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Rosiglitazone Modulates Insulin-Induced Plasma Membrane Area Changes in Single 3T3-L1 Adipocytes

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Abstract In this study we hypothesized that rosiglitazone, an antidiabetic high-affinity agonist for the peroxisome proliferator–activated receptor γ, affects the plasma membrane (PM) turnover in single 3T3-L1 adipocytes. To study the PM turnover, the patch-clamp electrophysiological method was used to measure changes in membrane capacitance (C_m), a parameter linearly related to the PM area. Microscopy results show that the presence of rosiglitazone in the differentiating medium significantly increased the differentiation of 3T3-L1 adipocytes in cell culture, based on oil red O–stained area (11.4 ± 1.2%) vs. controls (3.1 ± 0.5%). Moreover, rosiglitazone treatment significantly reduced the size of single 3T3-L1 adipocytes; their average radius of 21.1 ± 1.1 μm in controls was reduced to 17.5 ± 0.5 μm in rosiglitazone-treated cells. Consistent with this, insulin application increased the rate of C_m increase to 2.34 ± 0.10%/min, which was significantly different from controls (0.12 ± 0.08%/min). However, pretreatment of cells with rosiglitazone prior to the treatment with insulin resulted in an attenuated rate of C_m increase. These data support the involvement of insulin in the modulation of membrane area and show that treatment by rosiglitazone reduced the insulin-mediated membrane area increase in 3T3-L1 adipocytes.

Keywords 3T3-L1 adipocyte · Insulin · Rosiglitazone · Exocytosis · Endocytosis · Membrane area regulation

Introduction

Adipocytes vary greatly in their size. Large adipocytes are associated with diseased states such as the metabolic syndrome and diabetes type 2 (Danforth 2000). Along with skeletal muscle cells, adipocytes are the main target for insulin action to lower blood glucose level by stimulating the transport of glucose across the plasma membrane (PM) into the cell (Whitesell and Abumrad 1985).

White adipocytes constitute one of the most important target cells for insulin action in the body. Upon a rise of plasma glucose concentration, β-cells in the pancreas begin to secrete insulin, which is blood-borne and binds to receptors on target cells, mainly liver but also adipocytes and skeletal muscle cells. Insulin binding to receptors triggers an increase in the permeability of the PM to glucose (Whitesell and Abumrad 1985) by increasing the number of glucose transporter isoform 4 (GLUT4) molecules in the PM (Oka and Czech 1984), most likely by the process of exocytosis (Holman et al. 1990; Cain et al. 1992; Holman and Cushman 1994; Holman 1999; Randhawa

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et al. 2000; Neher and Marty 1982; Lee and Pappone 1997). Exocytosis represents the fusion of membrane-bound vesicular organelles with the PM and increases the PM surface area, whereas the process of endocytosis causes a decrease in the PM surface area. Net changes in PM surface area can be monitored directly by electrophysiological measurements of membrane capacitance (C_m), a parameter linearly proportional to the PM surface area (Neher and Marty 1982).

Tiazolidinediones (TZDs), a new class of oral antidiabetic agents, are high-affinity ligands for peroxisome proliferator–activated receptors (PPARs) and function as insulin sensitizers (Saltiel and Olefsky 1996). Many studies have shown that administration of TZDs to obese animal models of insulin resistance and type 2 diabetes patients can result in a substantial correction of hyperglycemia and/or hyperinsulinemia (Berger et al. 1996; Fujiwara et al. 1988). The TZD rosiglitazone is used in the treatment of type 2 diabetes mellitus (Yki-Jarvinen 2004). It has been proposed that the glucose-lowering action of TZDs is mainly mediated by activating PPARγ (Larsen et al. 2003; Olefsky 2000). PPARγ is most abundantly produced in adipose tissue, suggesting that this is the primary site of action of TZDs (Kintscher and Law 2005). It has been shown that PPARγ agonists are dominant regulators of adipocyte development and that PPARγ activation lowers insulin resistance (Camp et al. 2000; Vazquez et al. 2002). As a TZD, rosiglitazone is a potent agonist of PPARγ and could significantly improve the adipocyte differentiation in 3T3-L1 cells. Activation of PPARγ can increase the number of small adipocytes and reduce the number of large adipocytes in white adipose tissue (Rangwala and Lazar 2004). Because small adipocytes are more sensitive to insulin (Lowell 1999), an increased number of small adipocytes in white adipose tissue can alleviate insulin resistance (Lowell 1999). Furthermore, adipocyte differentiation leads to the expression of adipocyte-specific genes, such as *GLUT4* and insulin receptor substrate-1 (*IRS-1*), which are important components of the insulin receptor signal-transduction pathway (Ntambi and Young-Cheul 2000; Okuno et al. 1998; White and Kahn 1994; Wu et al. 1998).

