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Phospholipid methyltransferase activity in diabetic rat fat cells: effect of isoproterenol and insulin

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Abstract

The effects of isoproterenol and insulin on phospholipid methyltransferase (PLMT) activity were investigated in adipocytes from control and streptozotocin-diabetic rats. PLMT activity was assayed by measuring the rate of incorporation of ^{3}H-methyl groups from S-adenosyl-L-[methyl-^{3}H] methionine into phospholipids. Basal PLMT activity was higher in adipocytes from diabetic animals. Treatment of adipocytes with isoproterenol induced a concentration-dependent stimulation of PLMT activity. In control adipocytes, the maximal effect was obtained at 100 nM isoproterenol with 2.3 fold increase in PLMT activity and a half maximal effect at 25 nM. In adipocytes from diabetic rats, a lower dose of isoproterenol (10 nM), caused 1.2 fold increase with a half maximal effect at 4 nM. Addition of 100 nM insulin inhibited basal PLMT activity and the stimulatory effect of isoproterenol in both types of adipocytes. The β-adrenergic blocking agent propranolol inhibited the stimulatory effect of isoproterenol on PLMT activity in control and diabetic adipocytes. Intracellular concentration of cAMP was higher in diabetic adipocytes but decreased to normal values after incubation in the presence of insulin. (Mol Cell Biochem **115:** 97–103, 1992)

Key words: phospholipid methylation, phospholipid methyltransferase, adipocytes, diabetic rats

Introduction

The biological response to hormones such as modulation of enzyme activities [1] and secretory events [2] are affected by the phospholipid microenvironments within the cell. In rat adipocytes, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the two major phospholipids of the plasma membrane. Phosphatidylcholine can be synthesized by transcholination or by transmethylation. The first pathway involves the transfer of a phosphocholine group from CDP-choline to a diacyl-glycerol molecule [3]. The transmethylation pathway consists of the stepwise N-methylation of PE to give PC, where phosphatidyl-N-monomethylethanolamine (PME) and phosphatidyl-N,N-dimethylethanolamine (PDE) were identified as intermediate products and S-adenosyl-L-methionine (AdoMet) as the methyl donor [4]. These methylation reactions are thought to change several membrane functions, such as alteration of membrane fluidity [5] and coupling of the β-adre-

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nergic receptor with adenylate cyclase [6]. The methylation reactions are catalyzed by phospholipid methyltransferase (PLMT), which is an ubiquitous membrane bound enzyme. In adipocytes [7–9], hepatocytes [10, 11], and Leydig cells [12], the activation of PLMT occurs in response to hormones that increase cAMP. Insulin, the major anabolic hormone, inhibits basal PLMT activity, as well as that stimulated by hormones acting through cAMP [7–9, 13]. Decreased PLMT activity has been observed in liver microsomes from alloxan-diabetic rats [14, 15]. However, phospholipid methylation in hepatocytes isolated from normal and alloxan diabetic rats was the same when assayed under identical conditions [15]. The present experiments were undertaken to further study the state of activation of PLMT and its response to isoproterenol and insulin in adipocytes from control and streptozotocin-diabetic rats.

Materials and methods

Materials

Male Wistar rats (180–190 g), maintained on a standard laboratory diet and water ad libitum, were used in our study. The diabetic group was obtained by intravenous injection of streptozotocin (40 mg/kg). The animals were used 8 days after the injection, exhibited a blood glucose concentration of 400–600 mg/100 ml, and did not show any significant loss of body weight. When treated with insulin, the animals received subcutaneously 2 U/day insulin NPH (Eli Lily Co) two days after streptozocin injection.

