Protein kinase C-dependent antilipolysis by insulin in rat adipocytes

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Abbreviations: β-AR, β-adrenergic receptor; α-AR, α-adrenergic receptor; PKA, protein kinase A; PKC, protein kinase C; PKB, protein kinase B; PDK, phosphoinositide-dependent protein kinase; PI3K, phosphatidylinositol 3-kinase; PIP_3, phosphatidylinositol 3,4,5-triphosphate; HSL, hormone-sensitive lipase; PDE3B, phosphodiesterase 3B; IRS, insulin-receptor substrate; EGF, epidermal growth factor; RACK, receptor of activated C-kinase; C-KIP, PKC-interacting protein; PMA, phorbol 12-myristate 13-acetate; buffer A, 119 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 2.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 32.3 mM Hepes, pH 7.4, 2 mM glucose, 20 mg/ml BSA, and 200 nM adenosine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; SDS, sodium dodecylsulphate

Abstract

Recently, we have shown that protein kinase C (PKC) activated by phorbol 12-myristate 13-acetate (PMA) attenuates the β_1-adrenergic receptor (β_1-AR)-mediated lipolysis in rat adipocytes. Stimulation of cells by insulin, angiotensin II, and α_1-AR agonist is known to cause activation of PKC. In this study, we found that lipolysis induced by the β_1-AR agonist dobutamine is decreased and is no longer inhibited by PMA in adipocytes that have been treated with 20 nM insulin for 30 min followed by washing out insulin. Such effects on lipolysis were not found after pretreatment with angiotensin II and α_1-AR agonists. The rate of lipolysis in the insulin-treated cells was normalized by the PKCα- and β-specific inhibitor Gö 6976 and PKCβ-specific inhibitor LY 333531. In the insulin-treated cells, wortmannin increased lipolysis and recovered the lipolysis-attenuating effect of PMA. Western blot analysis revealed that insulin slightly increases membrane-bound PKCα, βI, and δ, and wortmannin decreases PKCβI, βII, and δ in the membrane fraction. These results indicate that stimulation of insulin receptor induces a sustained activation of PKC-dependent antilipolysis in rat adipocytes.

Keywords: adipocyte; lipolysis; β-adrenergic receptor; PKC; PMA; insulin; wortmannin

1. Introduction

Adipose tissues have important functions in the regulation of energy balance. Adipocytes store excess energy supply as triglyceride droplets, resulting in the development of obesity. During fasting and exercise, triglycerides stored in the cells are hydrolyzed producing glycerol and free fatty acids, which are important oxidative fuels for other tissues such as liver, skeletal muscle, kidney, and the myocardium. Different hormones govern the use of energy in the triglyceride depots (1, 2).

Rat adipocytes contain three β-adrenergic receptor (β-AR) subtypes: β_1, β_2, and β_3. The three β-ARs are coupled with Gs protein and transmit an activation signal to adenylylcyclase, leading to an increase in cAMP. The resulting activation of protein kinase A (PKA) mediates activation of hormone-sensitive lipase (HSL), which hydrolyzes triglycerides stored in the cells (1, 2). The fact that the protein level of β_2-AR is extremely low (3) and that lipolysis stimulated by norepinephrine and

isoproterenol is not significantly affected by a β_2-AR antagonist ICI 118551 (4) indicate a very minor role of the β_2-AR signaling for lipolysis in rat adipocytes. β_3-AR may represent the physiological receptor for high norepinephrine concentrations (100 nM), attained by sympathetic activity near adipose tissues in conditions such as fasting and cold exposure (5, 6). In rat adipocytes, norepinephrine at concentrations usually found in the circulation (1–25 nM) stimulates mainly the high-affinity β_1-AR and thereby induces lipolysis (5). In humans, circulating catecholamine stimulates the β_1- and β_2-ARs, which are critical determinants for the rate of lipolysis induced by submaximal exercise in subcutaneous adipose tissue (7) and lipolysis in response to insulin-induced hypoglycemia in skeletal muscle (8).

The rate of lipolysis is regulated not only by lipolytic hormones but also by antilipolytic hormones. Insulin is the most potent antilipolytic hormone in adipose tissues. It inhibits the activity of HSL by decreasing the cAMP level through phosphorylation and activation of phosphodiesterase 3B (PDE3B) (1). PDE3B stimulation by insulin requires the activities of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB) (1, 9, 10). Lipolysis is also inhibited by the activities of

α_2-AR and adenosine receptor that couple to Gi protein and inhibit adenylylcyclase, and by other activities of different effector molecules (1, 2).

Using rat adipocytes, we have shown that phorbol 12-myristate 13-acetate (PMA) attenuates lipolysis induced by submaximally stimulating concentrations of isoproterenol and norepinephrine (4). The decrease in lipolysis by PMA may be due to specific inhibition of the β_1-AR system by the activity of protein kinase C (PKC). In this study, we attempted to characterize the signaling pathway of PKC-dependent antilipolysis in rat adipocytes. It was found that stimulation of insulin receptor substitutes for the action of PMA. The effect of insulin on lipolysis was blocked by PKC inhibitors and wortmannin.

