0026-895X/89/030381-06$02.00/0

MOLECULAR PHARMACOLOGY, 35:381-386

Role of Hormone-Sensitive Low K_m cAMP Phosphodiesterase in Regulation of cAMP-Dependent Protein Kinase and Lipolysis in Rat Adipocytes

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Received July 11, 1988; Accepted December 1, 1988

SUMMARY

The time-courses of isoproterenol activation of rat adipocyte particulate low K_m cAMP phosphodiesterase (PDE) activity, cAMP-dependent protein kinase (A-kinase), and glycerol production were measured in the presence and absence of insulin. Isoproterenol (100 nM) alone rapidly activated A-kinase 8- to 10-fold and increased particulate cAMP PDE by ~100%. A-kinase and PDE activity remained relatively constant for at least 25 to 30 min. K_{act} values for isoproterenol activation of PDE and lipolysis were similar. In comparison with isoproterenol, insulin (0.1–0.3 nM) alone increased particulate cAMP PDE at a slower rate and to a lesser extent (by ~50% within 12 to 16 min) and without any change in A-kinase. With insulin plus isoproterenol there was a rapid, transient, and synergistic activation of particulate cAMP PDE, which temporally correlated with a decrease in A-kinase and reduction in lipolysis. These and other data suggest the following: 1) there is a close concentration-dependent and temporal relationship in isoproterenol activation of adenylate cyclase, of A-kinase, and of particulate cAMP PDE; 2) isoproterenol and insulin activate particulate cAMP PDE by two distinct mechanisms; 3) the temporal changes in PDE and A-kinase in the presence of insulin and isoproterenol suggest that insulin activation of the PDE does not require, but may be enhanced by, elevated cAMP and is important in the antilipolytic action of insulin.

In adipose tissue, cAMP is an important intracellular mediator in hormonal regulation of triacylglycerol hydrolysis. Lipolytic hormones, such as catecholamines, corticotropin, and glucagon, activate adenylate cyclase, increasing cAMP formation (1, 2). cAMP promotes lipolysis by activating cAMP-dependent protein kinase, which phosphorylates and activates hormone-sensitive lipase (3), resulting in hydrolysis of stored triglyceride to glycerol and free fatty acids. By catalyzing the hydrolysis of cAMP, cyclic nucleotide PDEs represent one potential mechanism for regulation of the effects of lipolytic hormones.

Insulin is a physiologically important and potent inhibitor of lipolysis (1). Although the precise mechanism(s) of the antilipolytic action of insulin is unknown, under certain conditions it correlates well with a reduction in A-kinase activity, which presumably closely reflects a decrease in cellular cAMP (4). Insulin-induced inhibition of adenylate cyclase or activation of cAMP PDE could reduce cAMP content (5) and in 3T3-L1, rat, and human adipocytes activation of a specific particulate cAMP PDE may be important in the antilipolytic action of insulin (6–8).

Investigation of the role of the particulate cAMP PDE in regulation of lipolysis is complicated by the fact that, in addition to insulin with its antilipolytic effects, lipolytic hormones (9–11), as well as cyclic AMP analogs that activate A-kinase and lipolysis (12), also increase particulate cAMP PDE activity. Activation of the PDE by lipolytic effectors appears to be secondary to changes in cAMP and activation of A-kinase, perhaps representing a type of feedback regulation resulting in PDE-mediated attenuation or termination of the cAMP signal generated by activation of adenylate cyclase (8, 10–13).

Although effects of isoproterenol and insulin on particulate cAMP PDE have been documented heretofore, in the studies reported here simultaneous measurements of hormone effects on particulate cAMP PDE, lipolysis, and A-kinase point to a close relationship or functional coupling between isoproterenol-induced activation of adenylate cyclase, of A-kinase, and of particulate cAMP PDE. Coordinate regulation of these enzymes may be important in regulation of intracellular cAMP and the activation "state" of A-kinase and hormone-sensitive lipase. In addition, cAMP may enhance effects of insulin on

C.J.S. was a Pharmacology Research Associate (1985–1987) in the National Institute of General Medical Sciences during the course of this work.

ABBREVIATIONS: PDE, phosphodiesterase; ADA, adenosine deaminase; Ado, adenosine; A-kinase, cAMP-dependent protein kinase; BSA, bovine serum albumin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PIA, N^6-phenylisopropyladenosine; TES, (*N*-(tris(hydroxymethyl)) methyl-2-amino-ethanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; MOPS, 3-[*N*-morpholino]propanesulfonic acid.

particulate cAMP PDE, and activation of the PDE may be important in insulin-induced reduction of isoproterenol-stimulated A-kinase activity and lipolysis.

