIO-treated and untreated KKAY mice. The mean value of PDE3B protein intensity of the untreated KKAY mice is defined as 1.0, and the fold induction is shown as means ± SE (fold). †Significant difference compared with untreated KKAY mice ($P < 0.01$, $n = 4$).

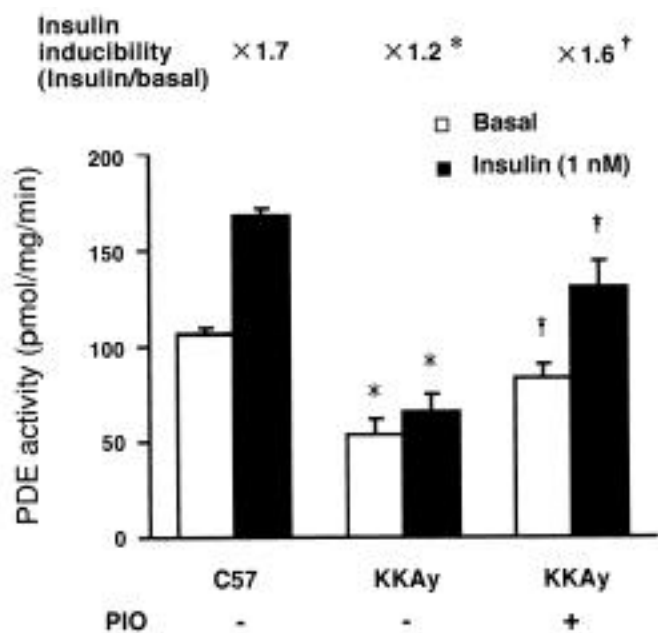


FIG. 3. Effect of PIO on basal and insulin-induced membrane-bound PDE activities in adipose tissues of KKAY mice. All mice were 10–12 weeks old. PIO (20 mg · kg⁻¹ · day⁻¹) was administered for 4 days in the case of treated KKAY mice. The basal and insulin-induced activities of the membrane-bound PDE were determined in epididymal adipocytes from C57BL/6J mice, KKAY mice, and PIO-treated KKAY mice as described in METHODS. Columns represent means ± SE of PDE catalytic activity (pmol · mg⁻¹ · min⁻¹). Numbers indicate insulin inducibility of PDE activity (insulin-treated/basal activities, fold induction). *Significant difference compared with C57BL/6J mice ($P < 0.01$, $n = 6$). †Compared with untreated KKAY mice ($P < 0.05$, $n = 6$).

brane-bound PDE (mainly PDE3B) activity is reduced in epididymal insulin-resistant diabetic KK mice (19).

The changes in PDE3B gene expression in adipocytes of the mice studied correlate with those of insulin resistance and serum FFA concentrations. Current evidence strongly suggests that an elevation in plasma FFA concentrations lowers peripheral insulin sensitivity in normal or diabetic subjects (17). Thus, the reduced PDE3B gene expression in adipocytes could be the primary event in the insulin resistance observed in the KKAY mice. Restoration of this reduced gene expression by PIO, with the parallel improvement of insulin resistance, supports this hypothesis. Likewise, in human type 2 diabetes, the reduced PDE3B gene expression in adipocytes could account for the development of insulin resistance.

Insulin-induced PDE activation is also reduced in adipocytes from KKAY mice, and PIO restores this reduction. Insulin-induced membrane-bound PDE (mainly PDE3B) activity is also reduced in insulin-resistant diabetic KK mice (19). Serum FFAs are high in these mice (38). An elevation in circulating FFA levels impairs the early process of insulin signal transduction (39,40), such as phosphatidylinositol 3-kinase inactivation, which is involved in the pathway leading to PDE3B activation in adipocytes (33,41). Thus, the decrease of insulin sensitivity in adipocytes by FFAs could account for the observed reduction in insulin-induced PDE3B activity. PIO may also directly improve insulin-signaling pathway by affecting molecules such as insulin receptor, insulin receptor substrate-1, or phosphatidylinositol 3-kinase (26,42).

A derivative of thiozolidinediones, PIO, induces PDE3B mRNA expression in white adipose tissues of KKAY mice.

Thiozolidinedione is thought to be a ligand for the peroxisome proliferator-activated receptor (PPAR)- γ . In fat tissues, adipogenesis is stimulated upon PPAR- γ activation by ligands (43). PIO increases the number of small size adipocytes without changes in overall fat pad weight, which suggests that adipocyte differentiation is induced (44,45). PDE3B mRNA is also induced during differentiation from preadipocyte to adipocytes in 3T3-L1 cells (46). The effect of PIO on PDE3B gene expression might therefore be related to the differentiation state of adipocytes.

In summary, PDE3B gene expression is reduced in adipocytes of KKAY mice, and PIO restores this altered gene expression with a parallel improvement in insulin resistance. It is not clear how PDE3B gene expression is regulated under these conditions and if the altered PDE3B gene expression is the primary event in the cause of insulin resistance observed with type 2 diabetes. Further experiments will be required to clarify these points.

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