Protein kinase CβI interacts with the β_1-adrenergic signaling pathway to attenuate lipolysis in rat adipocytes.

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Abbreviations: β-AR, β-adrenergic receptor; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; cPKC, conventional PKC; nPKC, novel PKC; aPKC, atypical PKC; PI3K, phosphatidylinositol 3-kinase; HSL, hormone-sensitive lipase; PDE3B, phosphodiesterase 3B; IRS, insulin-receptor substrate; PMA, phorbol 12-myristate 13-acetate; DAG, diacylglycerol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; SDS, sodium dodecylsulphate; buffer A, Dulbecco's modified Eagle's medium supplemented with 20 mM Hepes, pH 7.4, 20 mg/ml BSA, and 200 nM adenosine; buffer B, 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 C, 10 mM Tris, pH 7.4, 0.15 m NaCl, 1 mM EDTA, and 1 mM EGTA

Abstract

We have shown previously that insulin attenuates β_1-adrenergic receptor (β_1-AR)-mediated lipolysis via activation of protein kinase C (PKC) in rat adipocytes. This antilipolysis persists after removal of insulin and is independent of the phosphodiesterase 3B activity, and phorbol 12-myristate 13-acetate (PMA) could substitute for insulin to produce the same effect. Here, we attempted to identify the PKC isoform responsible for antilipolysis. Isolated adipocytes were treated with high and low concentrations of PMA for up to 6 h to degrade specific PKC isoforms. In the PMA-treated cells, the downregulation profiles of PKC isoforms α and βI, but not βII, δ, ε, or ζ, correlated well with a decrease of lipolysis-attenuating effect of PMA. After rats fasted for 24 h, adipocyte expression of PKC isoform α increased, while expression of PKCδ decreased. Fasting did not change the potency of PMA to attenuate lipolysis, however. The lipolysis-attenuating effect of PMA was blocked by the PKCβI/βII inhibitor LY 333531, but not by the PKCβII inhibitor CGP 53353 or the PKCδ inhibitor rottlerin. These data suggest that PKCβI interacts with β_1-AR signaling and attenuates

lipolysis in rat adipocytes.

Keywords: adipocyte; lipolysis; β-adrenergic receptor; PKC; PKCβI

1. Introduction

Adipose tissues are important for the regulation of energy balance. Adipocytes store an excess supply of energy as triglyceride droplets. During fasting and exercise, these triglycerides are hydrolyzed to produce glycerol and free fatty acids, which are important oxidative fuels for other tissues such as liver, skeletal muscle, kidney, and the myocardium (1). Defects in triglyceride metabolism may contribute to the development of obesity (2). Chronic elevation in plasma free fatty acid levels is closely linked to type 2 diabetes and cardiovascular complications (3). Thus, an accurate regulation of lipolysis to maintain a normal level of plasma free fatty acids is essential to the prevention of these disorders (1-3).

The cAMP-dependent pathway is one of the best-understood mechanisms for the

activation of lipolysis in adipocytes (1). Catecholamines are potent lipolytic agents, stimulating the β-adrenergic receptor (β-AR) and leading to an increase in intracellular cAMP. The rise in cAMP levels activates protein kinase A (PKA), which then phosphorylates cytosolic hormone-sensitive lipase (HSL) and the lipid droplet-associated protein, perilipin (1). This phosphorylation causes an increase in the hydrolysis of triglyceride by HSL and adipose triglyceride lipase, which works in concert with HSL (4). Phosphorylation of perilipin by PKA may be a critical step for the induction of lipolysis (5).

Insulin negatively regulates lipolysis by stimulating phosphodiesterase 3B (PDE3B) activity (1). The binding of insulin to its receptor triggers the activation of the receptor tyrosine kinase, which phosphorylates insulin-receptor substrates (IRSs). IRSs act as scaffolding proteins for several Src homology 2-containing proteins that recognize the IRS-phosphorylated tyrosine residues. Upon binding to the IRS, phosphatidylinositol 3-kinase (PI3K) initiates a series of events, including the activation of protein kinase B (PKB) (6). PKB acts as an insulin-sensitive PDE3B kinase (1, 6, 7). Phosphorylation and activation of PDE3B promotes degradation of cAMP and reduction

in PKA activity (1).