Methods

Animals. Male Sprague-Dawley rats (125–150 g), purchased from Taconic Farms (Germantown, NY) and maintained 1–3 weeks under National Institutes of Health animal care protocols, were fasted 16–18 hr before decapitation (between 9 and 10 a.m.).

Adipocyte preparation. Adipocytes were prepared from epididymal fat pads by the method of Rodbell (14), with inclusion of 200 nM Ado and 2 mM glucose in all buffers as suggested by Honnor *et al.* (15). All experiments were carried out with adipocytes prepared with two or three different lots of collagenase and BSA and were incubated in a Precision water bath set at 1.5-inch stroke length for 120 cycles shaking speed during collagenase digestion and 150 cycles for incubations with hormones. Cells were incubated, in Krebs Ringer buffer (pH 7.4) containing 4% BSA and 25 mM HEPES (BSA-KRH), at a final (packed) cell concentration of 2–3%, which was determined by centrifugation of the concentrated cell suspension, in heparinized microcapillary tubes, for 3 min in an IEC Capillary Microfuge (model MB). After incubation for 15 to 20 min at 37°, hormones or effectors were added (each in 1% of the final incubation volume) and the incubations were continued. For zero time samples, hormones were added after cells had been homogenized.

When measuring lipolysis, A-kinase, and PDE activity, incubations (3.5 ml) were terminated by homogenization of 0.8-ml samples in 0.2 ml of 2.5 mM RO-20-1724/50 mM Tris-EDTA (pH 7.4), as described (15). After centrifugation (14,000 × *g* for 30 min), the supernatant fraction was assayed for A-kinase (15, 16) and glycerol content (17). For assay of cAMP PDE, the remaining cells plus medium (2.7 ml) were poured into a 7-ml Dounce homogenizer containing 2.7 ml of TES buffer at 4° (50 mM TES, pH 6.7 at 25°/5 μg/ml pepstatin/1 μg/ml leupeptin/250 mM sucrose), homogenized with eight strokes of a B pestle (18), and centrifuged (35,000 rpm for 65 min with a 40.3 rotor in a Beckman LM-8 ultracentrifuge); the fat cake was discarded. Except where indicated, results are presented as the mean ± standard error of different (n) preparations. In several "large" experiments (> 13 conditions) with simultaneous assays of kinase/glycerol and PDE, e.g., two dose response curves or two different time-courses, basal/time-zero cell incubations were carried out in duplicate and all others were single incubations; these experiments were repeated with at least two different preparations.

Low K_m cAMP PDE assay. Samples of supernatant and particulate fractions (dispersed in 400–500 μl of 1:1 TES/BSA-KRH in a 2-ml Dounce homogenizer with B pestle) were assayed for cAMP PDE as described (19). Assays (in duplicate at two enzyme concentrations) contained 0.5 μM [^{3}H]cAMP (10,000–30,000 cpm), 58 mM Na·HEPES (pH 7.5), 9.1 mM $MgCl_2$, 0.1 mM EGTA, 31 mM NaCl, 1.2 mM KCl, 0.25 mM KH_2PO_4, 0.62 mM $CaCl_2$, 1% BSA, 12 mM TES, 1.2 μg/ml pepstatin, 0.25 μg/ml leupeptin, and 62 mM sucrose. Rates of cAMP hydrolysis (at 30°) were constant for at least 18 min. The sum of particulate and supernatant cAMP PDE activities accounted for at least 90% of initial homogenate activity under all conditions (i.e., basal, isoproterenol, ADA, insulin). Although data from assays of freshly prepared pellets and supernatants are presented in this report, preparations were stable to rapid freezing in methanol/CO_2 and storage at −20° for at least 3 weeks.

In seven experiments, particulate cAMP PDE activity varied < 15% from zero time values during incubations for 30 min. Specific activities of particulate low K_m cAMP PDE varied with different lots of BSA (e.g., lot 73F-9305, 140 ± 7 pmol/[min·ml of packed cells], n = 4; and lot 114F-9392, 223± 29 pol/[min·ml of packed cells], n = 5 but, with all lots, hormones produced the same relative increase in particulate cAMP PDE, with little or no effect on supernatant PDE activity. Cilostamide (2 μM), a selective inhibitor of cAMP PDE, inhibited particulate low K_m cAMP PDE activity from basal or hormone-treated adipocytes by > 80% and supernatant activity < 35%. The distribution of low K_m cGMP PDE activity (assayed with 0.5 μM [^{3}H]cGMP) was quite different from that of cAMP PDE; ~5% of total low K_m cGMP PDE was recovered in the particulate fraction and the specific activity of the supernatant was 2–4 times that of the particulate fraction. These observations support the conclusion that the hormone-sensitive cAMP PDE is highly concentrated in the particulate fraction.