The three aims of this study were to determine (1) whether the treatment of 3T3-L1 cells by rosiglitazone affects the rate of differentiation and the size of single 3T3-L1 adipocytes, (2) whether changes in C_m of insulin-treated single 3T3-L1 adipocytes exhibit an increase in PM area as shown previously for primary adipocytes (Chowdhury et al. 2005), (3) whether activation of PPARγ by the competitive PPARγ receptor agonist rosiglitazone (Teruel et al. 2005) significantly alters the specific insulin-induced changes in membrane surface area of single adipocytes.

Materials and Methods

Chemicals

Dulbecco's modified Eagle's medium (DMEM), dexamethasone, isobutylmethylxanthine (IBMX), *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), KCl, D-glucose, NaH_2PO_4, $NaHCO_3$, Na_2ATP and oil red O (ORO) were purchased from Sigma (St. Louis, MO). Insulin was purchased from Novo Nordisk (Bagsvaerd, Denmark). $CaCl_2 \times 6H_2O$ was obtained from Riedel De Haen (Seelze-Hannover, Germany). $MgCl_2$ was purchased from Kemika (Zagreb, Croatia). Fetal bovine serum (FBS) was obtained from Biochrom (Berlin, Germany). Rosiglitazone maleate was obtained from GlaxoSmithKline (Worthing, UK). Dimethyl sulfoxide (DMSO) was purchased from Merck Schuchardt (Hohenbrunn, Germany).

Cell Culture

3T3-L1 fibroblasts were grown in DMEM containing 10% FBS, as described previously (Rangwala and Lazar 2004). Briefly, fibroblasts were seeded onto poly-L-lysine-coated coverslips and allowed to grow to confluence. Conversion into adipocytes was induced on day 0 in DMEM containing 10% FBS, 0.25 mM dexamethasone, 0.5 mM IBMX and 174 nM insulin for 2 days (time zero to day 2), and then medium was replaced with DMEM containing 10% FBS and 174 nM insulin for a further 2 days (days 2-4). On day 6 most of the cells were differentiated and cells were transferred to DMEM containing 10% FBS. In the first part of our study conversion into adipocytes was induced as described above except that we applied an additional bolus of rosiglitazone (rosiglitazone-pretreated cells) or vehicle (control cells) from the beginning of the induction of differentiation (from day zero). During experiments, rosiglitazone was added to a final concentration of 10 μM, from a 1 mM stock prepared in DMSO. In the second part of this study differentiated 3T3-L1 cells were divided into three experimental groups: (1) control cells, vehicle addition; (2) cells treated with 0.2 μM insulin for 9 min (Ins); (3) cells pretreated with 10 μM rosiglitazone for 24 h and then treated with 0.2 μM insulin for an additional 7 min (Ins + Rosi).

ORO Staining

ORO staining was used to stain for lipid accumulation in differentiating 3T3-L1 cells (Ramirez-Zacarias et al. 1992). Cells were rinsed in phosphate-buffered saline prior to fixing with 4% paraformaldehyde for 2 min. After removing the fixation agent and rinsing with redistilled water, ORO working solution (six parts of ORO 0.5% stock in isopropanol mixed with four parts of distilled water) was

used to stain for lipid accumulation (1–2 h at room temperature). After rinsing with the redistilled water, transmitted-light images were obtained using the LSM 510 microscope (Zeiss, Oberkochen, Germany) on days 1 and 7 in control and rosiglitazone-treated cells.

Laser Scanning Confocal Microscopy

3T3-L1 cells on poly-L-lysine-coated coverslips were placed in a recording chamber on the LSM 510 microscope. The recording medium consisted of (in mM) NaCl 131.8, $CaCl_2$ 1.8, KCl 5, HEPES/NaOH 10, D-glucose 10, NaH_2PO_4 0.5, $NaHCO_3$ 5 (pH 7.2). The transmitted-light images of 3T3-L1 cells were acquired through a planapochromatic oil-immersion objective ($\times$40, numerical apreture (NA) = 1.2), excited by the 543 nm helium-neon laser line. Differentiated adipocytes were examined in two groups, control and rosiglitazone-treated, both on day 1 and on day 7. Using the LSM 510 software (Zeiss), ORO-stained cell images were examined by setting the intensity threshold value to 5% maximal intensity level (Fig. 1b), and we calculated the relative area of the dark, below-threshold level pixels relative to the area of all pixels on the image. The below-threshold pixels corresponded to the ORO-stained lipid droplets. For each set of experiments 10 images were analyzed.

Electrophysiological Recording

Using the whole-cell patch-clamp technique, the cells were voltage-clamped at a holding potential of −25 mV. Membrane capacitance was recorded using a two-phase lock-in amplifier (800 Hz, 1 mV peak to peak) incorporated into a patch-clamp amplifier (SWAM Cell, Celica, Slovenia) (Zorec et al. 1991). A DC current (low pass, 10 Hz, −3 dB), holding potential and real and imaginary admittance signals (low pass, 10 Hz, −3 dB) were used in calculations (Zorec et al. 1991). The plots of the passive cell parameters access conductance (G_a), parallel combination of leak and membrane conductance (G_m) and C_m were derived by computer-aided reconstruction following an analogue-to-digital conversion (NI PCI 6014; National Instruments, Austin, TX) using a compatible PC and the Cell software (Celica, Ljubljana, Slovenia). Recordings were made at room temperature with pipette resistance between 1 and 4 MΩ. The basic solution in the recording pipette consisted of (in mM) $MgCl_2$ 2, HEPES/KOH 10, KCl 150, Na_2ATP 2 (pH 7.2). The recording medium consisted of (in mM) NaCl 131.8, $CaCl_2$ 1.8, KCl 5, HEPES/NaOH 10, D-glucose 10, NaH_2PO_4 0.5, $NaHCO_3$ 5 (pH 7.2).

