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Increasing cAMP attenuates activation of mitogen-activated protein kinase

(ERK1/ERK2/insulin/forskolin/adipocyte)

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ABSTRACT **Activation of the mitogen-activated protein kinase (MAP kinase) isoforms ERK1 and ERK2 was investigated in rat adipocytes. Kinase activities were measured by using myelin basic protein as substrate after the isoforms were resolved by Mono Q chromatography or by immunoprecipitation with specific antibodies. Insulin increased the activity of both isoforms by 3- to 4-fold. The β-adrenergic agonist isoproterenol was without effect in the absence of insulin but markedly reduced the increases in ERK1 and ERK2 activities produced by the hormone. MAP kinase activation was also attenuated by forskolin and glucagon, which increase intracellular cAMP, and by dibutyryl-cAMP, 8-bromo-cAMP, and 8-(4-chlorophenylthio)-cAMP. Thus, increasing cAMP is associated with decreased activation of MAP kinase by insulin. Forskolin also inhibited activation of MAP kinase by several agents (epidermal growth factor, phorbol 12-myristate 13-acetate, and okadaic acid) that act independently of insulin receptors. Moreover, forskolin did not inhibit insulin-stimulated tyrosine phosphorylation of the insulin receptor substrate IRS-1. Therefore, the inhibitory effect on MAP kinase did not result from compromised functioning of the insulin receptor. The inhibitory effect was not confined to adipocytes, as forskolin and dibutyryl-cAMP inhibited the increase in MAP kinase activity by phorbol 12-myristate 13-acetate in wild-type CHO cells. In contrast, these agents did not inhibit MAP kinase activity in mutant CHO cells (line 10248) that express a cAMP-dependent protein kinase resistant to activation by cAMP. Our results suggest that activation of cAMP-dependent protein kinase represents a general counter-regulatory mechanism for opposing MAP kinase activation.**

Mitogen-activated protein kinase (MAP kinase) was first described as a microtubule-associated protein 2 kinase that was rapidly activated by insulin (1). cDNA cloning of MAP kinase led to the identification of three isoforms, ERK1, ERK2, and ERK3 (2), and to the discovery that the MAP kinases are homologous to KSS1 and FUS3 (3), two kinases that are intermediates in the response of *Saccharomyces cerevisiae* to α mating factor. Phosphorylation of ERK1 and ERK2 in two sites (one a threonine and one a tyrosine residue) is necessary for kinase activity (4, 5). Relatively little is known about the properties of ERK3.

MAP kinase is now known to be activated in response to a large number of mitogenic stimuli, and the enzyme is believed to be a key participant in the response to growth factors (see refs. 6–8). The kinase phosphorylates the transcription factors c-Myc (9) and c-Jun (10) and activates the protein-serine/threonine kinase Rsk-2 (11). In skeletal muscle the protein phosphatase that activates glycogen synthase, PP1G, is phosphorylated and apparently activated in response to insulin (12). The kinase catalyzing phosphorylation of PP1G is very similar, if not identical, to Rsk-2 (13). Thus MAP kinase has been implicated in the stimulation of glycogen synthesis by insulin, one of the most important actions of the hormone.

In the present report, we demonstrate that the activation of MAP kinase by insulin in rat adipocytes is inhibited by the β-adrenergic agonist isoproterenol. Moreover, the results presented indicate that the inhibitory effect involves activation of cAMP-dependent protein kinase (protein kinase A, PKA) and represents a general mechanism for attenuating MAP kinase activation. Given the central role of MAP kinase in mediating the actions of numerous hormones, growth factors, and mitogens, cAMP-dependent inhibition of the enzyme may be of broad physiological importance.