2. Materials and methods

2.1. Materials

The β-AR agonists, isoproterenol and dobutamine, and the β_1-AR antagonist, CGP 20712A, were purchased from Sigma (St. Louis, USA). Angiotensin II, phenylephrine,

and methoxamine were from Wako Pure Chemical Industries (Osaka, Japan). The PKC inhibitors, Gö 6976 and LY 333531, were from Calbiochem (Darmstadt, Germany) and Alexis (San Diego, USA), respectively. PMA, wortmannin, and protease inhibitor cocktail were from Sigma. The rabbit anti-peptide antibodies recognizing PKCα, βI, and βII were purchased from Santa Cruz Biotechnologies (Santa Cruz, USA) and the rabbit anti-peptide antibodies to PKCδ, ε, and ζ were from Sigma.

2.2. Animals and adipocyte preparation

Male rats of the Charles River CD strain weighing 200–240 g (7 weeks old) were used. Animals were fed a standard commercial diet *ad libitum* and allowed free access to water. The light cycle was 08:00–20:00. Isolated adipocytes were prepared from the epididymal fat pads by the method previously described (4). After collagenase digestion, cells were washed, suspended in Dulbecco's modified Eagle's medium supplemented with 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), pH 7.4, 20 mg/ml bovine serum albumin (BSA), and 200 nM adenosine, and incubated at 37ºC for 4 h with gentle agitation. If necessary, cells were treated with insulin for

the final 30 min of a 4-h-incubation. Then, cells were washed with a buffered solution containing 119 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 2.6 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 32.3 mM Hepes, pH 7.4, 2 mM glucose, 20 mg/ml BSA, and 200 nM adenosine (buffer A). After being washed, the volume of packed adipocytes was determined.

2.3. Lipolysis and glycerol measurement

The packed adipocytes were diluted 10-fold with buffer A, and incubated at 37ºC for 20 min in the presence of pharmacological agents. The concentration of glycerol in the incubation mixture was measured to determine the rate of lipolysis. Lipolysis, as assessed by glycerol release, linearly increased for at least 40 min (11).

2.4. Western blot analysis

Adipocytes were washed three times with a buffered solution containing 10 mM Tris, pH 7.4, 0.15 M NaCl, 1 mM EDTA, 1 mM EGTA, and 0.5% protease inhibitor cocktail. Then, cells were processed for separation of the cytosol and membrane fractions as

described previously (4).

The membrane fraction from adipocytes was mixed with the sample-loading buffer, and subjected to sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis followed by electrotransfer of protein onto a polyvinylidene difluoride membrane (4). After incubation of the membrane with the primary and secondary antibodies, immunoreactive bands were detected by ECL Plus (Amersham, UK). Samples that were compared from each experiment were analyzed on the same immunoblot, and relative changes in the density of the bands were determined by scanning densitometry.

2.5. Statistics

Each experiment was performed in duplicates and repeated three times or more. Results were mean values ± SEM. In some cases, data were expressed as the percentage of the activity in control cells (taken as 100%). If necessary, data were examined by Student's *t* test to evaluate the statistical significance.

3. Results

3.1. Effects of insulin, angiotensin II, and α_1-AR agonist on the lipolysis-attenuating effect of PMA

Rat adipocytes possess a PKC-dependent signaling pathway for antilipolysis (4). To explore the mechanism of PKC signaling, adipocytes were treated with insulin, angiotensin II, and α_1-AR agonist, which are known to activate the PKC pathway (2, 12–17). Adipocytes were treated with 20 nM insulin for 30 min, washed with an insulin-free buffer, and determined for the rate of lipolysis. As shown in Fig. 1, lipolysis stimulated by low concentrations of dobutamine (0.4 and 0.8 μM) and isoproterenol (8 and 16 nM) was lower in the insulin-treated cells than the control cells. PMA attenuated lipolysis stimulated by low concentrations of dobutamine and isoproterenol in the control cells, but not in the insulin-treated cells.

The concentration of insulin (20 nM) used for pretreatment of adipocytes was sufficient for the total inhibition of lipolysis induced by 0.4 and 0.8 μM dobutamine in rat adipocytes, and washing out insulin partially restored the lipolysis (Fig. 1). The restored lipolysis was again inhibited by insulin in the following assay of lipolysis.

Thus, insulin causes a reversible and an irreversible inhibition of lipolysis.

Next, adipocytes were treated with angiotensin II and α_1-AR agonists, phenylephrine and methoxamine, and lipolysis was induced in the presence and absence of PMA before washing out the agents. PMA was found to significantly attenuate lipolysis (Fig. 2). Methoxamine decreased lipolysis, but PMA further reduced it. The lipolysis-attenuating effect of PMA was detected in adipocytes that had been treated with angiotensin II, phenylephrine, and methoxamine for 30 min, and the agents were washed out (results not shown).