During electrophysiological recordings of C_m, cells were treated either with 200 or 70 nM insulin, with vehicle solution or with 30 nM IGF-I for 7 min. In separate experiments, cells were pretreated for 15 min with 20 nM wortmannin prior recording C_m and then stimulated with insulin during the recording of C_m. Secretory responses were measured as a change in C_m (%) relative to the resting C_m determined immediately after the establishment of a whole-cell recording.

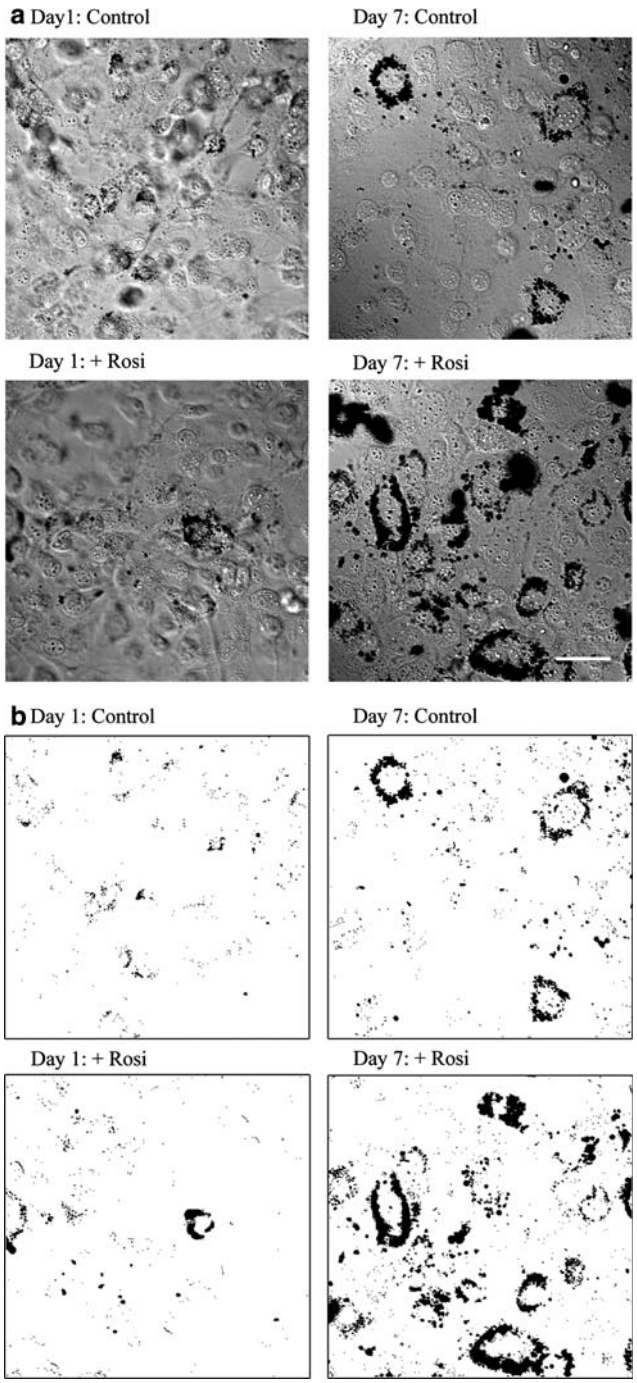


Fig. 1 (**a**) Transmitted-light images of control (*upper panels*) and rosiglitazone-treated (*lower panels*) 3T3-L1 cells stained with ORO on day 1 and day 7 of differentiation show an increased number of ORO-stained cells in the presence of rosiglitazone (day 7 + Rosi). Scale bar = 40 μm. (**b**) Corresponding mask images of **a**, showing only the dark ORO staining, with a threshold level of 5%