MATERIALS AND METHODS

Incubation of Adipocytes. Adipocytes were isolated by incubating rat (Sprague–Dawley, 180–220 g) epididymal adipose tissue with collagenase (*Clostridium histolyticum*, Worthington, CLS1 lot no. M1C271) (14), as described (15). Cells were suspended (1 g of cells per 10 ml of medium) in medium composed of buffer A (135 mM NaCl/5.4 mM KCl/1.4 mM $CaCl_2$/1.4 mM $MgSO_4$/0.2 mM NaP_i/10 mM Hepes, pH 7.4) containing 3% bovine serum albumin (fraction V, Sigma, lot no. 58F0581). After incubation at 37°C with insulin and other agents being tested, the cells were centrifuged at 500 × *g* for 15 sec and the medium was aspirated. The incubations were terminated in one of two ways. For detecting phosphotyrosine, cells were homogenized directly in SDS sample buffer (90°C). For measuring MAP kinase activity, buffer B [20 mM EGTA/15 mM $Mg(OAc)_2$/1 mM dithiothreitol/40 mM *p*-nitrophenyl phosphate/0.1 mM phenylmethylsulfonyl fluoride, 1 mM/20 mM Hepes, pH 7.0, with leupeptin (10 μg/ml) and aprotinin (10 μg/ml)] (0°C) was added and the cells were homogenized with a glass tissue grinder and Teflon pestle. The homogenates were centrifuged at 10,000 × *g* for 20 min to remove triacylglycerol and then at 100,000 × *g* for 45 min. The high-speed supernatants were retained for analysis.

Cultured Cells. Wild-type CHO cells (line 10001) and CHO cells which express a PKA resistant to activation by cAMP (CHO cell line 10248) (16) were obtained from Michael Gottesman (National Institutes of Health). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM)/10% fetal bovine serum. Confluent cells were incubated at 37°C in buffer A/5 mM glucose/0.1% bovine serum albumin (crystalline, Sigma) for 30 min before treatment with the appropriate agents. Cells were scraped from the culture dishes (60-mm diameter) with a rubber policeman and then homog-

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Abbreviations: Bt_2cAMP, dibutyryl-cAMP; EGF, epidermal growth factor; IRS-1, insulin receptor substrate 1; MAP kinase, mitogen-activated protein kinase; MBP, myelin basic protein; PMA, phorbol 12-myristate 13-acetate; PKA, protein kinase A.

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enized in 1.5 ml of buffer B. Homogenates were centrifuged at 100,000 × *g* for 45 min, and the supernatants were retained.

Immunoprecipitation of MAP Kinase Isoforms. Antibodies against ERK1 and ERK2 were generated by immunizing rabbits with synthetic peptides having sequences derived from the carboxyl termini of the respective enzymes. The ERK1 peptide was Cys-Gln-Glu-Thr-Ala-Arg-Phe-Gln-Pro-Gly-Ala-Pro-Glu-Ala-Pro. The ERK2 peptide was Cys-Ile-Phe-Glu-Glu-Thr-Ala-Arg-Phe-Gln-Pro-Gly-Tyr-Arg-Ser. The peptides were coupled to keyhole limpet hemacyanin in a two-step reaction by using the bifunctional reagent *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (17). Rabbits were immunized with a 1:1 emulsion of Freund's complete adjuvant and peptide conjugate and were given booster injections at monthly intervals with 1:1 emulsions prepared with incomplete adjuvant. Sera were harvested 1 week after the second booster injection. Antibodies were affinity-purified by using columns prepared with the respective peptides. The peptides were coupled to solid support (10 mg of peptide per ml of resin) by using Sulfolink reagent as directed by the supplier (Pierce).

For immunoprecipitation, affinity-purified antibodies or nonimmune IgG were coupled to protein G-Sepharose beads (Bio-Rad, 0.5 μg of antibodies per μl of beads) by incubation at 23°C for 60 min in phosphate-buffered saline (PBS: 140 mM NaCl/5.4 mM KCl/10 mM NaP_i, pH 7.4). The beads were then washed five times with PBS and once with buffer C (1 mM EDTA/1 mM dithiothreitol/0.15 mM Na_3VO_4/50 mM β-glycerophosphate, pH 7.3). Samples (30 μl) of extract or column fractions were incubated with beads (10 μl) for 30 min at 23°C with constant mixing. The beads were then washed twice and suspended in 10 μl of buffer C before MAP kinase activity was measured.