Collagenase was purchased from Worthington (Freehold, NJ), and bovine albumin (Fraction V) from U.S. Biochemicals (Cleveland, OH). Phosphatidylethanolamine (PE), phosphatidyl-N-mono-methylethanolamine (PME), phosphatidyl-N-N-dimythylethanolamine (PDE), phosphatidylcholine (PC), S-Adenosylmethionine (AdoMet) and DL-isoproterenol-HCl were obtained from Sigma Biochemical Co; DL-propranolol-HCl was from Imperial Chemical Industries Limited (Great Britain). S-Adenosyl-L-[methyl-^{3}H]methionine and the [^{125}I]cAMP RIA kit were purchased from New England Nuclear (Boston, MA).

Isolation and incubation of adipocytes

Isolated fat cells were obtained by enzymatic digestion of epididymal fat pad according to the procedure of Rodbell [16]. Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4, containing 2.5 mM calcium and 4% albumin was used. Fat cells (1×10^6 cells in 1.25 ml medium) were incubated with continuous shaking at 37° C in polyethylene vials gassed with 95% O^2-5% CO_2. After 30 min preincubation, aliquots of KRB buffer or buffer containing test agents were added, and the incubation was continued for an additional 15 min. After incubation with the test agents, cell suspensions were used for measurement of PLMT activity or cAMP concentration.

Measurement of PLMT activity

PLMT activity of adipocyte infranatant was assayed as previously described [13]. The suspension of adipocytes (1×10^6 cells) was transferred to a microfuge tube, and cells were separated from incubation medium by centrifugation. The incubation medium was removed by aspiration, and the cells were disrupted with the addition of 0.25 ml of homogenization buffer (125 mM Tris-HCl, 2 mM KF, 2 mM EGTA, 2 mM EDTA, pH 8.5). The cells were vigorously shaken, and the homogenate was microcentrifuged. The resultant infranatant was transferred to another tube and kept in ice. To measure PLMT activity, 0.125 ml fat cell infranatant was used in a reaction mixture that contained 0.125 mM Tris-buffer (pH 8.5), 5 mM $MgCl_2$, 0.5 mM KF, 4 mM dithiothreitol, 0.5 mM EGTA, 0.5 mM EDTA, 100 μM AdoMet, 2 μCi S-adenosyl-L-[methyl-^{3}H]methionine ([^{3}H]AdoMet; 15 Ci/mmol), and 40 μg PME (0.1 mM) in a final volume of 0.5 ml. PME was dispersed by sonication in 125 mM Tris-buffer (pH 8.5) containing 1% propylene glycol. The reaction was initiated by addition of the mixture of labeled and unlabeled AdoMet. Samples were incubated for 20 min at 37° C. Preliminary assays showed that the reaction was linear with time for at least 20 min. The reaction was stopped by the addition of 3 ml 0.05 N HCl in chloroform-methanol (1:2, vol/vol) and phospholipids were extracted by the method of Bligh and Dyer [17]. An aliquot of the chloroform phase was transferred to a scintillation vial, evaporated to dryness and resuspended in scintillation mixture for determination of radioactivity. The activity of PLMT was expressed in nmoles of [^{3}H]methyl groups incorporated into phospholipids per 10^6 cells/20 min of assay. Radioactivity extracted into the organic phase after incubation in the absence of tissue was subtracted from all

samples. Separation and quantification of the individual methylated phospholipids was performed by thin layer chromatography on Silica Gel G plates developed in a solvent of propionic acid : 1-propanol : chloroform : water (2:4:2:1, vol/vol). PE, PME, PDE and PC were identified chromatographically using authentic standards as described by Hirata *et al.* [6]. The radioactivity incorporated into PC and its intermediates was accomplished by visualizing the standards after exposure to iodine and scraping the fraction of silica gel corresponding to known standards. Each fraction was then eluted with 0.5 ml of the chromatography solvent. After a 6 h incubation at room temperature, 0.4 ml was pipetted into a counting vial, dried at 70° C, dissolved in scintillator, and counted. The total recovery of the radioactivity applied to thin layer plates was 80–90%. Activity of the enzyme was expressed either in pmoles or nmoles of [^{3}H]methyl groups incorporated into individual phospholipids per 10^6 cells/20 min of assay.