A-kinase assay. Assays in triplicate containing 20 μl of fat cell supernatant, 25 mM MOPS (pH 7.0), 16 mM $MgSO_4$, 125 μM [γ-^{32}P] ATP (8×10^5 cpm), 4.2 mM dithiothreitol, and 100 μM Kemptide with or without 16 μM cAMP (total volume, 60 μl) were incubated for 10 min at 30° before addition of 40 μl of 2.5% sodium dodecyl sulfate/1.2 mM ATP/175 mM dithiothreitol and heating (2 min, at 100°). Phosphorylated Kemptide was isolated for radioassay by the method of Egan *et al.* (16). Samples were assayed without and with cAMP to determine the ratio of A-kinase activation, i.e., activity without cAMP)/activity with cAMP); 1.0 reflects maximal activation and high intracellular cAMP content. Total A-kinase activity (i.e., assayed with 16 μM cAMP) did not vary significantly from zero time values during incubation of adipocytes (with or without hormones) for 30 min.

Preparation of solutions. PIA (100 μM in 10% ethanol), as well as 200 μM Ado and M glucose in 75% ethanol, was stored at −20° for up to 6 months. Cilostamide (provided by Dr. H. Hidaka of Mie University, Tsu City, Japan) was stored (10 mM in dimethyl sulfoxide at 4° for 10 months with no apparent loss of potency. Bovine insulin (Sigma I-5500), 0.9 mM in 10 mM HCl, was stored for up to 6 months at −20° in small portions, each used for a single experiment. All other stock solutions were prepared on the day of use. The final concentration of NH_4SO_4 contributed by ADA (Sigma A-1030, Type VIII, > 2500 units/ml) was 0.6–1 mM. The concentration of vehicle (ethanol, NH_4SO_4, or dimethyl sulfoxide) was equivalent to the amount added with the highest drug concentration.

Materials. $MgCl_2$ was purchased from Fisher Scientific (Pittsburgh, PA); glycerol and KH_2PO_4 from Mallinckrodt (St. Louis, MO); other salts for KRH solutions and acids from J. T. Baker (Phillipsburg, NJ); leupeptin and PIA from Boehringer-Mannheim (Indianapolis, IN); ultra-pure sucrose and electrophoresis-pure sodium dodecyl sulfate from Bethesda Research Laboratories (Rockville, MD); other reagents and enzymes for the glycerol, A-kinase, and PDE assays from Sigma Chemical Co. (St. Louis, MO); [^{3}H]cAMP (40 Ci/mmol) and [γ-^{32}P] ATP (40 Ci/mol) from New England Nuclear (Boston, MA). [^{3}H] cAMP was purified as described (19). Fatty acid-free BSA (A-6003) was from Sigma or from Armour (Bovuminar Cohn Fraction V); collagenase (CLS 4196; 130–200 units/mg) was from Worthington Biochemicals (Freehold, NJ).

Results

Basal conditions. Different adipocyte preparations exhibit variations in A-kinase ratios and consequently in basal lipolytic rates, presumably due in part to undefined contaminants in collagenase and BSA preparations as well as to variations in production/metabolism of endogenous Ado, a potent antilipolytic agent (15). As suggested by Honnor *et al.* (15), dilute suspensions of adipocytes were therefore incubated with 200 nM Ado or ADA, 0.5–1 units/ml, plus 3–100 nM PIA to maintain basal lipolysis and A-kinase at a minimum and keep adipocytes responsive to stimulation by isoproterenol (20). Ado has also been reported to enhance the responsiveness of adipocytes to insulin (4, 21). Cell suspensions were homogenized under conditions designed to prevent association/dissociation of the A-kinase (15). Under basal conditions, the A-kinase ratio was low (0.06 ± 0.01, n = 8) and glycerol production was usually at the limit of detection (< 60 nmol/ml). Total and specific activities of particulate and supernatant cAMP PDEs were

similar in fractions from cells incubated with Ado or PIA plus ADA (data not shown).