Anion-Exchange Chromatography. Extracts (2 ml) were diluted with water (5 ml) and applied at 21°C to a Mono Q HR 5/5 FPLC column (Pharmacia) equilibrated with buffer C. The flow rate was maintained at 1 ml/min and fractions were collected each minute. Proteins were eluted with the following gradient of NaCl: 0 mM for 10 min, 0–60 mM in 5 min, 60–180 mM in 40 min, 180–240 mM in 5 min, and 240–800 mM in 10 min.

Measurements of MAP Kinase Activity. MAP kinase activity was determined by using [γ-^{32}P]ATP and myelin basic protein (MBP) as substrates (18). The reaction was initiated by adding to 10 μl of sample (extract or column fraction) an assay mixture (20 μl) containing 75 mM β-glycerophosphate, 0.45 mM EDTA, 10.5 mM NaF, 15 mM $MgCl_2$, 2 mM dithiothreitol, 0.66 μM PKA-inhibitory peptide (19), MBP at 0.5 mg/ml, and 22.5 μM [γ-^{32}P]ATP (5–20 cpm/fmol). When soluble samples were analyzed, the reactions were terminated after 20 min at 30°C by spotting 15 μl of the reaction mixtures onto phosphocellulose papers (1 × 2 cm, Whatman P81) and immersing the papers in 175 mM phosphoric acid (20). When immunoprecipitated enzymes were analyzed, 10 μl of beads was added to 20 μl of an assay mixture having the same composition as that described above, except that the $MgCl_2$ and [γ-^{32}P]ATP concentrations were 22 mM and 5 μM, respectively. The reactions were terminated by adding 10 μl of 0.1 M EDTA (pH 7.0). After centrifugation at 12,000 × *g* for 5 min to pellet the beads, samples (20 μl) of the supernatants were spotted onto papers. Papers were washed five times with phosphoric acid and then dried under a heat lamp. The amount of ^{32}P incorporated into MBP was determined by scintillation counting.

Electrophoretic Analyses. Samples were subjected to electrophoresis in the presence of sodium dodecyl sulfate (SDS) by the method of Laemmli (21). Proteins were electrophoretically transferred to nitrocellulose sheets (Schleicher & Schuell) (22). For detection of ERK1 and ERK2, the sheets were immersed in PBS containing 5% powdered milk (Carnation). Sheets were then incubated with the appropriate antibody (1 μg/ml) and washed as described (15). Antibody binding was detected by enhanced chemiluminescence (ECL kit, Amersham) with donkey anti-rabbit IgG conjugated to horseradish peroxidase (1:3000 dilution). For detection of species containing phosphotyrosine, nitrocellulose sheets were immersed in PBS/1% bovine serum albumin and then incubated with RC20 (1:5000 dilution), a recombinant antibody based on PY20 (23), that is directly coupled to horseradish peroxidase. Antibody binding was detected after the sheets were incubated and washed as instructed by the supplier of RC20 (Transduction Laboratories, Lexington, KY).

RESULTS

Resolution of MAP Kinase Isoforms ERK1 and ERK2 by Anion-Exchange FPLC. Two major peaks of MBP kinase activity were observed when extracts of rat adipocytes were fractionated by Mono Q chromatography (Fig. 1*A*). Insulin increased the sizes of the peaks by approximately 3-fold