cAMP assay

cAMP concentration in adipocytes was determined as previously described [18]. After incubation, 125 μl of 100% trichloroacetic acid was added and the cell suspension homogenized at 4° C. The homogenate was centrifuged at 2000 × g at 4° C for 15 min and 0.4 ml aliquots of the supernatant were extracted four times with 5 vol of water-saturated ether. The ether phase was discarded and the samples were lyophilized and redissolved in 0.3 ml of 0.05 M sodium acetate buffer pH 6.2. Recovery during extraction was determined by the addition of a tracer quantity (5000 cpm) of [^{3}H]cAMP to the trichloroacetic extract. The cAMP was determined by the radioimmuno-assay involving acetylation of the samples as supplied by New England Nuclear.

Statistical analysis

Statistical analysis of the results were done by ANOVA. A P value < 0.05 was considered significant.

Results

Treatment of adipocytes from control and diabetic rats with isoproterenol caused a concentration-dependent stimulation of PLMT activity (Fig. 1). The maximal effect of isoproterenol occurred at 100 nM and resulted in a 2.3 fold increase in PLMT activity of adipocytes from control animals; the half-maximal effect was seen at 25 nM. In adipocytes from diabetic rats, the concentration-dependent stimulation of PLMT activity was shifted to the left; the maximal effect was lower (1.2 fold) and occurred at 10 nM isoproterenol, with the half-maximal effect found at 4 nM. At 10 nM isoproterenol, PLMT activity was higher in adipocytes from diabetic rats. Addition of 100 nM insulin decreased the stimulatory effect of isoproterenol in adipocytes from both control and diabetic animals. It also decreased basal PLMT activity in control and diabetic adipocytes, the latter exhibiting greater activity than the control (Fig. 2). PLMT activity in adipocytes from diabetic rats incubated in the presence of 100 nM insulin was the same as that in control adipocytes incubated without insulin. Both basal and isoproterenol-stimulated PLMT activity returned to control values in streptozocin-diabetic rats treated with insulin for 6 days (Fig. 3).

Intracellular cAMP concentration in adipocytes from control and diabetic rats incubated for 15 min under basal conditions was 190 ± 22 and 320 ± 35 pmoles/10^6 cells, respectively. Incubation for 15 min in the presence of 100 nM insulin produced a decrease in cAMP content in both preparations (control: 120 ± 15 and diabetic: 210 ± 28 pmoles/10^6 cells). The level of cAMP in adipocytes from diabetic rats after incubation with insulin, was similar to that in control adipocytes without insulin.

Figure 4 shows the pattern of methylated phospholipids in adipocytes from control and diabetic rats. Under our assay conditions, the [^{3}H]methyl group was mainly incorporated into PDE. This is consistent with previously reported data on PLMT activity measured under similar assay conditions [7, 13]. Basal incorporation of [^{3}H]methyl group into PDE and PC was significantly higher in adipocytes from diabetic rats.

Table 1 shows the overall results of methylated phospholipids in adipocytes from control and diabetic rats incubated for 15 min in the absence and presence of 100 nM insulin, 10^{-8} M isoproterenol, 100 nM insulin plus 10^{-8} M isoproterenol, 10^{-8} M isoproterenol plus 10^{-5} M propranolol and 10^{-5} M propranolol, respectively. Incubation in the presence of 100 nM insulin caused inhibition of basal and isoproterenol stimulated PLMT activity in control and diabetic adipocytes. The stimulatory effect induced by 10^{-8} M isoproterenol was also higher in adipocytes from diabetic rats. That is, the higher total incorporation of [^{3}H-methyl] groups into phospholipids (PC + PDE + PME) confirmed the re-