Isoproterenol activation. A maximally effective concentration of isoproterenol caused a rapid increase in particulate cAMP PDE activity and the A-kinase activity ratio. Both were maximal in 2 min and remained relatively constant for ~25 min thereafter (data not shown). Isoproterenol-stimulated glycerol production proceeded at a constant rate for at least 30 min after, in some instances, a delay of 1–2 min (data not shown). Very similar effects of isoproterenol on A-kinase and glycerol production have been previously reported (20). In nine experiments (with Ado or ADA plus PIA), incubation for 15 min with isoproterenol (100–300 nM) increased basal PDE activity by 99 ± 14% (also see Fig. 2B); glycerol production was increased by 380 ± 60% (n = 6) (also Fig. 2C). The concentration dependency for isoproterenol activation of PDE was virtually identical to that for activation of lipolysis (Fig. 1; Table 1), indicating a very close relationship or coupling between activation of adenylate cyclase and the particulate cAMP PDE. Increasing PIA from 3 to 100 nM decreased sensitivity to isoproterenol and increased the K_{act} for isoproterenol activation of lipolysis and PDE (Table 1). At all PIA concentrations, activation of PDE and lipolysis was maximal at an A-kinase ratio of ~0.50 (Table 1; Fig. 1).

Insulin activation. A maximally effective concentration (0.1–0.3 nM) of insulin increased particulate cAMP PDE activity by ~50 ± 10% (n = 15) in 12 to 16 min, whereas the A-kinase activity ratio and basal lipolysis were unaltered (data not shown). The K_{act} for insulin activation of particulate PDE was ~6 pM (Table 2). Thus, the rate and extent of insulin activation of the particulate PDE were somewhat less than that produced by isoproterenol. Similar effects of insulin on fat cell particulate cAMP PDE have been reported (9–11, 13, 19).

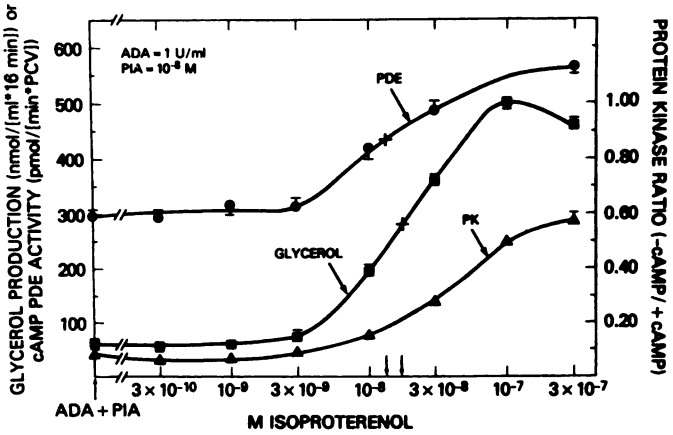

Fig. 1. Concentration dependence for isoproterenol activation of particulate low K_m cAMP PDE, glycerol production, and A-kinase. Adipocytes (47 μl of packed cells/3.5 ml of buffer) were incubated for 16 min with 10 nM PIA plus 1 unit/ml ADA in the absence and presence of the indicated concentrations of isoproterenol. ●, Particulate low K_m cAMP PDE; ▲, A-kinase; ■, glycerol production, *PCV*, packed cell volume (ml). The isoproterenol K_{act} values (+) for cAMP PDE and lipolysis are 13 and 18 nM respectively. Results are from a representative experiment, repeated several times. Incubations without hormone and at 300 nM isoproterenol were in duplicate; single incubations were at all other hormone concentrations. PDE activities are values of mean ± standard error of quadruplicate assays of each incubation. A-kinase activity was assayed in triplicate in the presence and absence of cAMP. Results for glycerol production are the mean ± half the range of values for duplicate assays.

TABLE 1

Isoproterenol activation of particulate cAMP PDE, lipolysis, and A-kinase

Cells were incubated for 12–16 min with five to seven concentrations (0.1–300 nM) of isoproterenol and other additions as indicated. Incubations with PIA also contained ADA, 1 unit/ml. K_{act} is the concentration of isoproterenol required for half-maximal activation of PDE or lipolysis. Maximal effects were produced with 3–10 nM isoproterenol in the presence of 0–3 nM PIA, 100 nM with 10 nM PIA, and 200 nM with 100 nM PIA; ADA was maximally effective at 0.5–1 unit/ml. Data are mean ± standard error of values from the number of preparations shown in parentheses, most of which were used for assays of cAMP PDE, glycerol, and A-kinase.