3.2. Effects of PKC inhibitor and β_1-AR antagonist on lipolysis

In the following experiments, lipolysis was stimulated by 0.8 μM dobutamine, as the effect of insulin was apparent (Fig. 1). The results shown in Fig. 1 indicate that insulin may substitute for PMA and attenuate lipolysis. To assess the significance of PKC activity, the effects of the PKCα- and β-specific inhibitor Gö 6976 (18) and the PKCβ-specific inhibitor LY 333531 (19) were determined in the insulin-treated adipocytes. In the previous study (4), Gö 6976 was found to increase lipolysis in the

untreated adipocytes in the presence of PMA in a dose-dependent manner, and the lipolysis-attenuating effect of PMA disappeared in the presence of 0.3–3 μM Gö 6976. Similarly, the effect of PMA was suppressed by LY 333531 at concentrations of 0.5 μM or more (results not shown). Fig. 3 shows that lipolysis in the insulin-treated cells is increased by 3 μM Gö 6976 much more than that in the control cells, resulting in a comparable level to the control. LY 333531 at concentrations of 0.5 μM or more increased lipolysis in the insulin-treated cells to the control levels (Fig. 3).

The β_1-AR antagonist CGP 20712A greatly decreased lipolysis induced by dobutamine in both control and insulin-treated cells, and abolished the difference in the rate of lipolysis between two cell preparations (Fig. 4). The lipolysis-attenuating effect of PMA in the control cells as well as the increase of lipolysis by Gö 6976 in the insulin-treated cells were not found in the presence of CGP 20712A.

3.3. Effects of wortmannin on the lipolysis-attenuating effect of PMA

Biological actions of insulin in the adipose tissues are mediated by the cell-surface receptor with intrinsic tyrosine kinase activity. One of the immediate targets of the

insulin receptor is the insulin-receptor substrates (IRSs), and phosphorylation of IRSs on specific tyrosines creates binding sites for *src* homology-2 (SH2) domain-containing proteins, including PI3K (10, 20). Recruitment and activation of PI3K initiates a series of events leading to biological actions of insulin (9, 10, 20). Wortmannin, a PI3K inhibitor (21), inhibits many of insulin's effects on glucose and lipid metabolism (9, 10, 20). In the present study, wortmannin increased lipolysis in a dose-dependent manner in the insulin-treated cells as well as lipolysis, to a lesser extent, in the control cells (Fig. 5). With 40 nM or more wortmannin, no difference in the rate of lipolysis between the two preparations of cells was found. Lipolysis in the control and insulin-treated cells determined in the presence of PMA also showed an upward tendency as the concentration of wortmannin increased. The lipolysis-attenuating effect of PMA was not detectable in the insulin-treated cells in the presence of ≤8 nM wortmannin, but became apparent at higher concentrations, reaching a 43.7 ± 5.1% inhibition by PMA at 80 nM. Inhibition of lipolysis by PMA in the control cells was always found within the range 42.3–49.9%, irrespective of the presence and absence of wortmannin.

3.4. Western blot analysis of PKC isoforms

In the previous report (4), we have shown that rat adipocytes express PKCα, βI, βII, δ, ε, and ζ. PKCα, βI, and βII showed translocation from cytosol to membrane in response to acute treatment with PMA. In this study, the amounts of membrane-bound PKC isoforms were determined before and after the treatment of adipocytes with 20 nM insulin for 30 min by Western blot analysis. As shown in Fig. 6, insulin caused slight increases of the membrane-bound PKCα, βI, and δ. An increase of 19% was found in the membrane-bound PKCβII; however, this was not significant.

The data shown in Fig. 5 indicate that the active form of PKC responsible for antilipolysis in this study is rapidly decreased by wortmannin. To examine this, adipocytes were treated with insulin followed by incubation with wortmannin for 10 and 30 min, and the amounts of membrane-bound PKC isoforms were determined (Fig. 6). PKCβI and βII decreased after 10 min of treatment with wortmannin (Fig. 6). A decrease of PKCδ was found after 30 min of treatment with wortmannin. No marked

change by wortmannin was found in the membrane-bound PKCα, ε, and ζ.

4. Discussion

Recently, we have shown that PKC is a regulator of lipolysis induced by submaximally stimulating concentrations of β_1-AR agonists (4). The present study shows that exposure of rat adipocytes to insulin followed by washing out insulin decreases lipolysis induced by low concentrations of the β_1-AR agonist dobutamine. This effect of insulin was not detected in the presence of a β_1-AR antagonist CGP 20712A. The PKC inhibitors, Gö 6976 and LY 333531, normalized the rate of lipolysis in the insulin-treated cells. PMA could not inhibit lipolysis in the insulin-treated cells. Wortmannin abolished the effect of insulin. These data indicate that insulin delivers an antilipolytic signal by using the activity of PKC. Insulin also promotes diacylglycerol production (13, 14, 22), which may substitute for the action of PMA.