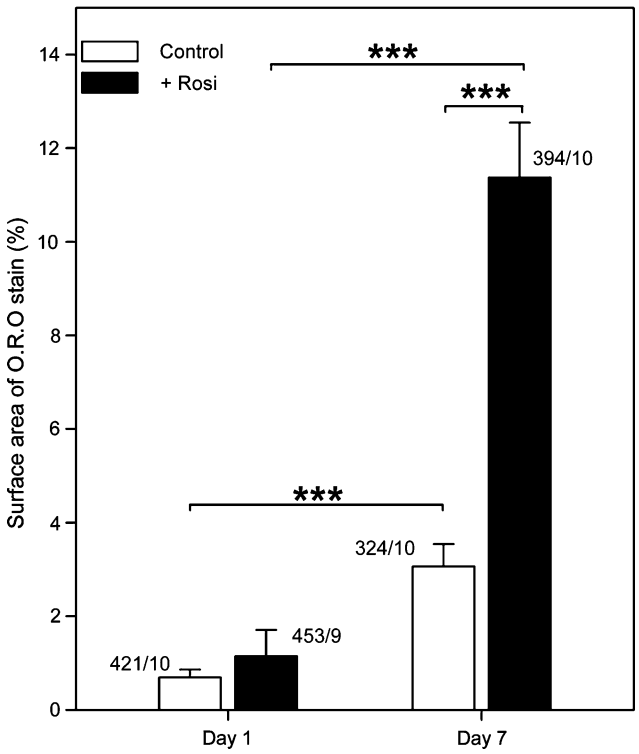


Fig. 2 The relative area of ORO-stained 3T3-L1 adipocytes expressed as a percent vs. entire area of the field of view on day 1 and day 7 in control (*open bars*) and in rosiglitazone-treated cells (*filled bars*). Numbers above columns indicate number of cells counted/number of images examined; error bars show SEM. *Asterisks* indicate significant differences (*** $P < 0.001$)

Statistical Analysis

Unless stated otherwise, statistics are in the format of mean ± SEM and differences between samples were tested by Student's *t*-test. Linear regression analysis was carried out by standard techniques (Sigma Plot software; SPSS, Inc., Chicago, IL).

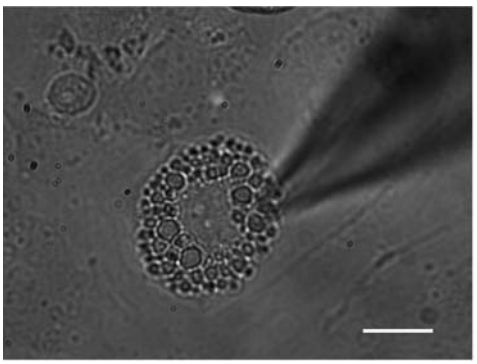
Results

Rosiglitazone Treatment Increases Lipid Accumulation in Differentiated Cells

It has been reported previously that treatment with PPARγ agonists improves insulin resistance and increases the number of small adipocytes in fat tissue (Rangwala and Lazar 2004). To study whether rosiglitazone, a high-affinity ligand for the PPARs, would have a similar effect on clonal 3T3-L1 adipocytes, we treated our cells with rosiglitazone (10 μM) for a period of 7 days, starting on day 0, the day of the induction of differentiation. Figure 1a shows differentiating cells on day 1 and fully differentiated adipocytes (stained with ORO for lipid droplets) on day 7 in control and rosiglitazone-treated conditions. The average ORO-stained area increased significantly from day 1 to day 7 in control (from 0.7 ± 0.2% to 3.1 ± 0.5%, $P < 0.001$) as well as in rosiglitazone-treated cells (from 1.19 ± 0.6% to 11.4 ± 1.2%, $P < 0.001$) (Fig. 2). Furthermore, the ORO-stained area in control and rosiglitazone-treated cells was not significantly different on day 1 but was significantly different on day 7 ($P < 0.001$). Based on this result, we have selected fully differentiated 3T3-L1 adipocytes exhibiting multiple lipid droplets for patch-clamp experiments.

C_m of 3T3-L1 Adipocytes Normalized to Square Radius

We determined C_m normalized to square radius from the regression between the resting membrane capacitance,



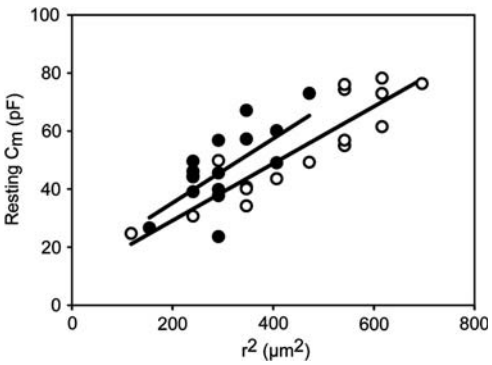


Fig. 3 Micrograph of a patch-clamped 3T3-L1 adipocyte (*left panel*) tacked to the glass coverslip; scale bar = 20 μm. *Right panel* shows correlation between squared radius (μm^2) and resting C_m (pF) of single control cells (*open circles*, $n = 15$) and rosiglitazone-treated cells (*filled circles*, $n = 19$). Cells were treated with 10 mM Rosi for 24 h. Regression lines (obtained using SPSS Sigma Plot software) are drawn according to the equations of the form C_m (pF) = (0.098 ± 0.014) (pF/μm^2) * (square radius) (μm^2) + (9.5 ± 6.6) (pF) for control and C_m (pF) = (0.111 ± 0.029) (pF/μm^2) * (square radius) (μm^2) + (13.1 ± 9.2) (pF) for rosiglitazone-treated cells. Note that both intercepts are not different from zero, while the slope coefficients are significantly different from zero ($P < 0.01$) and reflect the C_m normalized to squared radius of 3T3-L1 adipocytes. Mean cell squared radius (r^2) of control and rosiglitazone-treated cells was 462.2 ± 42.4 and 310.1 ± 18.8 μm^2, respectively. Mean resting C_m of patch-clamped control cells and rosiglitazone-treated cells was 54.9 ± 4.6 and 47.4 ± 3.1 pF, respectively