Additions	Isoproterenol K_{act}		A-Kinase Ratio
	cAMP PDE	Lipolysis	
	nM		
Ado, 200 nM	11 ± 4 (2)	13 ± 2 (5)	ND*
PIA, 3 nM	2.5 ± 0.7 (4)	3.8 ± 1.4 (3)	0.55 ± 0.05 (3)
PIA, 10 nM	12 ± 1 (2)	16 ± 2 (2)	0.50 ± 0.00 (2)
PIA, 100 nM	32 ± 6 (3)	31 ± 6 (3)	0.54 ± 0.13 (3)

* ND, not determined.

TABLE 2

Insulin activation of particulate low K_m cAMP PDE

Cells were incubated for 12–15 min with five to seven concentrations of insulin (0.1 pM to 10 nM) and other additions as indicated. K_{act} is the concentration of insulin required for half-maximal activation. Data are mean ± standard error of values from the number of preparations shown in parentheses.

Additions	Insulin K_{act}
	pM
Ado, 200 nM	3 (1)
PIA, 3 nM	4 (1)*
PIA, 3 nM, plus isoproterenol, 100–300 nM	0.9 ± 0.7 (3)
PIA, 100 nM	6 ± 4 (5)

* In this experiment, K_{act} for insulin in the presence of 100 nM isoproterenol was 0.4 pM.

Effects of insulin in the presence of isoproterenol. In the presence of isoproterenol (100 nM) and insulin (0.1 nM), isoproterenol-activated A-kinase decreased to a new steady state from 2 to 16 min (Fig. 2A) and lipolysis was reduced (Fig. 2C). Similar effects of insulin on epinephrine-stimulated A-kinase in epididymal fat pads have been reported (22). Within 2 min in the presence of isoproterenol and insulin together, particulate cAMP PDE activity was greater than with insulin or the β agonist alone (Fig. 2B). Insulin and isoproterenol produced a transient synergistic activation of the PDE, which was maximal at 9 min and 50–80% greater that the expected additive effects of each agonist alone (Fig. 2B) and which temporally coincided with the fall in A-kinase ratio (Fig. 2A). PDE activity then declined. After 12–15 min with isoproterenol plus insulin, PDE activity was at least equal to the added effects of insulin and β agonist alone, i.e., basal activity increased by 180 ± 14% (n = 12; data not shown).

Insulin increased the K_{act} for isoproterenol activation of A-kinase (Fig. 3A) and lipolysis (Fig. 3B). In other experiments in which adipocytes were incubated with hormones for 15 min, insulin (0.3 nM) with ADA plus 3 nM PIA increased the K_{act} for isoproterenol for activation of both lipolysis (2.5 ± 0.7 versus 14 ± 6 nM, n = 2) and particulate cAMP PDE (2.5 ± 0.7 versus 8.2 ± 1.7 nM, n = 4). With ADA plus 100 nM PIA, lipolysis stimulated by 100 nM isoproterenol was inhibited by insulin (IC_{50} 8 ± 3 pM, n = 9); similar results have been reported by others (4). The concentration dependency for insulin inhibition of lipolysis was comparable to that for activation of the particulate cAMP PDE (Table 2). As also seen in Fig. 3B and has been reported by others (4), at A-kinase ratios