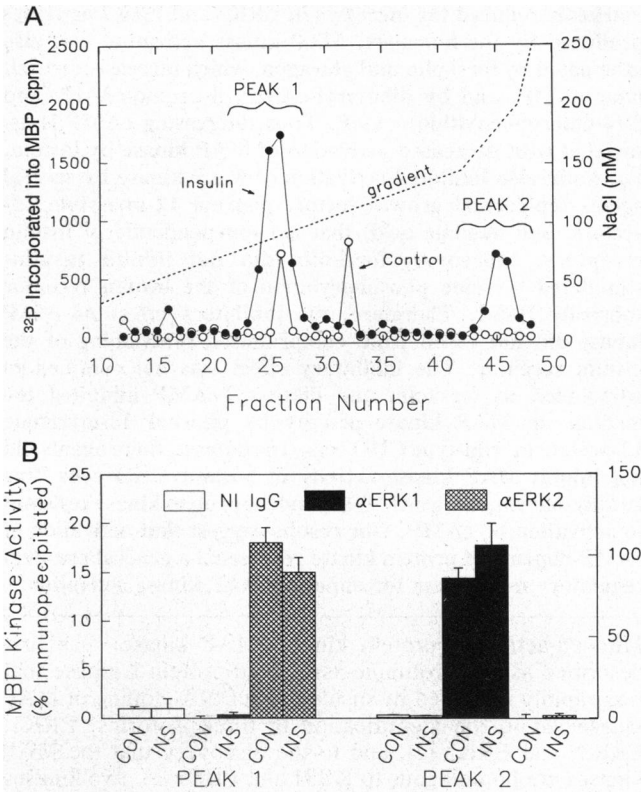

FIG. 1. Resolution of the MAP kinase isoforms ERK1 and ERK2 by Mono Q FPLC. Adipocytes (3 g per treatment) were incubated at 37°C for 5 min in the absence or presence of insulin (2.5 milliunits/ml) before homogenization. After centrifugation at 100,000 × *g* for 45 min, samples of the supernatant were diluted 1:2.5 with water and applied to a Mono Q column, and MBP kinases were eluted with an increasing gradient of NaCl. (*A*) MBP kinase activity was measured in 10-μl samples of 1-ml column fractions by using [γ-^{32}P]ATP and MBP as substrates. (*B*) Peak fractions of MBP kinases (peaks 1 and 2 in Fig. 1) from control (CON) and insulin-stimulated (INS) cells were pooled, and samples (30 μl) were incubated at 23°C for 30 min with protein G-Sepharose (10 μl) to which nonimmune rabbit IgG (NI IgG, 5 μg), ERK1 antibody (αERK1, 5 μg), or ERK2 antibody (αERK2, 5 μg) had been bound. Other samples were incubated at 23°C for 30 min without beads. The beads were collected by centrifugation and washed twice before the kinase activities were measured by using [γ-^{32}P]ATP and MBP. Results represent the percentages of MBP kinase activities recovered in the beads (mean ± SEM from three immunoprecipitations).

(peak 1) and 10-fold (peak 2). In extracts, by comparison, MBP kinase activity was increased by only 50% after adipocytes were incubated with insulin (results not shown). After Mono Q chromatography a relatively large peak of activity was observed between peak 1 and peak 2 in samples from control cells. This peak of activity was abolished by insulin treatment. Thus, the presence of insulin-inhibited MBP kinase activity explains, at least in part, the relatively small insulin-dependent increase in MBP kinase activity in whole extracts.

To identify the kinases present in peaks 1 and 2, immunoprecipitations were conducted with isoform-specific antibodies (Fig. 1*B*). Activity in peak 1 was partly immunoprecipitated with ERK2 antibody, but no activity in this peak was recovered with ERK1 antibody. In contrast, essentially all of the MBP kinase activity in peak 2 was recovered with ERK1 antibody, but no activity was immunoprecipitated with ERK2 antibody. Activity in neither peak was immunoprecipitated when nonimmune IgG was used. These results indicate that peaks 1 and 2 represent ERK2 and ERK1, respectively.