Insulin provokes rapid changes in phospholipid metabolism and thereby generates biologically active lipids that serve as intracellular signaling factors. Conventional, novel, and atypical protein kinase Cs (cPKCs, nPKCs, and aPKCs) that are activated by this insulin signaling cascade have key roles in the regulation of insulin's metabolic effects (8, 9). We have previously shown that insulin interacts with the β_1-AR signaling pathway via activation of PKC in rat adipocytes (10). Isolated adipocytes that were treated with insulin, followed by an insulin wash-out, exhibited attenuated lipolysis when induced with the β_1-AR agonist dobutamine. The PI3K inhibitor wortmannin blocked insulin's effect on lipolysis, while the PDE3B inhibitor cilostamide had no effect (10). Phorbol 12-myristate 13-acetate (PMA) could substitute for the action of insulin (10, 11). The PKC inhibitors, GF 109203X, Gö 6976 and LY 333531, blocked the antilipolytic effects of both insulin and PMA (10, 11). In this study, we attempted to identify the PKC isoform responsible for antilipolysis in rat adipocytes. Isolated adipocytes were treated with PMA under various experimental conditions to induce downregulation of PKCs. Alternatively, the cellular expression of PKCs was altered by

allowing the rats to fast for 24 h prior to the preparation of isolated adipocytes. The PKC isoform present in these adipocytes was measured and correlated with the lipolysis-attenuating effect of PMA. We also examined the effect of PMA in the presence of specific PKC inhibitors. In all, the data obtained suggested that PKCβI is a negative regulator of the β_1-AR-mediated lipolysis.

2. Materials and methods

2.1. Materials

The β_1-AR agonist dobutamine, the PKCβII inhibitor CGP 53353, the PKCδ inhibitor rottlerin, and PMA were purchased from Sigma (St. Louis, USA). The PKCα/β inhibitor Gö 6976 and the PKCβI/βII inhibitor LY 333531 were obtained from Calbiochem (Darmstadt, Germany) and Alexis (San Diego, USA), respectively. The rabbit anti-peptide antibodies recognizing PKCs α, βI, and βII were purchased from Santa Cruz Biotechnologies (Santa Cruz, USA) and the rabbit anti-peptide antibodies to PKCs δ, ε, and ζ were from Sigma. Anti-rabbit IgG linked with horseradish peroxidase

was from Calbiochem.

2.2. Downregulation of PKCs with PMA and modified expression of PKCs with fasting

The epididymal fat pads were collected from male rats of the Charles River CD strain (7 weeks old), and the adipocytes were isolated as described previously (10, 11). To degrade PKC, adipocytes were treated with 0.1 to 2 μM PMA 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 (buffer A) at 37ºC for up to 6 h.

To examine the effect of fasting, food was withheld from the rats for 24 h, starting at 08:00. Control rats were fed a standard commercial food *ad libitum*. Free access to water was given to both feeding and fasting rats. The light cycle was 08:00-20:00. Rats were euthanized by decapitation at 08:00, and isolated adipocytes were prepared. The cells were then incubated in buffer A with gentle agitation at 37ºC for 5 h.

2.3. Lipolysis

Prior to measurement of lipolysis, adipocytes were washed three times 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 B). Packed cells obtained with the final centrifugation were diluted 10-fold with buffer B. Aliquots (0.25 ml) of the cell suspension were mixed with an equal volume of buffer B containing the β_1-AR agonist dobutamine and other pharmacological agents, and incubated at 37°C for 30 min (10, 11). After incubation, the concentration of glycerol in the mixture was measured to determine the rate of lipolysis (12).

2.4. Western blot analysis

Adipocytes were washed five times with a buffered solution containing 10 mM Tris, pH 7.4, 0.15 M NaCl, 1 mM EDTA, and 1 mM EGTA (buffer C). Packed cells obtained with the final centrifugation were added buffer C containing 2 % sodium dodecyl sulphate (SDS) and 0.5% protease inhibitor cocktail, kept at 60°C for 30 min, and centrifuged at 15,000*g* for 10 min. The resulting supernatants were mixed with the

sample-loading buffer (11). Aliquots containing equal amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis. After electrotransfer of protein onto a polyvinylidene difluoride membrane, the membrane was incubated with the primary and secondary antibodies. Immunoreactive bands were detected by ECL Plus (11). Samples from each experiment were analyzed on the same immunoblot, and relative changes in band density were determined by scanning densitometry (10).

