Activation of cAMP-guanine exchange factor confers PKA-independent protection from hepatocyte apoptosis

Kimberly A. Cullen, John McCool, M. Sawkat Anwer, and Cynthia R. L. Webster. Activation of cAMP-guanine exchange factor confers PKA-independent protection from hepatocyte apoptosis. Am J Physiol Gastrointest Liver Physiol 287: G334–G343, 2004. First published March 25, 2004; 10.1152/ajpgi.00517.2003.—cAMP has previously been shown to promote cell survival in a variety of cell types, but the downstream signaling pathway(s) of this antiapoptotic effect is unclear. Thus the role of cAMP signaling through PKA and PKA-regulated guanine nucleotide exchange factors (cAMP-GEFs) in cAMP’s antiapoptotic action was investigated in the present study. cAMP’s protective effect against bile acid-, Fas ligand-, and TNF-α-induced apoptosis in rat hepatocytes was largely unaffected by the selective PKA inhibitor, Rp-8-(4-chlorophenylthio)-cAMP (Rp-cAMP). In contrast, a novel cAMP analog, 8-(4-chlorophenylthio)-cAMP, which activated cAMP-GEFs in hepatocytes without activating PKA, protected hepatocytes against apoptosis induced by bile acids, Fas ligand, and TNF-α. The role of cAMP-GEF and PKA on activation of Akt, a kinase implicated in cell survival signaling, was investigated. Inhibition of PKA with Rp-cAMP had no effect on cAMP-mediated Akt phosphorylation, whereas cAMP-2-Me-cAMP, which did not activate PKA, induced phosphatidylinositol 3-kinase (PI3-kinase)-dependent activation of Akt. Pretreatment of hepatocytes with the PI3-kinase inhibitor, Ly-294002, prevented cAMP-2-Me-cAMP’s protective effect against bile acid and Fas ligand, but not TNF-α-mediated apoptosis. Glucagon, CPT-cAMP, and CPT-2-Me-cAMP all activated Rap 1, a downstream effector of cAMP-GEF. These results suggest that a PKA-independent cAMP/cAMP-GEF/Rap pathway exists in hepatocytes and that activation of cAMP-GEFs promotes Akt phosphorylation and hepatocyte survival. Thus a cAMP/cAMP-GEF/Rap/PI3-kinase/Akt signaling pathway may confer protection against bile acid- and Fas-induced apoptosis in hepatocytes.

bile acid apoptosis; death receptor apoptosis; phosphatidylinositol 3-kinase; Akt; Rap 1

CAMP transduces survival signals in a diverse array of cell types (18, 19, 28, 31, 36, 55, 60). In hepatocytes, cAMP protects against apoptosis induced by hydrophobic bile acids, TNF-α, and Fas ligand (11, 14, 27, 44, 55). The downstream effectors of cAMP’s antiapoptotic action have not been fully characterized. In classic signaling cascades, cAMP activates PKA, a serine threonine kinase, which in turn regulates intracellular pathways via phosphorylation of signaling intermediates (25). CAMP activation of PKA alone, however, cannot account for cAMP’s survival effect in all cell types. Whereas in neurons and gastric epithelial cells, cAMP’s antiapoptotic effect is PKA dependent (19, 28), in neutrophils and pancreatic beta cells the survival effect of cAMP is PKA independent (8, 31). In hepatocytes, cAMP-mediated survival in bile acid and TNF-α-mediated apoptosis is only partially PKA dependent (14, 27, 55). These studies suggest that the antiapoptotic effect of cAMP may be mediated primarily through PKA-independent signaling pathways in hepatocytes.