Conventional and novel PKCs after activation by diacylglycerol bind to membrane

through interaction with receptors of activated C-kinase (RACKs) and other PKC-interacting proteins (C-KIPs) for biological functions (23, 24). In adipocytes, insulin promotes translocation of PKCα and β to membrane (14, 25, 26). However, no consistent changes of intracellular distribution of PKCδ, ε and ζ by insulin have been reported (14, 25, 26). In this study, we found small increases in the membrane-bound PKCα, βI, and δ after treatment of adipocytes with 20 nM insulin for 30 min. It is known that the insulin-induced translocation of the PKC isoforms is very rapid and transient, and the membrane-bound PKC isoforms are maximal at 2–10 min of insulin treatment and then decrease in a time-dependent manner (14, 25, 26). Because of prolonged treatment in the present study, no marked increase of the membrane-bound PKC by insulin could be detected. Even with such small increases, lipolysis in the insulin-treated cells apparently responded to the PKC inhibitors, but not to PMA. Wortmannin suppressed the effect of insulin and recovered the lipolysis-attenuating effect of PMA. These data suggest that the PKC isoform responsible for antilipolysis was activated in the insulin-treated cells.

There are reports suggesting that activation and translocation of PKC by insulin are

insensitive to wortmannin (12, 27). However, other studies have shown that wortmannin inhibits both insulin-mediated translocation of PKC (14, 28) and glucose uptake (14). In this study, wortmannin inhibited the PKC-dependent antilipolysis by insulin and decreased the levels of PKC isoforms in the membrane fraction. Insulin may acts through PI3K to activate phospholipase C and phospholipase D, leading to production of diacylglycerol (13, 14, 22). Binding of diacylglycerol to PKC results in a high-affinity interaction of PKC with the specific membrane sites (29), which is essential for PKC to accomplish biological functions (23, 24). Wortmannin has been shown to inhibit diacylglycerol production (14, 22). Wortmannin does not directly inhibit PKC or affect translocation of PKC isoforms or glucose uptake by PMA (14, 28). Accordingly, wortmannin did not inhibit the effect of PMA on lipolysis in the control and insulin-treated adipocytes. Translocation of PKCα, βI, and βII to membrane by PMA was not inhibited by wortmannin (results not shown).

It is well known that, upon interaction with the specific membrane sites, PKC is irreversibly activated and plays a role in signaling after Ca^{2+} and diacylglycerol have returned to basal levels (23). This sustained activation of the enzyme may account for

the PKC-dependent antilipolysis found after washing out insulin. Alternatively, insulin tightly bound to the insulin receptor, which is not immediately removed by washing with an insulin-free buffer (30, 31), may sustain signaling for the activation of PKC. Adipocytes that are treated with insulin followed by washing out insulin still show the acute effects of insulin (30, 31). Insulin, present in the incubation mixture, provokes a prolonged increase of phosphatidylinositol 3,4,5-triphosphate (PIP_3), a PI3K by product, in adipocytes that persists at least for 1 h with no marked decrease (32, 33), which contrasts with a rapid and transient increase of PIP_3 by epidermal growth factor (EGF) (32).

LY 333531 exhibits 40-fold or more selectivity for inhibition of PKCβ compared with PKCα, δ, ε, and ζ (19). In cultured cells, LY333531 inhibits the PKCβ-mediated pathway with IC_{50} values of about 0.2 μM (34, 35). However, even at 10 μM, LY 333531 does not inhibit PKCδ in mouse adipocytes (36). The fact that the effect of insulin is inhibited by 0.5 μM LY 333531 in the present study might suggest an involvement of the activity of PKCβ in the antilipolytic pathway. Further studies are in progress to identify the PKC isoform responsible for antilipolysis.

For full activation, PKB is phosphorylated at two sites by 3-phosphoinositide-dependent kinase-1 (PDK1) and PDK2 (37). Activation and translocation of PDK1 and PDK2 depend on PIP_3. Recently, PKCβII was shown to act as a PDK2 activity in some cases for activation of the catalytic domain of PKB (38). Thus, it is possible that PKCβII stimulated by insulin activates PKB, which then causes phosphorylation and activation of PDE3B. To assess this possibility, the effect of a PDE3B inhibitor cilostamide (10) on the lipolysis-attenuating effect of PMA was determined. In the untreated adipocytes, the effect of PMA was clearly detected in the presence of cilostamide (4). Cilostamide at a concentration of 1 μM increased lipolysis in the insulin-treated adipocytes to a level higher than the control, although the mechanism is unclear. Moreover, addition of PMA to the mixture containing cilostamide decreased lipolysis with a 29.2 ± 3.6 % inhibition (mean ± SEM, $p < 0.01$, $n = 6$). The results suggest that rat adipocytes possess a PKC-dependent antilipolytic pathway, which is independent of the PDE3B activity.

In contrast to the results obtained with insulin, angiotensin II and α_1-AR agonists failed to affect the lipolysis-attenuating effect of PMA, though the angiotensin II

receptor and α_1-AR are coupled with activation of PKC (15-17). Stimulation of α_1-AR by norepinephrine results in translocation of PKCδ and ε to membrane (15). However, lipolysis induced by norepinephrine was clearly decreased by PMA (4). At present, no evidence suggesting that stimulation of angiotensin II receptor or α_1-AR induces PKC-dependent antilipolysis has been obtained.