Inhibition of Insulin-Stimulated MAP Kinases by Isoproterenol and Dibutyryl-cAMP (Bt_2cAMP). Incubation of adipocytes with isoproterenol alone did not affect the amount of MBP kinase activity recovered in either peak 1 or peak 2 (Fig. 2). However, isoproterenol reduced the insulin-dependent increases in peaks 1 and 2 by approximately 60% and 90%, respectively. Thus, activation of β-adrenergic receptors markedly attenuated activation of MAP kinase by insulin. Bt_2cAMP alone was without effect on MBP kinase activity in peaks 1 and 2 but reduced the insulin-stimulated increases in both peaks by ≈50%, suggesting that increasing cAMP opposes the activation of MAP kinase isoforms by insulin.

To investigate further the inhibition of MAP kinase activity, ERK1 activity was measured after immunoprecipitation. Incubating cells with either isoproterenol or Bt_2cAMP inhibited insulin-stimulated ERK1 activity by 30–40% (Table 1), confirming the observations made when Mono Q was used to resolve the isoforms (Fig. 2). 8-bromo-cAMP and 8-(4-chlorophenylthio)-cAMP inhibited basal and insulin-stimulated ERK1 activities by 40–50%, as did forskolin and glucagon, two agents that increase cAMP in adipocytes (24, 25). In contrast, 1,9-dideoxyforskolin, which is inactive in stimulating adenylate cyclase, had little effect on ERK1 activity.

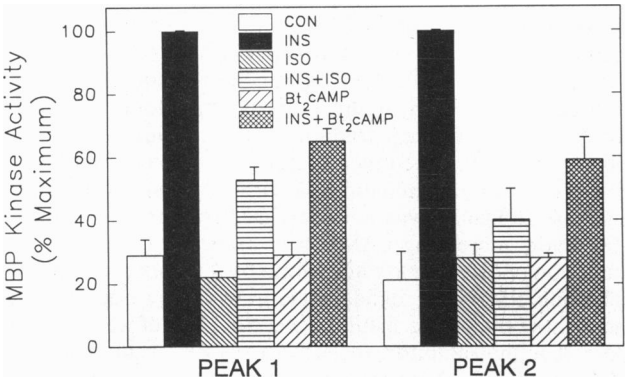

FIG. 2. Effects of isoproterenol and Bt_2cAMP on MBP kinase activity in peaks 1 and 2. Adipocytes (3 g per treatment) were incubated at 37°C without (control, CON) or with insulin (2.5 milliunits/ml) (INS), 1 μM isoproterenol (ISO), or the combination of insulin plus isoproterenol for 5 min. For Bt_2cAMP treatment cells were incubated for 15 min before a second 5-min incubation with or without insulin. The cells were homogenized and extracts were prepared and subjected to Mono Q chromatography. The amounts of MBP kinase activity in peaks 1 and 2 were then determined. Insulin-stimulated activities in peaks 1 and 2 were 12.0 ± 3.0 and 4.9 ± 2.5 pmol/min per g of cells, respectively. Results are expressed as a percentage of the maximum (insulin-stimulated) values of the respective peaks (mean ± SEM of three to five experiments).

Table 1. Inhibitory effects of cAMP derivatives and agents that increase cAMP on the ERK1 isoform of MAP kinase

Addition	% inhibition of ERK1	
	− insulin	+ insulin
Isoproterenol (1 μM)	39 ± 14	30 ± 10
Glucagon (0.1 μM)	40 ± 12	26 ± 8
Forskolin (100 μM)	50 ± 6	55 ± 5
Dideoxyforskolin (100 μM)	−3 ± 27	15 ± 6
Bt_2cAMP (5 mM)	36 ± 7	34 ± 2
8-Bromo-cAMP (5 mM)	40 ± 7	53 ± 11
8-(4-Chlorophenylthio)-cAMP (2 mM)	49 ± 5	42 ± 1

Adipocytes were incubated with the indicated additions in either the absence or the presence of insulin (2.5 milliunits/ml) for 5 min. ERK1 activity was measured after immunoprecipitation. Insulin increased ERK1 activity by 295 ± 21% in the experiments presented. Results represent percent inhibition of ERK1 activity produced by the additions (mean ± SEM of three experiments).