2.5. Statistics

Each experiment was repeated three times or more. Results are shown as mean ± SEM. If necessary, statistical significance was assessed with a Student's *t* test.

3. Results

3.1. Dobutamine-induced lipolysis

The β_1-AR agonist dobutamine causes a dose-dependent increase in lipolysis, with maximal lipolysis occurring upon addition of 16 μM dobutamine (11). With

submaximal concentrations of dobutamine (0.4-1.6 μM), the lipolysis-attenuating effect of PMA is apparent and inhibition of lipolysis by the β_1-AR antagonist CGP 20712A is clearly detected (11). Here, lipolysis was induced with 0.8 and 1.6 μM dobutamine to assess the effect of PMA. The rate of lipolysis in untreated adipocytes (control cells) was 13.0 ± 1.3 and 16.4 ± 1.1 μmol glycerol/ml packed cells/h (mean ± SEM, n = 3) for 0.8 and 1.6 μM dobutamine, respectively. To evaluate the effect of PMA in multiple sets of experiments, lipolysis rates were expressed as a percentage of the control.

3.2. Effect of PMA-induced downregulation of PKC on the lipolysis-attenuating effect of PMA

PMA treatment activates cPKCs and nPKCs, but not aPKCs. Upon prolonged treatment, these PMA-responsive PKCs are proteolytically degraded (13, 14). This PMA-induced downregulation has been used to study the role of specific types of PKCs during insulin signaling and other biological functions (8, 14-17). In our previous study (11), we found that treating rat adipocytes with 2 μM PMA for 6 h decreases cellular expression of PKCs α, βI, βII, δ, and ε and abolishes the lipolysis-attenuating effect of

PMA. In this study, the rate of downregulation was examined during early timepoints of PMA treatment. As shown in Fig. 1-A and B, western blots on total cell lysates revealed a marked decrease in PKCs α, βI and δ after 2 h of 2 μM PMA treatment. PKCs βII and ε significantly decreased after 6 h while PKC ζ remained unchanged.

After treatment with 2 μM PMA for 15 min, the rate of lipolysis stimulated by dobutamine was reduced, and the lipolysis-attenuating effect of PMA was greatly diminished (Fig. 1-C). No lipolysis-attenuating effect of PMA was found after treatment with PMA for 1 h or more. It is possible that the failure to detect PMA's effect on lipolysis was due to a sustained activation of PKC in the PMA-treated cells. To test this possibility, we measured the rate of lipolysis in the presence of the PKC inhibitor Gö 6976, which inhibits the lipolysis-attenuating effect of PMA (11). As shown in Fig. 2, Gö 6976 increased lipolysis in adipocytes that were treated with PMA for 15 min or 1 h, suggesting that the PKC isoform responsible for antilipolysis had been present in the cells in its active form. Gö 6976 did not increase lipolysis in the cells treated with PMA for 2 h or more. The results shown in Fig. 1 and 2 suggest that the PKC isoform responsible for antilipolysis is largely degraded after a 2 h treatment with 2 μM PMA.

To investigate the mode of PKC downregulation in more detail, adipocytes were treated with low doses of PMA for 2 h (Fig. 3-A). The amount of PKCδ greatly decreased, even with 0.1 μM PMA. In contrast, PKCs α and βI gradually decreased as the concentration of PMA increased. The levels of these PKCs decreased with a slope that could be superimposed on the descending slope of the lipolysis-attenuating effect of PMA in the PMA-treated cells (Fig. 3-B). In these experiments, lipolysis in the presence of PMA was compared to that in the presence of Gö 6976 to determine the degree of the lipolysis-attenuating effect of PMA.

3.3. Effect of modifying PKC expression with fasting on the lipolysis-attenuating effect of PMA

The expression, phosphorylation and intracellular distribution of PKCs are regulated by nutritional conditions (18-22). Serum starvation in cultured cells increases the amount of PKCα (23). Thus, we compared the levels of PKC expression to the PMA's lipolysis-attenutaing effect in adipocytes from rats that had fasted for 24 h. Western blot analysis revealed that after fasting, PKCα levels increased, PKCδ levels decreased (Fig.