The β_1-AR is the dominating lipolytic AR subtype in rat adipocytes and responds to low plasma concentrations of norepinephrine (5). Adipocytes from transgenic mice overexpressing β_1-AR show an increased sensitivity of lipolytic response to isoproterenol and dobutamine, which may be important for preventing diet-induced obesity (39). The present study indicates that insulin-mediated PKC signaling regulates the sensitivity of lipolysis to low concentrations of the β_1-AR agonist. In contrast to the reversible inhibition mediated by other effector molecules, PKC-dependent antilipolysis is active even after washing out insulin. These results suggest a unique role for PKC signaling in the metabolism of triglycerides.

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Figure Legends

Fig. 1 Effects of PMA on dobutamine- and isoproterenol-stimulated lipolysis. The isolated adipocytes were treated without (control) and with 20 nM insulin for 30 min.

The cells were washed with an insulin-free buffer, and mixed with increasing concentrations of dobutamine (A) and isoproterenol (B) in the presence (■) and absence (●) of 0.8 μM PMA. After being incubated at 37°C for 20 min, the amount of released glycerol was determined. The results were expressed as mean values ± SEM of ten (A) and four (B) experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, compared to no PMA.

Fig. 2 Effects of angiotensin II and α_1-AR agonist on the lipolysis-attenuating effect of PMA. The isolated adipocytes were incubated in buffer A in the absence (None) and presence of 1 μM angiotensin II (AngII), 10 μM phenylephrine (Phe), and 10 μM methoxamine (Methox) at 37°C for 30 min. Then, the mixtures were added 0.8 μM dobutamine with and without 0.8 μM PMA. After being incubated at 37°C for 20 min, the amount of released glycerol was determined. The results were mean values ± SEM of four to seven experiments, and were expressed as the percentage of the lipolytic activity determined without PMA or other agent. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, compared to no PMA. +++ $p < 0.001$, compared to control (None).

Fig. 3 Effects of Gö 6976 and LY 333531 on lipolysis. Lipolysis was stimulated in the control (●) and insulin-treated (■) adipocytes by 0.8 μM dobutamine in the presence of Gö 6976 (A) and LY 333531 (B) at 37ºC for 20 min. The results were mean values ± SEM of eight experiments, and were expressed as the percentage of the lipolytic activity in the control cells determined in the absence of the PKC inhibitor. *** $p < 0.001$, compared to control cells.

Fig. 4 Effects of CGP 20712A on lipolysis. The control and insulin-treated adipocytes were stimulated by 0.8 μM dobutamine in the presence and absence of 16 nM CGP 20712A at 37ºC for 20 min. The mixtures also contained 0.8 μM PMA or 3 μM Gö 6976. The results were mean values ± SEM of four experiments, and were expressed as the percentage of the lipolytic activity determined without PMA or other agent in the control cells. ** $p < 0.01$, compared to value determined with no agent.

Fig. 5 Effects of wortmannin on lipolysis. The control (A) and insulin-treated (B)

adipocytes were stimulated by 0.8 μM dobutamine in the presence (■) and absence (●) of 0.8 μM PMA at 37°C for 20 min. The mixtures also contained wortmannin in the concentrations indicated. The results were mean values ± SEM of four experiments, and were expressed as the percentage of the lipolytic activity determined without PMA or wortmannin in the control cells. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, compared to value determined without PMA.

Fig. 6 Effects of insulin and wortmannin on membrane-bound PKC isoforms. (A), the isolated adipocytes were treated without (control, C) and with 20 nM insulin (I) at 37°C for 30 min. Aliquots of the insulin-treated cells were washed with an insulin-free buffer and incubated with 80 nM wortmannin for 10 min ($I + W_{10}$) or 30 min ($I + W_{30}$). The control-, insulin-, and insulin/wortmannin-treated cells were washed and subjected to preparation of the membrane fractions. Western blot analysis was performed by using the antibobies specific to PKCα, βI, βII, δ, ε, and ζ as described in Materials and methods. The results shown were a representative from four experiments. (B), the isolated adipocytes were treated as in (A). Immunoblots were scanned and quantified

by a densitometer. The results were mean values ± SEM of four experiments, and were expressed as the percentage of the control cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to control cells. + $p < 0.05$, ++ $p < 0.01$, compared to insulin-treated cells.