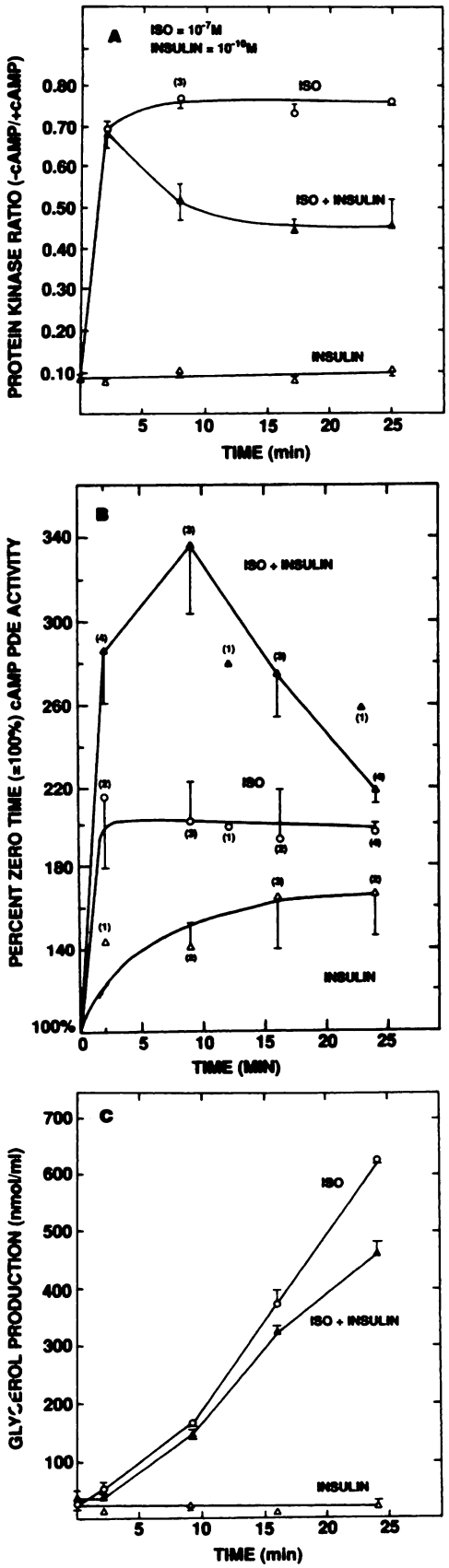

Fig. 2. Time-course of isoproterenol (*ISO*) activation of particulate cAMP PDE, A-kinase, and lipolysis in the presence and absence of insulin. Adipocytes (56–60 μl of packed cells/3.5 ml of buffer) were incubated in duplicate with 3 nM PIA plus 1 unit/ml ADA and 100 nM isoproterenol (○), 0.1 nM insulin (△), or isoproterenol plus insulin (▲) for the indicated times. Complete time-courses (five time-points) were compared for at least two different conditions (e.g., insulin versus insulin plus isoproterenol) in each experiment. A, A-kinase ratio as a function of time. Results are the mean ± half the range from two (isoproterenol, insulin) or mean ± standard error for three (isoproterenol plus insulin) different preparations in which glycerol (C) and cAMP PDE activity were also assayed (B). B, Particulate cAMP PDE activity as a function of time. Results are the mean ± half the range of two or mean ± standard error of *N* preparations (numbers in parentheses). Particulate cAMP PDE activity was normalized relative to the specific activities of time-zero values, which were 144 ± 9 (n = 3 preparations; BSA lot A94107) and 269 (n = 1 preparation; BSA lot 114F-9392) pmol of cAMP hydrolyzed /[min·ml of packed cells] in particulate fractions from cells prepared in two different BSA lots. C, Glycerol production as a function of time. Same cell preparations as in A.

> 0.70 isoproterenol-stimulated lipolysis was not inhibited by insulin, perhaps because synergistic activation of PDE was not sufficient to reduce cAMP enough to drive the A-kinase ratio below ~0.5 and reduce lypolysis. In other experiments in which adipocytes were incubated with hormones for 15 min, the loss of the antilipolytic effect of insulin was also associated with A-kinase ratios (0.83 ± 0.06, n = 4) much higher than those required for maximal activation of lipolysis by isoproterenol (i.e., ~0.5; cf. Figs. 1 and 3).

Discussion

In intact rat adipocytes incubated with isoproterenol, regulation of particulate cAMP PDE was assessed in concert with A-kinase and lipolysis. Measurement of the A-kinase ratio is presumed to reflect cellular cAMP concentrations (22) and our studies were carried out under conditions in which activation of A-kinase apparently reaches a steady state (20). In intact adipocytes, isoproterenol rapidly activates adenylate cyclase and increases cAMP, resulting in maximal activation of A-kinase and particulate cAMP PDE within 2 min. The concentration dependencies for isoproterenol activation of triglyceride lipase (lipolysis) and particulate cAMP PDE are identical. The antilipolytic agent PIA, which inhibits adenylate cyclase (23), increases the concentration of isoproterenol required for activation of both lipolysis and PDE. Thus, as has been suggested previously (8–13), activation of cAMP PDE and of lipolysis appear to be secondary to changes in cAMP and activation of A-kinase. Previous reports, however, have not demonstrated or emphasized that activation occurs over virtually the entire range of hormonal activation of adenylate cyclase and triglyceride lipase. Apparently, in adipocytes there exists a close relationship (perhaps tight functional coupling via activation of a-kinase) between activation of cyclase and of particulate PDE. Although representing a "feedback" mechanism for attenuating or terminating the cAMP signal generated during hormonal activation of cyclase, activation of particulate PDE would appear to be not merely a response to excess cAMP. In fact, maximal activation of particulate PDE [and, as has also been previously reported, triglyceride lipase (15)] is associated with A-kinase ratios of ~0.5, i.e., presumably at cAMP concentrations well below saturation of A-kinase. The coordinate regulation of adenylate cyclase, protein kinase, and PDE activities may be important in the physiological regulation of cAMP turnover [cf. Goldberg *et al.* (24)], steady state concentrations of cAMP, and, hence, the activation state of A-kinase (12, 24, 25) and consequently triglyceride lipase.