measured immediately after the establishment of the whole-cell configuration, and squared radius of cells. Therefore, we measured the diameter of cultured 3T3-L1 adipocytes prior to patching them (Fig. 3, left). The radius of the rosiglitazone-treated group was significantly smaller (17.5 ± 0.5 μm, $n = 19$) vs. controls (21.1 ± 1.1 μm, $n = 15$). The average resting C_m was not significantly different in rosiglitazone-treated cells (47.4 ± 2.9 pF) vs. controls (54.8 ± 4.6 pF). From the squared radius and resting C_m, regression lines were determined separately for control and rosiglitazone-treated cells. The correlation coefficient for control cells was 0.90, and that or rosiglitazone-treated cells was 0.68. The slope coefficient of the regression line indicates the C_m normalized to square radius, as shown in Fig. 3 (right). The normalized C_m corresponds in control cells to 0.10 ± 0.01 pF/μm^2 ($n = 15$, open symbols) and in rosiglitazone-treated cells to 0.11 ± 0.03 pF/μm^2 ($n = 19$, filled symbols), both significantly different from zero ($P < 0.002$) but not different from each other. These values are comparable to the value of specific C_m reported previously (Chowdhury et al. 2005). The line intercepts are 9.5 ± 6.6 pF and 13.1 ± 9.2 pF for control and rosiglitazone-treated cells, respectively; both parameters are not different from zero.

Insulin- and Rosiglitazone-Induced Changes in C_m

It has been shown previously that in primary white adipocytes insulin addition results in an increase in C_m (Chowdhury et al. 2005); therefore, we first tested whether an insulin-induced increase in C_m is present also in 3T3-L1 adipocytes. Furthermore, we also questioned whether the activation of PPARγ by rosiglitazone significantly alters the specific insulin-induced change in C_m of single adipocytes. Rosiglitazone was applied to fully differentiated cells on day 9 (i.e., 3 days after the estimated time of completed differentiation), and after 24 h we measured C_m. Approximately 2 min after the establishment of the whole-cell configuration, insulin was applied (final concentration of 200 nM, for 7 min) into the recording medium in culture dish with 3T3-L1 cells. Representative time-dependent traces in C_m are shown in Fig. 4. In control cells C_m was increased with a slow, steady rate (Fig. 4a, top). However, over time the average increase was not statistically significant, as evidenced by the regression analysis (Fig. 5a). The slope of the regression line was not significantly different from zero. G_a did not change during recordings (Fig. 4a, bottom trace), nor did G_m (not shown). Importantly, the application of insulin resulted in a significant increase in C_m (Fig. 4b, top), which was not correlated with changes in G_a (Fig. 4b, bottom). In control cells, the regression line through the data is characterized with a slope coefficient not significantly different from zero