4-A), and levels of PKCs βI, βII, ε, and ζ were unchanged (data not shown). The ratio of PKC amount in fasting rats to that in feeding rats was 1.55 ± 0.08 for PKCα and 0.57 ± 0.06 for PKCδ (mean ± SEM, $p<0.05$ or less, n = 4). The ratios determined for PKCs βI, βII, ε, and ζ, whose levels did not change significantly after fasting, were found within the range of 0.97-1.21. Importantly, no marked difference was found in the potency of PMA to attenuate lipolysis in adipocytes from fasting versus feeding rats (Fig. 4-B). Therefore, fasting did not alter the level of the PKC isoform required for antilipolysis, ruling out a possible contribution of PKCs α and δ.

3.4. Effect of PKC inhibitors on the lipolysis-attenuating effect of PMA

GF 109203X specifically inhibits cPKCs and nPKCs, while Gö 6976 inhibits PKCs α and β (24). These inhibitors have been previously shown to block the lipolysis-attenuating effect of PMA (11). In this study, the effect of other PKC inhibitors, the PKCβI/βII inhibitor LY 333531 and the PKCβII inhibitor CGP 53353, were examined. At 1 μM, LY 333531 inhibits PKCβ-mediated pathways (25, 26) and CGP 53353 blocks PKCβII-mediated cellular events (19, 22). As shown in Fig. 5-A, 1

μM LY 333531 abolished the lipolysis-attenuating effect of PMA while CGP 53353 did not.

Rottlerin inhibits PKCδ-mediated signaling in mouse adipocytes (27). Here, we found that the lipolysis-attenuating effect of PMA could be observed in both low and high concentrations of rottlerin (Fig. 5-B). In addition, we found that rottlerin dose-dependently decreases lipolysis. Lipolysis was totally abolished in adipocytes that had been pretreated with 2 μM rottlerin for 2 h.

4. Discussion

We have previously shown that insulin attenuates the β_1-AR-mediated lipolysis in rat adipocytes, independently of PDE3B activity (10). This antilipolytic effect was insensitive to a PKB inhibitor (Akt inhibitor, 1L-6-hydroxymethyl-*chiro*-inositol 2-(R)-2-O-methyl-3-O-octadecylcarbonate, J. Nakamura, unpublished observation), ruling out a possible contribution of the PKB activity. PI3K has an important role for insulin signaling, as wortmannin inhibits the insulin-induced antilipolysis (10). An

essential role of the PMA-responsive PKC for insulin signaling has been verified (10, 11). To determine the PKC isoform responsible for antilipolysis, the lipolysis-attenuating effect of PMA was assessed in rat adipocytes that had been treated with PMA to degrade specific PKC isoforms. It was found that PMA triggers the downregulation of PKC sioforms at different rates, with PKCδ>PKCα and βI>PKCβII and ε. PMA does not cause a change in PKCζ levels. The downregulation profiles of PKCs α and βI, but not those of PKCs βII, δ, ε, and ζ, correlated with a decrease in the potency of PMA to attenuate lipolysis in the PMA-treated cells (Fig. 1, 2, and 3). The antibodies to PKCs γ and η used in this study do not detect any band that decreases in response to PMA (11). It is known that in adipocytes, PKCs θ and μ remain unchanged or decrease only minimally with PMA treatment (15, 17). Although a 24 h fast modified expression of PKCs α and δ in adipocytes, it did not affect the lipolysis-attenuating effect of PMA (Fig. 4). The effect of PMA was blocked by inhibitors of PKCβI, such as LY 333531 (Fig. 5-A) as well as GF 109203X and Gö 6976 (11), but not by CGP 53353 (Fig. 5-A) or rottlerin (Fig. 5-B). The observed decrease in lipolysis by rottlerin may be due either to inhibition of PKA or to depletion of ATP (28). Gö 6976 and LY 333531

have been shown to effectively inhibit the PKC-dependent antilipolysis triggered by insulin (10). In all, these data suggest that PKCβI is the specific isoform that mediates the signal transduction cascade initiated by PMA and insulin, leading to antilipolysis.