It is now well established that cAMP controls intracellular signaling by regulating proteins other than PKA (25). cAMP can bind to a novel class of guanine nucleotide exchange factors (GEFs) known as exchange proteins regulated by cAMP (Epacs) or cAMP-GEFs (3, 4). These cAMP-GEFs link cAMP production to activation of small GTPases, Rap 1 and Rap 2. Rap GTPases, members of the Ras subfamily of GTP binding proteins, exist in an inactive GDP-bound form and an active GTP-bound conformation. GEFs catalyze the release of GDP allowing GTP to bind. In the active GTP-bound state, Rap interacts with target proteins to promote cellular responses.

The biological significance of cAMP-mediated Rap activation is beginning to emerge (3, 4). Recent studies (22, 30) show that Rap activation accounts for cAMP-regulated secretion of insulin and amyloid precursor protein from pancreatic beta cells and neurons, respectively. In addition to its role in exocytosis, Rap activation plays a pivotal role in mediating inside-out integrin signaling and in this context modulates lymphocyte adhesion and migration in response to integrin binding (24, 42). cAMP-mediated Rap activation also potentiates the response to mitogenic stimuli in thyroid follicular cells and promotes neurite outgrowth (6, 52).

Several studies suggest that a cAMP-GEF/Rap pathway may mediate cell survival. Selective activation of cAMP-GEF in pancreatic beta cells protects against free fatty acid-induced apoptosis in a pancreatic beta cell line (26). Genetic deletion of Rap 1 is embryonic lethal in Drosophila and mice and makes it difficult to establish viable mammalian and slime mold cell lines (4, 23). Rap activation is necessary for growth factor-mediated survival in hematopoietic cells (49) and mediates intracellular trafficking of survival receptors in neurons (2, 35, 61). These studies made it logical to hypothesize that the PKA-independent antiapoptotic effects of cAMP may be linked to cAMP-GEF/Rap activation.

Two protein kinases linked to hepatocyte survival, ERK and Akt, are proposed downstream effectors of Rap. Depending on cell types studied, Rap-GTP can activate or inhibit Akt or ERK (3–5, 29). The role of Rap in modulating these kinases in hepatocytes remains uncharacterized. It is known that cAMP
can activate Akt and inhibit ERK in hepatocytes (12, 14, 27, 48, 55, 56, 58). In hepatocytes, ERK inhibition has been reported to be both a PKA-dependent and -independent event (14, 48), although in nonhepatic cells, cAMP modulation of ERK is PKA dependent (9). The role of PKA in Akt activation in hepatocytes has not been well characterized.

Modulation of both the Akt and ERK signaling pathways has been implicated in hepatocyte survival (16, 41, 50, 55, 57). Several studies have demonstrated that cAMP activates phosphatidylinositol 3-kinase (PI3-kinase)/Akt (10, 21, 40), and the results of our work show that this activation is necessary for the survival effect of cAMP in bile acid-induced hepatocyte apoptosis (58). In our previous studies (55, 57), we demonstrated that ERK inhibition had a weak protective effect in bile acid-induced hepatocyte apoptosis; however, other studies (41) demonstrate that growth factor-mediated ERK activation is cytoprotective.

In the present study, the role of cAMP-GEF and PKA activation in cAMP’s antiapoptotic action in hepatocytes and in cAMP’s modulation of ERK and Akt were investigated. These studies show that cAMP-mediated protection from apoptosis induced by various stimuli is largely independent of PKA and that cAMP-induced activation of Akt and inhibition of ERK are PKA-independent and PKA-dependent events, respectively. Furthermore, a novel cAMP-GEF binding cAMP analog, which activates Rap 1 but not PKA in hepatocytes, promotes hepatocyte survival and PI3-kinase/Akt activation, but does not affect Akt activity.