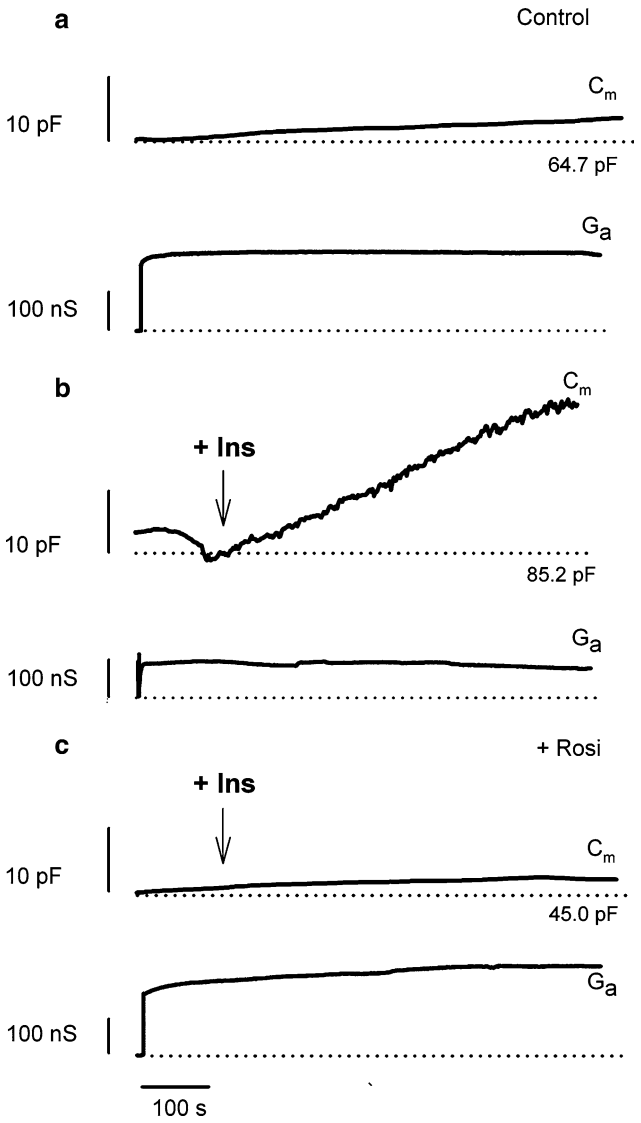


Fig. 4 Representative time-dependent changes in C_m (*top traces*) and G_a (*bottom traces*) in a control, nonstimulated cell (**a**), in a cell stimulated with 0.2 μM insulin (**b**) and in a rosiglitazone-treated cell stimulated with 0.2 μM insulin (**c**). *Arrows* indicate the addition of insulin (Ins). Numbers (pF) adjacent to C_m traces and the *dotted lines in top traces* of each panel indicate resting C_m recorded at the time of whole-cell recording establishment. *Dotted lines in bottom traces* of each panel indicate zero level (nS)

(Fig. 5a). However, during the 7 min of insulin application an increase in C_m of about 20% (20.7 ± 3.6, $n = 4$) was recorded (Fig. 5b), which was significantly higher than in control cells ($P < 0.01$). The corresponding regression line revealed a steady increase with a slope coefficient of 2.34 ± 0.10%/min ($n = 32$), over one order of magnitude higher than the slope coefficient of 0.12 ± 0.08%/min ($n = 64$) as was recorded in controls. It is unlikely that the effect of insulin is due to an artifact since the application of a bolus of a vehicle without insulin resulted in no change of the time course of C_m (Chowdhury et al. 2005). Similar

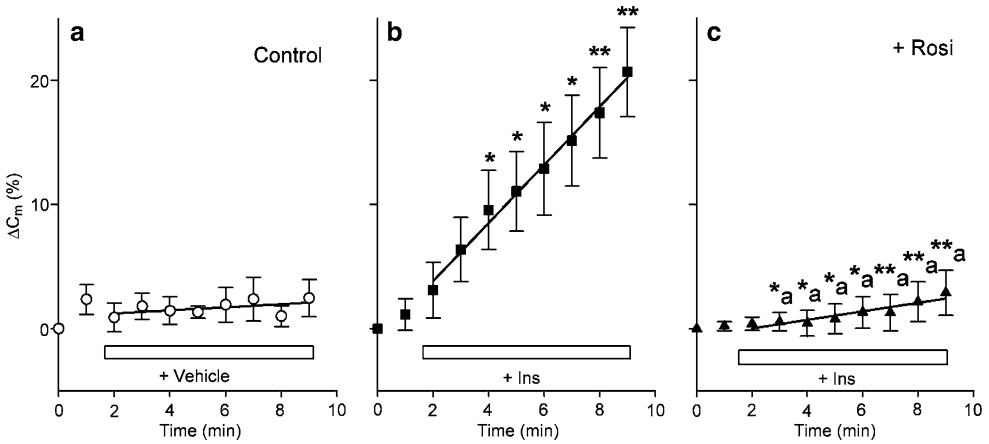


Fig. 5 Average time-dependent changes in C_m (ΔC_m) measured relative to resting C_m (expressed as 0%) after establishment of the whole-cell configuration (**a**) in control cells (*open circles*, $n = 8$), (**b**) in cells treated with insulin (*full squares*, $n = 4$) and (**c**) in cells pretreated with 10 μM rosiglitazone for 24 h and then treated with 0.2 μM insulin for an additional 7 min (*full triangles*, $n = 4$). *Horizontal bars* indicate the presence of vehicle or insulin in recording medium. Regression lines (*solid lines*) have the following forms: (**a**) C_m (%) = (0.12 ± 0.08) (%/min) * t (min) + (0.98 ± 0.50) (%), (**b**) C_m (%) = (2.34 ± 0.10) (%/min) * t (min) – (0.86 ± 0.58) (%) and (**c**) C_m (%) = (0.34 ± 0.05) (%/min) * t (min) – (0.66 ± 0.33) (%). The slope coefficient of the regression lines is significantly different from zero in graph (**b**) ($P << 0.001$) and in graph (**c**) ($P < 0.001$) but not in graph (**a**). *Asterisks* indicate significant differences (*$P < 0.05$ Ins vs. control, **$P < 0.01$ Ins vs. Control, *a $P < 0.05$ Ins vs. Ins + Rosi, **a $P < 0.01$ Ins vs. Ins + Rosi)

results were obtained in cells stimulated with IGF-I, with −3.0 ± 1.5% ($n = 4$) change in C_m (Fig. 6), which was not significantly different from controls but was significantly different from insulin-stimulated cells ($P < 0.01$). Wortmannin pretreatment resulted in a 3.0 ± 1.0% ($n = 4$) C_m increase after insulin stimulation for 7 min, which was also not significantly different from controls and was significantly different from insulin-stimulated cells ($p < 0.01$). Furthermore, in cells pretreated with 10 μM rosiglitazone for 24 h, subsequent insulin application for 7 min resulted in an attenuated steady time-dependent increase in C_m (Figs. 4c, top, and 5c), with a slope coefficient of the regression line of 0.34 ± 0.05%/min ($n = 32$), significantly different from zero ($P < 0.001$). Therefore, these results show that treatment of cells with rosiglitazone affects insulin-mediated membrane turnover in 3T3-L1 adipocytes.