There is evidence to suggest a functional modification of β_1-AR signaling via molecular crosstalk with PKC. In HEK 293 cells that co-express different PKC isoforms with β_1- or β_2-AR, β_1-AR is modulated by the PKCs to a much greater extent than β_2-AR (29). Different PKCs can lead to a lower accumulation of cAMP following stimulation of β_1-AR, with PKCβII having the largest effect, followed in descending order by PKCα, PKCε, then PKCζ (29). Activation of PKCα causes a rightward shift in the EC_{50} for agonist stimulation of β_1-, but not β_3-, AR-mediated activation of adenylylcyclase in SK-N-MC cells (30). In these cultured cells, only PKCs α and ζ were detected by western blot analysis (30). The PKC isoform that causes negative regulation of β_1-AR signaling may be different among cell types. The present work shows that PKCβI interacts with β_1-AR signaling.

At present, the mechanism that allows molecular crosstalk between the PKCβI and β_1-AR signaling pathways in rat adipocytes remains unknown. It is known that PKC

promotes coupling between β-AR and the G-protein Gi/o, which inhibits adenylylcyclase, though β-AR is primarily associated with Gs (31). PKC activates G-protein coupled receptor kinase, which phosphorylates β-AR and thereby uncouples the β-AR and the G-protein (32). Alternatively, PKC directly phosphorylates β-AR and interferes with its activation of G-protein (32). A downstream point in the β-AR signaling pathway, such as PDE, may be a PKC target (33).

Insulin signaling involves the activity of PKC, and PI3K is essential for PKC activation (6, 8, 9). Insulin activates phospholipase C and/or D via activation of PI3K, leading to production of diacylglycerol (DAG) (8, 9, 35). An increase in DAG concentration induces the activation and membrane translocation of cPKCs and nPKCs, which are cytosolic in their mature phosphorylated forms (8, 9, 34). Elevation of intracellular Ca^{2+}, which is necessary for the cPKC activation, also requires the PI3K activity (9). An increase in membrane-associated cPKCs and nPKCs is generally used to indicate the activation of these enzymes (8, 9, 18, 20-22, 34). The product of PI3K, phosphatidylinositol 3,4,5-triphosphate, may facilitate the activation of aPKCs and colocalize the enzymes with substrates (6, 8).

The results of this study, together with those presented previously (10), suggest that the mode of insulin activation of PKCβI in rat adipocytes is dependent on PI3K. Levels of membrane-associated PKCβI increased upon insulin stimulation of adipocytes. Wortmannin treatment caused a rapid decrease in the amount of enzyme in the membrane fraction and a simultaneous disappearance of PKC-dependent antilipolysis (10). There is also evidence to suggest that the mode of activation of PKCs α and δ by insulin does not involve PI3K. These enzymes are activated by insulin upstream of PI3K, probably through tyrosine phosphorylation, and wortmannin does not block their insulin-stimulated activation and membrane translocation (8, 9, 36). We observed that wortmannin does not affect the level of membrane-associated PKCα in insulin-treated adipocytes (10). Wortmannin did cause a fairly slow decrease in the membrane-associated PKCδ, in contrast to the rapid decline of PKCβI (10). Because the PKC-dependent antilipolysis by insulin is highly sensitive to wortmannin (10), PKCs α and δ can not be regulators of the β_1-AR-mediated lipolysis in adipocytes.

Rat adipocytes contain three β-AR subtypes: β_1, β_2, and β_3. Since β_2-AR protein levels are extremely low (37) and norepinephrine- and isoproterenol-stimulated lipolysis

is not significantly affected by the β_2-AR antagonist ICI 118551 (11), β_2-AR signaling is likely to play only a very minor role in lipolysis in rat adipocytes. The β_3-AR may be the physiological receptor for the high norepinephrine concentrations that are attained during sympathetic activity near adipose tissues in conditions such as fasting and exposure to cold (38, 39). Therefore, the β_1-AR is the primary lipolytic AR subtype that responds to low concentrations of norepinephrine in the plasma of rat adipocytes (39). Circulating catecholamines are important for the exercise-induced increase in adipose tissue lipolysis (40) and for lipolysis in response to insulin-induced hypoglycemia (41). An increase in the sensitivity of lipolysis to β_1-AR agonists is effective in preventing obesity (42). In earlier work (11), we have shown that PKC regulates the rate of lipolysis induced by submaximally stimulating concentrations of β_1-AR agonists. These results, together with the present findings, suggest that PKCβ_1 regulates the sensitivity of lipolysis to β_1-AR.