Figure 1-A

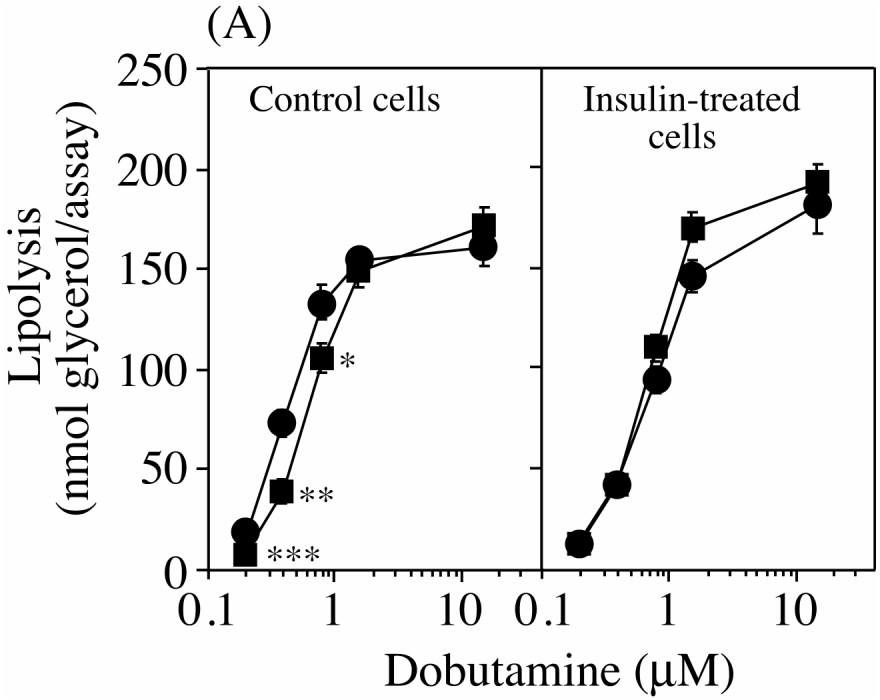

(A)
Control cells
Insulin-treated cells
Lipolysis (nmol glycerol/assay)
250
200
150
100
50
0
0.1
1
10
0.1
1
10
Dobutamine (μM)
*
**
***

Figure 1-B

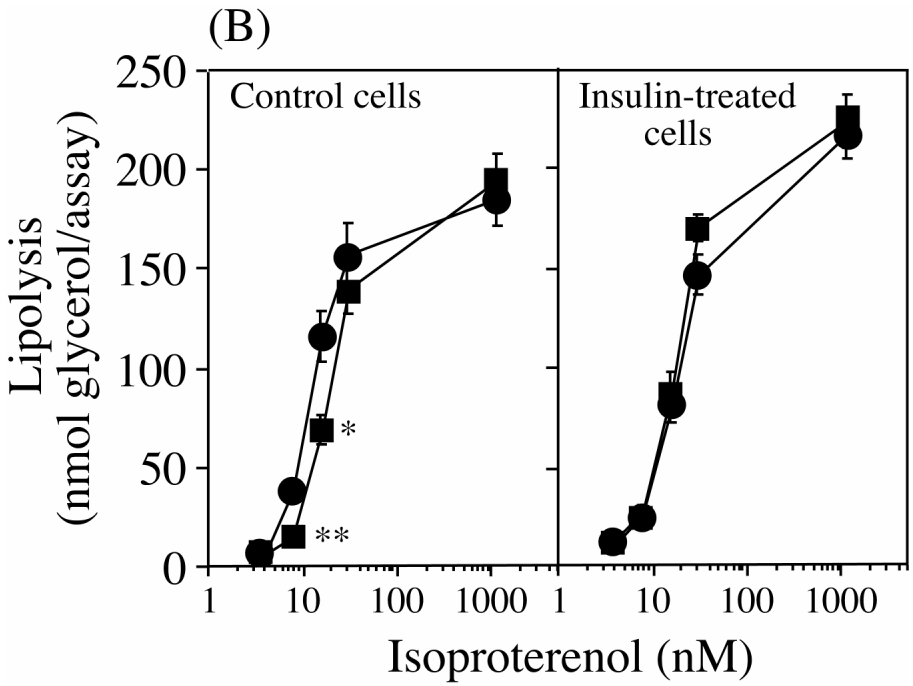

(B)
Control cells
Insulin-treated cells
Lipolysis (nmol glycerol/assay)
250
200
150
100
50
0
*
**
1
10
100
1000
Isoproterenol (nM)

Figure 2

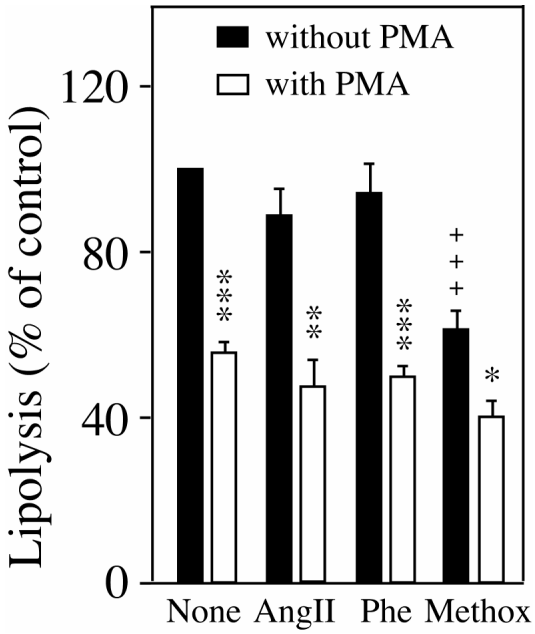

without PMA
with PMA
Lipolysis (% of control)
120
80
40
0
***
**
***
+++
*
None
AngII
Phe
Methox