cAMP-Dependent Inhibition of MAP Kinase Activation Occurs Downstream of the Insulin Receptor. As previously observed (26, 27), incubation of adipocytes with insulin dramatically increased the tyrosine phosphate content of a number of proteins (Fig. 3). Species of particular note are the M_r 90,000 β subunit of the insulin receptor and IRS-1, a M_r 185,000 species which is thought to be the major insulin receptor substrate in cells (28). Insulin also stimulated tyrosine phosphorylation of species having mobilities identical to that of ERK1 or ERK2, although the intensities of these species were considerably less than that of the insulin receptor or IRS-1. Incubating cells with forskolin alone had little effect on the levels of phosphotyrosine detected in the various proteins. In the presence of insulin, forskolin had little effect on labeling of bands corresponding to ERK1 and

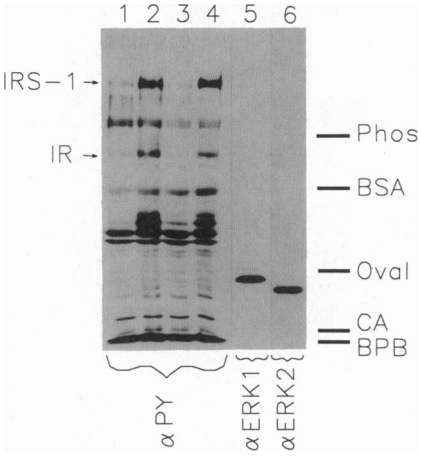

FIG. 3. Relative levels of phosphotyrosine in proteins from adipocytes treated with insulin and forskolin. Adipocytes were incubated for 5 min without additions (lane 1), with insulin (2.5 milliunits/ml) (lanes 2, 5, and 6), with 100 μM forskolin (lane 3), or with the combination of insulin plus forskolin (lane 4). The cells were then homogenized in SDS sample buffer that had been heated to 90°C. Samples were subjected to SDS/PAGE using a 7.5% acrylamide resolving gel. After electrophoresis, proteins were transferred to nitrocellulose and phosphotyrosine-containing proteins were detected by using the RC20 antibody (lanes 1–4). Map kinase isoforms were identified by using ERK1 (lane 5) and ERK2 (lane 6) antibodies. Positions of insulin receptor substrate 1 (IRS-1) and insulin receptor (IR) are indicated. Size markers were phosphorylase (Phos), bovine serum albumin (BSA), ovalbumin (Oval), carbonic anhydrase (CA), and bromophenol blue (BPB).

ERK2 but decreased the phosphotyrosine content of several other species, including the insulin receptor and a species migrating midway between the bovine serum albumin and ovalbumin standards (Fig. 3). No decrease in the phosphotyrosine content of IRS-1 was observed.

The ability of forskolin to inhibit MAP kinase activity in the presence of agents that do not signal through the insulin receptor was investigated. Cells were incubated with epidermal growth factor (EGF), phorbol 12-myristate 13-acetate (PMA), and okadaic acid, three agents previously shown to activate MAP kinase (6). EGF was much more effective than insulin in increasing ERK1 and ERK2 activities (Fig. 4). Forskolin reduced the effects of EGF on the MAP kinase isoforms by >75%. Forskolin also markedly attenuated the increases in ERK1 and ERK2 produced by okadaic acid, an inhibitor of protein phosphatases 1 and 2a (29), and by PMA, a protein kinase C activator (30).

Inhibition of MBP Kinase Activity in CHO Cells. Inhibition of MAP kinase activity by increasing cAMP was not confined to adipocytes. In normal CHO cells, both Bt_2cAMP and forskolin inhibited the stimulation of MAP kinase activity in response to PMA by >50% (Fig. 5). In contrast, no inhibition was observed in CHO cells of line 10248. These cells express a mutant regulatory subunit of PKA that binds cAMP with lower affinity than the wild-type subunit (16). Consequently, ≈10 times higher concentrations of cAMP are required to activate PKA in 10248 cells than in normal CHO cells.