Discussion

In this study we examined the signaling crosstalk between insulin and PPARγ in the regulation of differentiation and C_m in 3T3-L1 adipocytes. Specifically, we examined (1) the effect of rosiglitazone treatment during differentiation on the cell size and lipid droplet accumulation in 3T3-L1 cells, (2) the effect of insulin on membrane area dynamics in single 3T3-L1 differentiated adipocytes and (3) the effect of acute (24 h) rosiglitazone treatment on insulin-induced changes in membrane area dynamics in single 3T3-L1 adipocytes. The principal new finding of the present study is that PPARγ receptor activation by rosiglitazone reduced insulin-stimulated C_m in single 3T3-L1 adipocytes.

The current investigation shows that insulin increases C_m in 3T3-L1 adipocytes. Recent studies, including our previously published data (Chowdhury et al. 2005), demonstrate that insulin induces changes in PM surface area of rat white adipocytes, which are influenced by both exocytosis and endocytosis. The current investigation also shows that exposure of 3T3-L1 adipocytes to insulin for 7 min increases C_m by about 20% (Figs. 4b, top, and 5), similar to the increase elicited by insulin in primary adipocytes (Chowdhury et al. 2005). Furthermore, our data with wortmannin show that insulin increases surface area via phosphatidylinositol 3-kinase, as is the case for GLUT4 translocation. These findings are of interest because it is well accepted that insulin treatment of primary adipocytes increases membrane area in adipocytes (Chowdhury et al. 2002; Coster et al. 2004), indicating a dominant role of exocytosis over endocytosis in insulin-stimulated 3T3-L1 adipocytes.

TZDs (e.g., troglitazone, pioglitazone and rosiglitazone) are a class of antidiabetic drugs that act as insulin sensitizers by decreasing insulin resistance in human and animal models (Olefsky 2000; Saltiel and Olefsky 1996). TZDs decrease the circulating levels of insulin, free fatty acids and triglycerides and increase insulin-stimulated glucose uptake and utilization (Olefsky 2000; Saltiel and Olefsky 1996). PPARγ is a member of a larger family of ligand-activated nuclear receptor transcription factors (Willson et al. 2000). Furthermore, in vivo efficacy in rodents