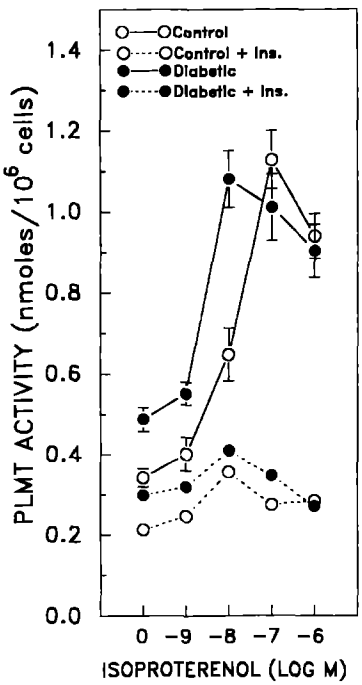

Fig. 1. Effect of isoproterenol on PLMT activity. Adipocytes (1×10^6 cells) from control and diabetic rats were incubated for 15 min at 37° C without and with increasing concentration of isoproterenol in the absence and presence of 100 nM insulin. PLMT activity was determined as described in Methods. Each value in the figure represents the mean ± SE of four experiments performed in duplicate. Where not shown, SE were smaller than symbol used.

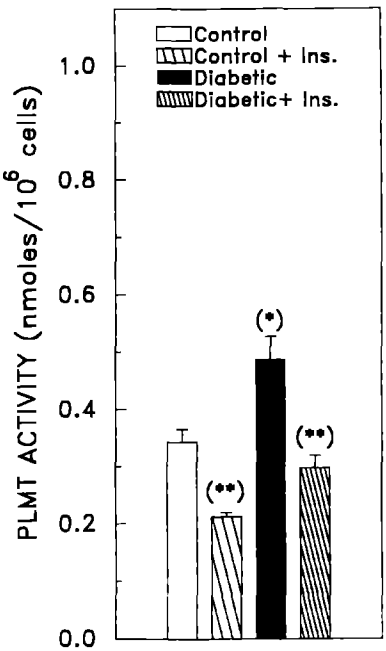

Fig. 2. PLMT activity in adipocytes from control and diabetic rats. Adipocytes (1×10^6 cells) were incubated during 15 min at 37° C in the absence and presence of 100 nM insulin. PLMT activity was determined as described in Methods. Each value in the figure represents the mean ± SE of four experiments performed in duplicate. (*) Diabetic versus control, $P < 0.05$; (**) control and diabetic (+ Ins.) versus (− Ins.), $P < 0.05$.

sults shown in Fig. 1 at 10^{-8} M isoproterenol where PLMT activity was increased in adipocytes from diabetic rats. Addition of the β-adrenergic blocking agent, propranolol, inhibited the stimulatory effect of isoproterenol without affecting basal PLMT activity in both types of adipocytes.

Discussion

The activity of PLMT and its response to isoproterenol and insulin in adipocytes from normal and streptozotocin-diabetic rats were examined. The dose-response curve in which PLMT activity was examined at varying isoproterenol concentration revealed a maximal effect of the hormone at 100 nM, consistent with previous studies using control adipocytes [9]. It has been proposed that the increase in PLMT activity induced by catecholamines, ACTH and glucagon in rat adipocytes is mediated by cAMP [7–9, 13]. This is supported by studies showing that incubation of adipocytes in the presence of cAMP and forskolin increases PLMT activity [9]. The increase in PLMT activity is associated with an elevation in the phosphorylation state of the enzyme, mediated by cAMP-dependent kinase [11, 20–21]. Compared to controls, adipocytes from diabetic rats showed a higher response to low doses of isoproterenol (Fig. 1). Although the maximal effect of the β-agonist on PLMT activity was obtained at lower doses (10 nM), this maximal effect was lower than that obtained with control adipocytes. The data also showed that basal PLMT activity is increased in diabetic rat adipocytes. Moreover, basal PLMT activity, as well as the response to isoproterenol in adipocytes from control and diabetic rats, were inhibited by insulin. The inhibitory effect of insulin on basal and hormone-stimulated PLMT activity in adipocytes from normal rats has been previously reported [7–9, 13] and has been attributed to inhibition of PLMT phosphorylation [22].