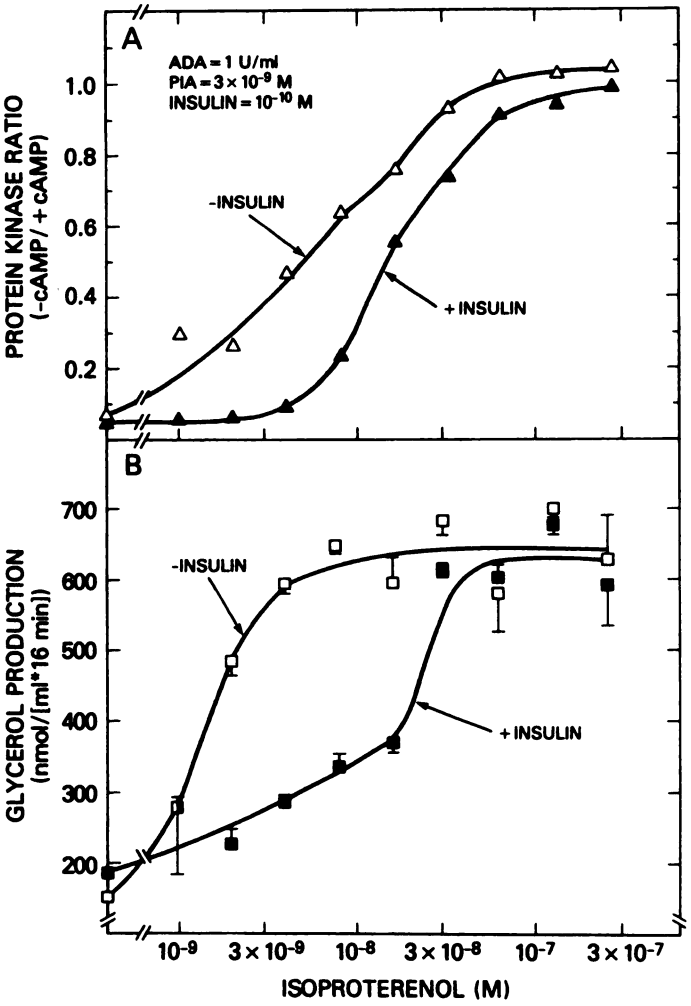

Fig. 3. Effect of insulin on isoproterenol-dependent activation of A-kinase and lipolysis. Adipocytes (20.8 μl of packed cells in 0.8 ml of buffer) were incubated for 15 min with 3 nM PIA plus 1 unit/ml ADA in the absence (open symbols) and presence (closed symbols) of 0.1 nM insulin and indicated concentrations of isoproterenol before assay of the A-kinase activity ratio (A) or glycerol production (B). Results are from a representative experiment, repeated several times; statistical analysis of A-kinase and glycerol assays are presented as in Fig. 1.

The hormone-sensitive triglyceride lipase is a substrate for A-kinase (3). The increase in particulate cAMP PDE induced in intact fat cells by lipolytic hormones or cAMP analogs can be assayed after homogenization, subcellular fractionation (9, 10), and solubilization and partial purification of the particulate cAMP PDE (1, 8, 26, 27); the A-kinase catalytic subunit has been recently reported to increase PDE activity in isolated particulate fractions (28). Although PDE activation presumably reflects covalent modification mediated by A-kinase, phosphorylation of the adipocyte particulate cAMP PDE has not been demonstrated. In platelets, a similar (but apparently cytosolic) low K_m cAMP PDE is activated and phosphorylated, during incubation of intact platelets with PGE_1, or in broken-cell preparations, by the catalytic subunit of A-kinase (29).

The mechanisms whereby insulin [presumably by receptor-mediated activation of a phosphorylation/dephosphorylation "cascade" (30) and/or generation of intracellular mediators (31)] regulates the particulate cAMP PDE are unknown. Activation by insulin alone was not accompanied by changes in A-kinase and is slower, with maximal effects observed in 12–16 min as compared with 2 min with isoproterenol. At maximally effective concentrations of either agent alone, the extent of activation was almost always greater (by ~50%) with isoproterenol than with insulin.