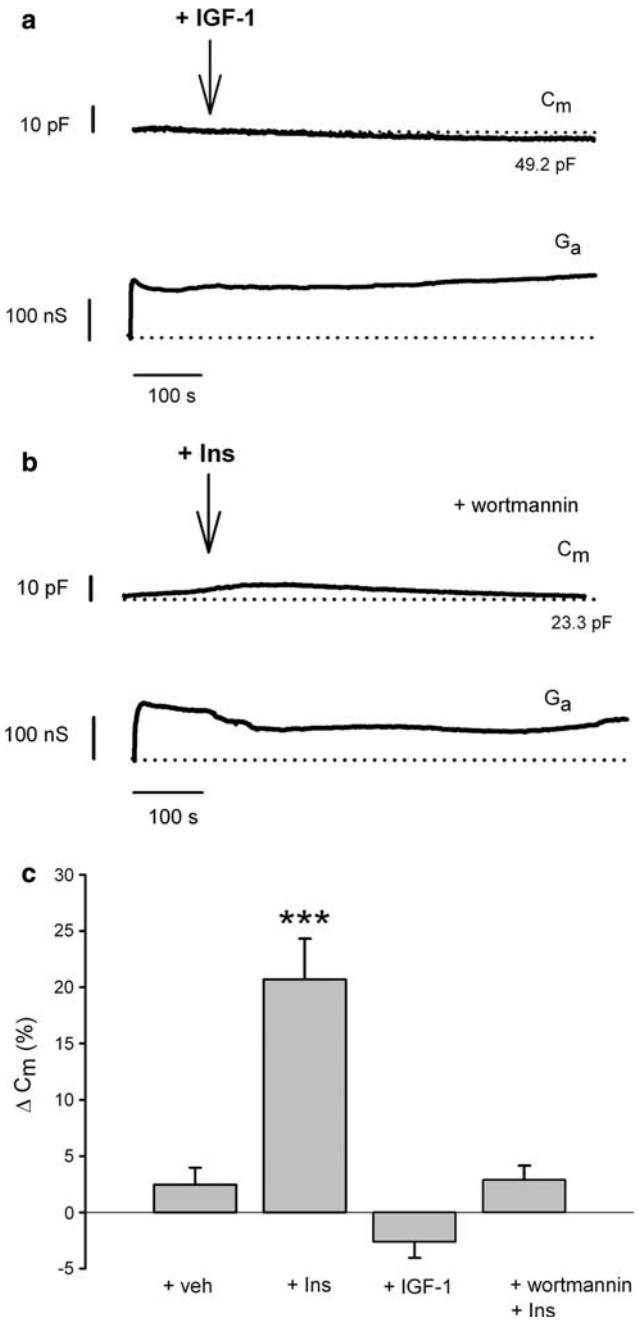


Fig. 6 (**a**) Representative time-dependent changes in C_m (*top trace*) and G_a (*bottom trace*) in a cell stimulated with 30 nM IGF-1. (**b**) Representative time-dependent changes in C_m (*top trace*) and G_a (*bottom trace*) in a cell pretreated with 20 nM wortmannin and stimulated with 0.2 μM insulin. *Arrows* indicate addition of IGF-1 or insulin (Ins). *Dotted lines in top traces* of each panel indicate resting C_m recorded at the time of whole-cell recording establishment, while *dotted lines in bottom traces* of each panel indicate zero level (nS). (**c**) Histogram showing C_m changes (%) relative to resting C_m 7 min after adding vehicle in control cells (+ veh, $n = 8$), 0.2 μM insulin in insulin-stimulated cells (+ Ins, $n = 4$), 30 nM IGF-I in IGF-I-stimulated cells (+ IGF-I, $n = 4$) and 0.2 μM insulin in cells pretreated with 20 nM wortmannin (+ wormannin + Ins, $n = 4$) ($P < 0.001$ for control vs. insulin-stimulated cells)

generally correlates with in vitro PPARγ activity, suggesting that PPARγ activation is the predominant mechanism for the antidiabetic efficacy of the PPARγ agonists (Berger et al. 1996; Saltiel 2001; Willson et al. 2000; Zhang and Moller 2000). Activation of PPARγ can increase the number of small adipocytes but reduce the number of large adipocytes in white adipose tissue (Rangwala and Lazar 2004). Normally, small adipocytes are more sensitive to insulin; an increased number of small adipocytes and a decreased number of large adipocytes in white adipose tissue can alleviate insulin resistance. In the present study we show that treatment of 3T3-L1 cells with rosiglitazone during differentiation resulted in an increased accumulation of lipids (Figs. 1 and 2) and smaller cell size, as determined by a smaller cell diameter (Fig. 3). However, the present results also show that differentiated 3T3-L1 adipocytes treated with PPARγ agonist rosiglitazone exhibit an attenuated insulin-stimulated increase in C_m.

Since administration of TZDs to obese animal models of insulin resistance and type 2 diabetes patients can result in a substantial correction of hyperglycemia and/or hyperinsulinemia (Berger et al. 1996; Fujiwara et al. 1988; Young et al. 1995), we further tested whether the action of rosiglitazone involves specific effects on membrane area of single adipocytes induced by insulin. Data from the literature (Awara et al. 2005; Kramer et al. 2001) indicate that rosiglitazone, as a PPARγ agonist, lowered basal plasma insulin level in obese rats and substantially corrected insulin resistance based on results from hyperinsulinemia euglycemic clamp studies (Saltiel and Olefsky 1996). Because, as a PPARγ agonist, rosiglitazone potentiates insulin signaling in adipose tissue (Ahima and Flier 2000), we were surprised that it attenuated the insulin-stimulated increase in C_m (Figs. 4 and 5).

To explain these results, we propose the following model: In rosiglitazone-treated cells insulin stimulates a rapid increase in exocytosis that is associated with a similar increase in the rate of endocytosis since both processes appear to be controlled by insulin (Chowdhury et al. 2005). Thus, secretory function (Ahima and Flier 2000) and/or transporter density regulation (Czech 2002) would be preserved under such conditions, while the insulin-induced increase in C_m is attenuated (Figs. 4b, top, and 5b). Cells treated with rosiglitazone are smaller (Fig. 3, left) but have a similar C_m normalized to square radius, indicating that the cell membrane is folded to a similar extent as in controls. In order to decrease the cell size and to concomitantly preserve the extent of membrane folding, rosiglitazone-treated cells have to be submitted to extensive endocytosis to retrieve the redundant membrane area.

However, we cannot exclude that PPARγ may also block GLUT4 recycling since it has been shown that ros-

iglitazone blocks the release of hormones and cytokines from adipocytes (McTernan et al. 2002; Sharma and Staels 2007). It remains possible, though unlikely, that rosiglitazone may exert its major effect independently of PPARγ (Furnsinn et al. 1999).

In conclusion, the current study suggests that activation of PPARγ by a competitive PPARγ receptor agonist, rosiglitazone, significantly attenuates the specific insulin-induced time-dependent changes in membrane area of single adipocytes.

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