The increase in the intracellular concentration of cAMP found in adipocytes from diabetic rats, which decreased to normal values after incubation in the presence of insulin, has been reported earlier by this laboratory [18]. Moreover, an increase in adenylate cyclase activity in adipose tissue from diabetic subjects [23] and

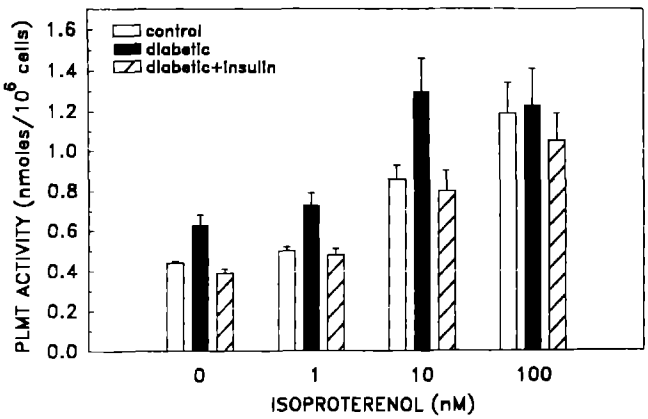

Fig. 3. Effect of isoproterenol on PLMT activity in adipocytes from control, diabetic and insulin-treated diabetic rats. Adipocytes (1×10^6 cells) were incubated during 15 min at 37° C in the absence and presence of increasing concentration of isoproterenol. PLMT activity was determined as described in Methods. Each value in the figure represents the mean ± SE of three experiments performed in duplicate.

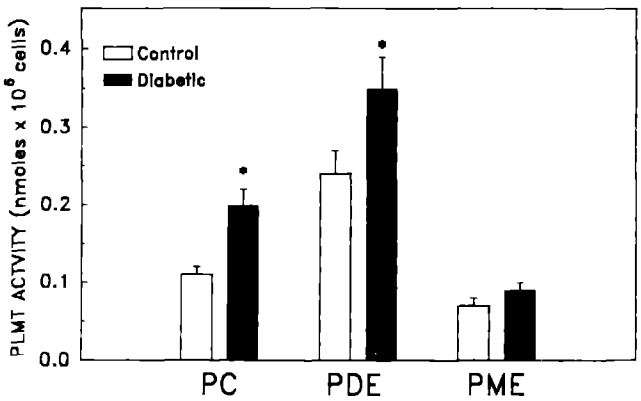

Fig. 4. PLMT activity in adipocytes from control and diabetic rats. Adipocytes (1×10^6 cells) were incubated for 15 min at 37° C. [^{3}H]-Methyl groups incorporated into PC, PDE and PME were determined as described in Methods. PLMT activity is expressed as nmoles/20 min. Results are the mean ± SE of four experiments performed in duplicate. (*) Statistically significant difference ($P < 0.05$) between control and diabetic adipocytes.

rats [24] has been previously reported. It has been also shown by this and other laboratories [24–26], greater response to β-adrenergic stimulation of adenylate cyclase activity and the cAMP-protein kinase system of adipocytes from diabetic rats. Insulin treatment of diabetic rats reduced the response of adenylate cyclase to epinephrine [26]. Thus, the present results with adipocytes from diabetic rats showing higher basal PLMT activity, as well as greater response to low doses of isoproterenol, which returned to control values following the incubation with insulin, could be attributed to the levels of cAMP in these adipocytes.