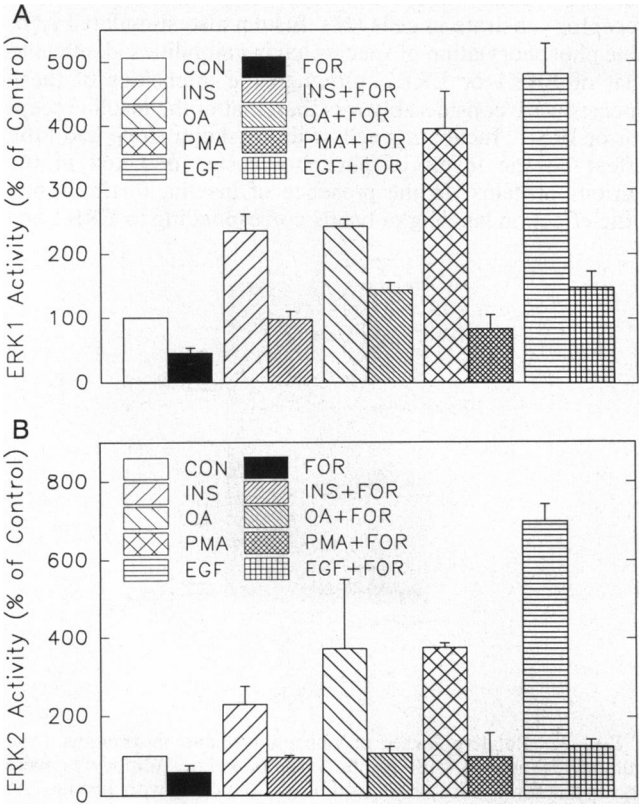

FIG. 4. Forskolin inhibition of ERK1 activation by insulin, EGF, PMA, and okadaic acid. Adipocytes were incubated for 5 min with no additions (control, CON) or with insulin (2.5 milliunits/ml) (INS), 100 nM EGF, 100 nM PMA, or 5 μM okadaic acid (OA). Other cells were incubated under identical conditions but with 100 μM forskolin (FOR). After 5 min, the incubations were terminated. ERK1 and ERK2 activities were measured after immunoprecipitation. ERK1 and ERK2 activities in samples from control cells were 0.46 ± 0.17 and 0.13 ± 0.07 pmol/min per g of cells, respectively. Results are expressed as percentages of the respective control activities (mean ± SEM from three experiments).

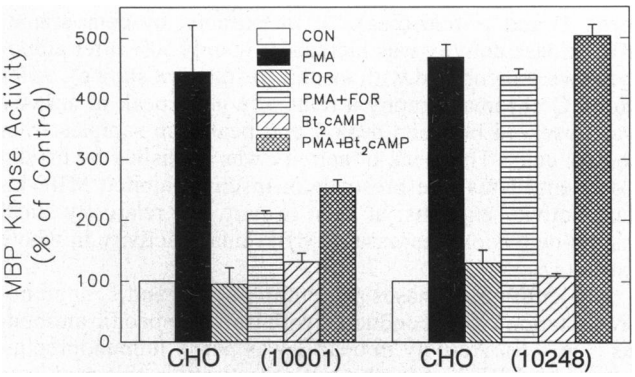

FIG. 5. Effects of forskolin and Bt_2cAMP on PMA-stimulated MAP kinase activation in CHO cells. Confluent cultures of normal (clone 10001) and mutant (clone 10248) CHO cells were incubated without additions (control, CON) or with 0.1 μM PMA, 30 μM forskolin (FOR), 5 mM Bt_2cAMP. Cells were also incubated with forskolin or Bt_2cAMP plus PMA. After 5 min the incubations were terminated and MBP kinase activity was measured in extracts. The results are expressed as the percentage of control kinase activity of the respective cell line (mean ± half the range of two experiments).