Figure 3

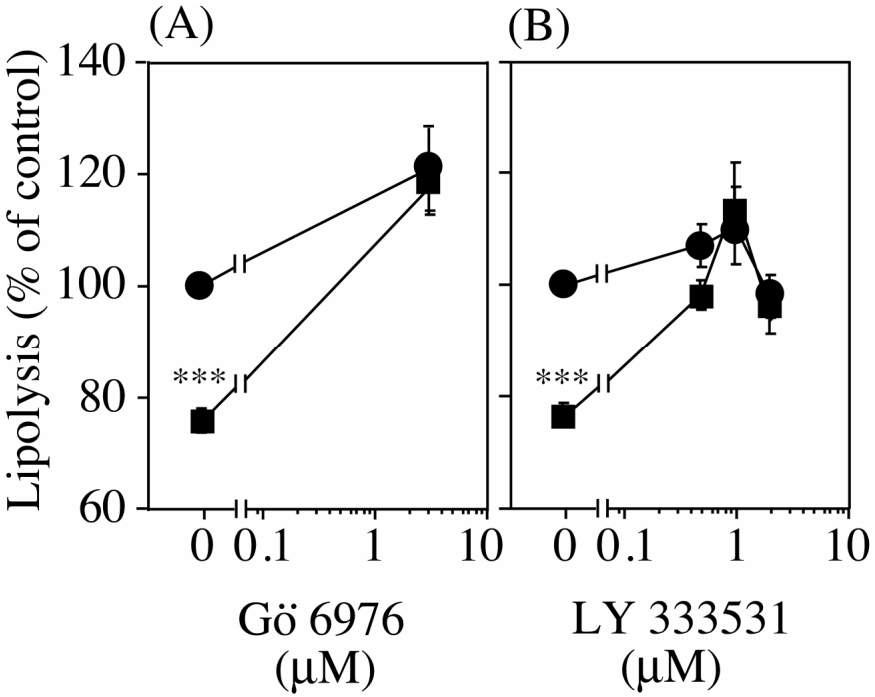

(A)
(B)
Lipolysis (% of control)
140
120
100
80
60
***
***
0
0.1
1
10
0
0.1
1
10
Gö 6976
(μM)
LY 333531
(μM)

Figure 4

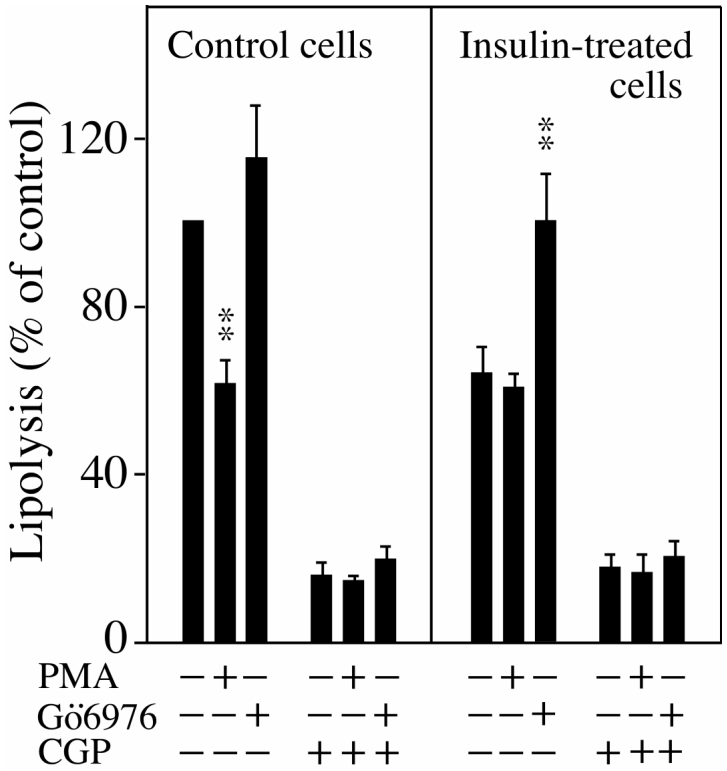

Control cells
Insulin-treated cells
Lipolysis (% of control)
120
80
40
0
**
**
PMA − + − − + − − + − − + −
Gö6976 − − + − − + − − + − − +
CGP − − − + + + − − − + + +