Our results indicate, however, that, despite these differences in mechanisms of activation, an apparent interaction between insulin and isoproterenol in regulation of particulate cAMP PDE may be of functional importance in the antilipolytic action of insulin. Incubation of adipocytes with both insulin and isoproterenol is associated with a rapid, synergistic, and transient activation of particulate cAMP PDE. The increased hydrolysis of cAMP perhaps results in the reduction of A-kinase, which in turn leads to a decline in the cAMP- and A-kinase-dependent component of PDE activation and triglyceride lipase activity (lipolysis). Because interaction of insulin with its specific cell surface receptor is accompanied by the activation of several specific intracellular serine protein kinases and alterations in phosphorylation/dephosphorylation of certain intracellular proteins (32) and because cAMP and insulin can regulate phosphorylation (and enzymatic activity) of the same proteins (33), perhaps isoproterenol-induced activation of the adipocyte particulate cAMP PDE by A-kinase sensitizes the PDE to activation by insulin. An analogous mechanism has been postulated for insulin-dependent activation of an hepatocyte particulate cAMP PDE (34). With the availability of antibodies to the purified rat adipocyte particulate cAMP PDE,[1] regulation of the adipocyte PDE by cAMP- and insulin-dependent phosphorylation/dephosphorylation can now be assessed in intact and broken-cell preparations.

In 3T3-L1 and rat adipocytes, similar concentrations of insulin are required to activate the particulate cAMP PDE and inhibit lipolysis (Ref. 8 and this work). In 3T3-L1 adipocytes, the antilipolytic action of insulin was inhibited by cilostamide, a specific inhibitor of the hormone-sensitive particulate cAMP PDE, but not by RO 20-1724, a specific inhibitor of supernatant low K_m cAMP PDE activity (8). Of a series of cAMP analogues that activated A-kinase and stimulated lipolysis in rat adipocytes, insulin effectively inhibited lipolysis (presumably via activation of particulate cAMP PDE) stimulated by those analogues that served as substrates for the particulate cAMP PDE (6). Similar observations have been made in human fat cells (7). Taken together, these results and our current studies strongly suggest that insulin-enhanced activation of the particulate cAMP PDE may be very important in (if not responsible for) the reduction of A-kinase that is associated with the antilipoytic effect of insulin (4). Other proposed effects of insulin, i.e., inhibition of adenylate cyclase (35), alterations of A-kinase sensitivity (36, 37), and activation of phosphatase (4, 32), may also contribute to its antilipolytic action.

Although it seems paradoxical that both lipolytic hormone and the antilipolytic agent insulin may activate the same PDE and increase cAMP hydrolysis, PDE activation may reflect part of those mechanisms whereby both hormones regulate A-kinase activity. With isosproterenol, the close coupling of activation of adenylate cyclase, of PDE, and of A-kinase may regulate steady state concentrations of cAMP and consequently the activation state of A-kinase and triglyceride lipase (lipolysis). With insulin plus isoproterenol, insulin-enhanced activation of

[1] E. Degerman, C. Smith, P. Belfrage, and V. Manganiello, unpublished observation.

cAMP PDE (and perhaps inhibition of adenylate cyclase) might decrease cAMP sufficiently to trigger a reduction in A-kinase to a new steady state, which results in a reduction in lipase activity and inhibition of lipolysis. Thus, in both instances, activation of the adipocyte particulate cAMP PDE may be functionally coupled to regulation of A-kinase activity and a specific A-kinase-regulated effector system (hormone-sensitive triglyceride lipase).

Acknowledgments

We thank Dr. M. Vaughan for support and helpful discussions and Mrs. B. Mihalko for excellent secretarial assistance; Ms. Eva Degerman and Dr. Per Belfrage (University of Lund, Sweden) for help with initial PDE experiments and discussions of these studies; Ms. Vivien Kao, Mr. Andrew Kim, and Mr. Thomas Webb for careful technical assistance; Dr. John J. Egan and Dr. C. Londos (National Institute of Diabetes and Diseases of the Kidney, National Institutes of Health) for providing us with details of the kinase assay before publication and for many helpful discussions; and Dr. Ian Simpson and Ms. Mary-Jane Zarnowski (National Institute of Diabetes and Diseases of the Kidney) for generously providing us with test lots of insulin, collagenase, and BSA, advice on adipocyte preparation, and discussion of the present studies.

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