The effect of isoproterenol and insulin on individual methylated phospholipids confirms the results shown in Fig. 1. Insulin decreases the methylation of individual phospholipids in adipocytes from both control and diabetic rats and inhibits the stimulatory effect of isoproterenol. The stimulatory effect of isoproterenol was blocked by propranolol, indicating that it was a β-agonist-mediated event in both type of adipocytes. This is in agreement with previously reported findings which showed that the stimulatory effect of isoproterenol in rat hepatocytes was blocked by propranolol [8].

It has been suggested that in rat liver, PLMT does not operate maximally because of nonsaturating cellular levels of AdoMet [15]. Therefore, increases in the concentration of AdoMet will stimulate PLMT activity. In this regard, it is significant that both the activity of

Table 1. [^{3}H]-Methyl groups incorporated into phospholipids

	Control			Diabetic		
	PC	PDE	PME	PC	PDE	PME
Basal	110 ± 13	241 ± 20	73 ± 7	200 ± 15[1]	350 ± 38[1]	90 ± 8
Ins	32 ± 3[2]	70 ± 8[2]	50 ± 4[2]	40 ± 7[2]	98 ± 8[2]	42 ± 4[2]
Iso	210 ± 20[3]	470 ± 70[3]	110 ± 10[3]	400 ± 25[3]	878 ± 90[3]	140 ± 13[3]
Iso + ins	120 ± 11	250 ± 33	80 ± 9	190 ± 30	370 ± 75	102 ± 15
Iso + prop	140 ± 13	260 ± 22	60 ± 5	210 ± 16	360 ± 72	100 ± 16
Prop	99 ± 10	240 ± 31	75 ± 9	190 ± 11	320 ± 25	70 ± 7

Adipocytes (1×10^6 cells) from control and diabetic rats were incubated for 15 min in the absence and presence of 100 nM insulin, 10^{-8} M isoproterenol, 10^{-8} M isoproterenol + 100 nM insulin, 10^{-8} M isoproterenol + 10^{-5} M propranolol and 10^{-5} M propranolol respectively. [^{3}H]-Methyl groups incorporated into PC, PDE and PME were determined as described in Methods. PLMT activity is expressed as pmoles/20 min. (1) Diabetic versus control, $P < 0.05$; (2) Control and diabetic (+ ins) versus (− ins), $P < 0.05$; (3) Control and diabetic (+ isoproterenol) versus (− isoproterenol), $P < 0.05$. Results are the mean ± SE of 4 experiments performed in duplicate.

AdoMet-synthetase and AdoMet levels increase in hepatocytes of alloxan-diabetic rats [15]. The same authors have found no difference in phospholipid methylation in whole hepatocytes although PLMT activity is lower in liver microsomes from diabetic animals. This apparent discrepancy is explained by the increased intracellular concentration of AdoMet in hepatocytes from diabetic animals, which could cause a concomitant increase in the rate of phospholipid methylation. The present data showing increased basal PLMT activity, as well as greater response to low doses of isoproterenol in diabetic adipocytes, cannot be attributed to an increased intracellular level of AdoMet since this is fixed (100 μM) in the enzymatic assay, but could be related to differences in the experimental conditions and in the severity of the diabetic state.

The lower maximal effect of isoproterenol on PLMT activity in adipocytes from diabetic rats might be caused by a deficiency in the number of β-receptors. Although it has been reported that diabetes decreases both the response of adenylate cyclase activity to isoproterenol and the number of β-receptors in rat adipocytes [27], this is not universally accepted [24–26]. Another possible explanation for the lower maximal effect of isoproterenol in diabetic adipocytes is that diabetes might repress synthesis of the enzyme. Consequently, the maximal response would be lower than that found in adipocytes from non-diabetic animals.

Insulin-treated diabetic rats exhibited normal basal and isoproterenol-stimulated PLMT activity. These results indicate that the effect of isoproterenol treatment on PLMT activity is insulin-dependent and not due to toxic effects of the drug.

It is concluded from these studies that multiple mechanisms may be involved in the state of activation of PLMT in adipocytes from diabetic rats.

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