Figure 5

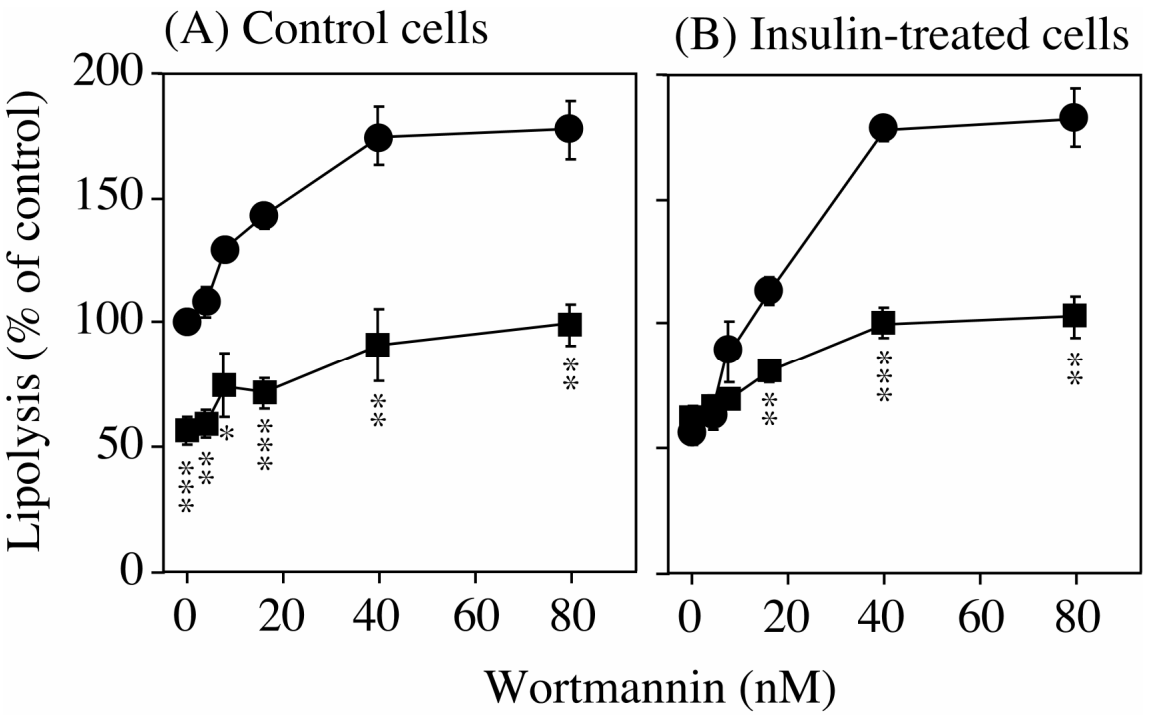
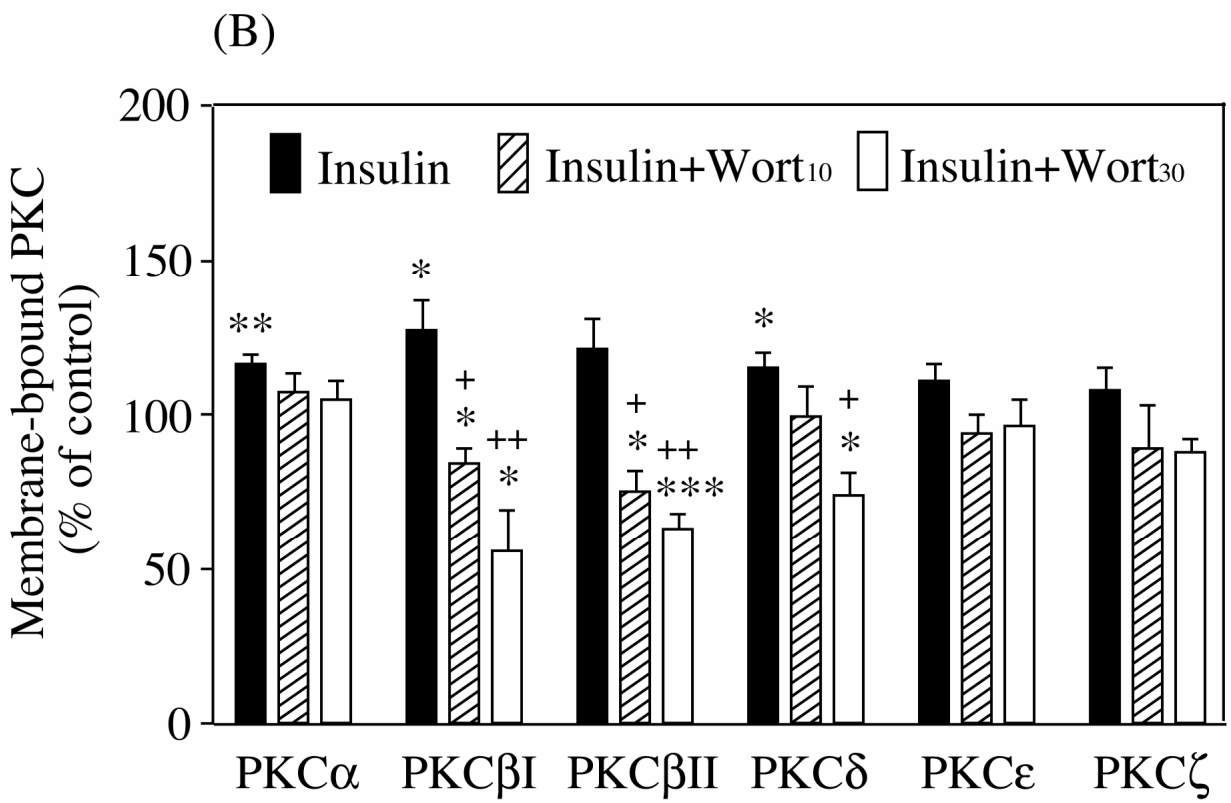

(A) Control cells
(B) Insulin-treated cells
Lipolysis (% of control)
200
150
100
50
0
0
20
40
60
80
Wortmannin (nM)

Figure 6-A

Figure 6-B


(B)
Membrane-bpound PKC
(% of control)
200
150
100
50
0
Insulin
Insulin+Wort10
Insulin+Wort30
PKCα
PKCβI
PKCβII
PKCδ
PKCε
PKCζ