DISCUSSION

In the present report several lines of evidence have been presented in support of the conclusion that increasing cAMP inhibits MAP kinase activation. ERK1 and ERK2 activation was inhibited by isoproterenol and glucagon, two agents that increase intracellular cAMP by activating receptors coupled to adenylate cyclase (Fig. 2 and Table 1). Moreover, marked inhibition was observed with forskolin (Figs. 4 and 5), which directly activates adenylate cyclase (31), and with several cAMP derivatives (Table 1 and Fig. 2). Finally, forskolin and Bt_2cAMP inhibited PMA-stimulated MAP kinase activity in normal CHO cells, but not in CHO cells expressing a PKA resistant to activation by cAMP (Fig. 5).

The findings in CHO cells also support the conclusion that the inhibition of MAP kinase is mediated by PKA. There is no evidence that PKA directly phosphorylates and inactivates MAP kinase; however, insulin receptors may be phosphorylated *in vitro* by PKA, resulting in decreased tyrosine kinase activity (32). Also, increasing cAMP in IM-9 lymphoblasts increased the serine/threonine phosphorylation of the receptor and decreased its tyrosine kinase activity (33). The phosphotyrosine content of the receptor β subunit was decreased by forskolin in the present experiments (Fig. 3). However, only a small fraction of the insulin receptors in adipocytes need to be functional to produce a full biological effect (34), and stimulation of IRS-1 phosphorylation in response to insulin was not decreased by forskolin (Fig. 3). Moreover, increasing cAMP was associated with marked inhibition of MAP kinase activation by a number of agonists, including PMA and okadaic acid, which presumably act independently of the insulin receptor. Therefore, it seems clear that the inhibitory effects of cAMP on activation of ERK1 and ERK2 occur after the receptor in the signal transduction pathway.

The intervening steps between the receptor and MAP kinase have not been fully defined. MAP kinase is phosphorylated and activated by MEK (35, 36), which in turn appears to be activated by either of two kinases, Raf-1 (37) or MEKK (38). There is increasing evidence that Ras mediates the actions of growth factors that signal via receptor protein-tyrosine kinases (39, 40), and recent findings link Ras to the activation of Raf-1 and MEK (41). Elements of this signaling pathway appear to have been conserved across evolution, as Ras and homologues of each of the kinases, save Raf-1, have been identified in *S. cerevisiae* (42). Interestingly, increased

cAMP in yeast is associated with increased phosphorylation of CDC25, the guanine nucleotide exchange factor (43). Phosphorylation appears to promote relocation of CDC25 to the cytoplasm, where it cannot activate Ras (42). It will be interesting to determine whether such a mechanism is operative in mammalian cells, since inhibition of Ras activation would presumably decrease the activity of the downstream kinases, including ERK1 and ERK2.

Although the mechanism by which cAMP opposes activation of MAP kinases is undefined, the present findings indicate that increasing cAMP inhibits the action of a variety of agonists in adipocytes and of PMA in CHO cells. We recently learned that Graves, Krebs and coworkers have observed cAMP-mediated inhibition of MEK and MAP kinase activation by platelet-derived growth factor in human aortic smooth muscle cells (personal communication; see also ref. 44, which immediately precedes this paper). Taken together, these findings indicate that increasing cAMP is a general counter-regulatory mechanism for attenuating the MAP kinase activation pathway.

Note Added in Proof: E. Van Obberghen and coworkers have recently observed activation of MAP kinase in response to increased cAMP in PC12 cells (personal communication). Thus, attenuation of the MAP kinase pathway by cAMP appears to be dependent on cell type.

We thank Drs. Lee Graves and Edwin Krebs for providing details of their unpublished work on MAP kinase regulation by platelet-derived growth factor and forskolin in smooth muscle cells. We thank Dr. Timothy Haystead for information concerning the chromatographic behavior of adipocyte MAP kinases, Dr. Melanie Cobb for providing antibodies to ERK2, and Ms. Mary Fang for technical assistance. This work was supported in part by National Institutes of Health Grant DK28312 and by the Washington University Diabetes Research and Training Center.

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