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Figure Legends

Fig. 1. PKC expression and lipolysis-attenuating effect of PMA in adipocytes pretreated with PMA. (A), adipocytes were incubated in buffer A at 37ºC without PMA for 6 h, with 2 μM PMA for the final 0.25, 1, and 2 h of a 6 h incubation, and with 2 μM PMA over the period of a 6 h incubation. After incubation, the cells were washed, and the total cell lysates were subjected to western blot analysis with antibodies recognizing PKCs α, βI, βII, δ, ε, and ζ. The results shown are a representative from three experiments. (B), immunoblots obtained in (A) were scanned and quantified by a densitometer. The results are expressed as mean ± SEM of three experiments and were expressed as the percentage of control cells (pretreated without PMA). *p<0.05 or less, compared to control. (C), adipocytes pretreated as in (A) were washed with buffer B, and were treated with 0.8 and 1.6 μM dobutamine in the presence and absence of 0.8

μM PMA. The results are expressed as mean ± SEM of seven experiments and were expressed as the percentage of lipolytic activity determined without PMA in control cells. *p<0.05 or less, compared to no PMA. +p<0.05 or less, compared to control cells.

Fig. 2. Effect of Gö 6976 on lipolysis in adipocytes pretreated with PMA. Adipocytes were treated with PMA as in Fig. 1-A, and determined for lipolysis induced by 0.8 and 1.6 μM dobutamine in the presence and absence of 3 μM Gö 6976. The percent increase in lipolysis by Gö 6976 was determined in each experiment and is expressed as mean ± SEM of six experiments. *p<0.05 or less, as compared to no Gö 6976.

Fig. 3. PKC expression and lipolysis-attenuating effect of PMA in adipocytes pretreated with low doses of PMA. (A), adipocytes were incubated in buffer A at 37ºC without PMA for 6 h and with 0.1, 0.2, and 0.3 μM PMA for the final 2 h of a 6 h incubation. The amounts of PKC isoforms in the cells were analyzed by western blot using the antibodies recognizing PKCs α, βI, and δ. The result shown is a representative from four experiments. (B), immunoblots obtained in (A) were scanned and quantified by a

densitometer. The results are expressed as mean ± SEM of four experiments and were expressed as the percentage of control cells (pretreated without PMA). Lipolysis was stimulated with 0.8 μM dobutamine in the presence of 3 μM Gö 6976 and 0.8 μM PMA in adipocytes pretreated with PMA as in (A). The lipolysis-attenuating effect of PMA was determined by comparing between lipolysis measured in the presence of PMA and Gö 6976. The results are mean ± SEM of four experiments and were expressed as the percentage of the lipolysis-attenuating effect of PMA in control cells.

Fig. 4. PKC expression and lipolysis-attenuating effect of PMA in adipocytes from rats that had fasted for 24 h. (A), adipocytes were prepared from rats fed and fasted for 24 h. The total cell lysates were subjected to western blot analysis using the antibodies recognizing PKCs α, βI, δ, and ζ. The results shown are a representative from four experiments. (B), lipolysis was stimulated by dobutamine with and without 0.8 μM PMA in adipocytes from rats fed and fasted for 24 h. The results are mean ± SEM of three experiments and were expressed as the percentage of lipolysis induced by 16 μM dobutamine in the absence of PMA. *p<0.05 or less, compared to no PMA.

Fig. 5. Effect of PKC inhibitors on lipolysis-attenuating effect of PMA. Adipocytes were stimulated by 0.8 μM dobutamine in the presence and absence of 0.8 μM PMA in a mixture with LY 333531 and CGP 53353 (A) or rottlerin (B). The results are mean ± SEM of six (A) or four (B) experiments and were expressed as the percentage of lipolysis induced by 0.8 μM dobutamine alone. *p<0.05 or less, compared to no PMA.