**MATERIALS AND METHODS**

**Reagents.** Collagenase, workman, 8-(4-chlorophenylthio)-cAMP (CPT-cAMP), glugagon, Hoechst 33258, glycochenodeoxycholate (GCDC), actinomycin D, and all tissue culture reagents were purchased from Sigma-Aldrich (St. Louis, MO). The cAMP-GEF-specific cAMP analog CPT-2′-O-methyl (CPT-2-Me)-cAMP, the PKA inhibitor Rp-8-(4-chlorophenylthio)-cAMP (Rp-cAMP), and human Fas ligand were from Alexis Biochemical (San Diego, CA). PKA inhibitor Rp-α (7μM) and Rp-β (10μM) were from Sigma-Aldrich (St. Louis, MO). cAMP-GEF inhibitors, Ral and Rap 1, were from Upstate Biotechnology (Lake Placid, NY). Caspase 3 antibodies were from Cell Signaling Technology (Beverly, MA). Actin antibodies for Actin (120 kD), actinomycin D (25 ng/ml) and actinomycin D (200 ng/ml) were obtained from Cell Signaling Technology (Beverly, MA). Actin and caspase 3 antibodies were from Cellbiochem and Santa Cruz Biotechnology (Santa Cruz, CA), respectively.

**Primary cultures of rat hepatocytes.** Rat hepatocytes were isolated from male Wistar rats (200–250 g) as previously described (55). Animal studies were conducted in accordance with National Institutes of Health policy on Care and Use of Animals in Research and were granted approval by the university’s Institutional Animal Care and Use Committee. Hepatocytes were plated at 5 × 10^6 cells/cm^2 on 35-mm tissue culture dishes or coverslips coated with Type I rat tail collagen in MEM with l-glutamine, 100 mM insulin, and 10% heat-inactivated fetal calf serum and were incubated at 37°C in a humidified atmosphere of 5% CO₂. After 1 h was allowed for cell attachment, cultures were washed, and media without insulin or serum was added. After an additional 3 h, experiments were initiated by adding the hydrophobic bile acid GCDC (50 μM), Fas ligand (50 ng/ml), or the combination of TNF-α (25 ng/ml) and actinomycin D (200 ng/ml). Unless otherwise noted, modulators were added at the indicated concentration 30 min before adding apoptotic stimuli.

**Assessment of hepatocyte apoptosis.** Morphological evaluation of apoptotic cell death was done at various times after treatment with GCDC or TNF-α/Fas as previously described (55). Brieﬂy, coverslips were stained with Hoechst 33258 and apoptosis was evaluated with ﬂuorescent microscopy. Apoptotic cells were identiﬁed as those whose nuclear exhibits brighty stained condensed chromatin or nuclear fragmentation. Five hundred cells were counted by an observer blinded to the treatment conditions, and the number of apoptotic cells were expressed as a %total number of cells counted.

**RESULTS**

cAMP-mediated protection against bile acid-induced apoptosis is PKA independent. The speciﬁc PKA inhibitor, Rp-cAMP, was used to assess the role of PKA in cAMP cytopro-
tection (6, 13, 47). In rat hepatocytes, pretreatment with Rp-cAMP minimally reversed cAMP’s antiapoptotic effect in GCDC-induced cell apoptosis (~10%) (Fig. 1A). The role of PKA in cAMP-mediated protection in two physiologically relevant models of death receptor-mediated apoptosis mediated by Fas ligand and TNF-α was evaluated. In both cases, pretreatment with Rp-cAMP had only a marginal effect on the protective effect of cAMP (Fig. 1, B and C). Two strategies were used to verify that RP-cAMP inhibited cAMP-mediated activation of PKA: 1) direct assay of PKA and 2) inhibition of PKA-mediated phosphorylation of CREB^{ser133}. PKA activity was determined in cytosolic extracts with a commercially available kit (Promega’s SignaTECH), which utilizes a biotinylated Kemtide substrate. Specificity of this assay in rat hepatocytes was verified by using a synthetic peptide derived from the 6–22 amino acid sequence of the endogenous PKA inhibitor PKA-I. When this inhibitor was directly added to cytosolic extracts, no cAMP-stimulated PKA activity could be measured with the assay kit (data not shown). Phosphorylation of CREB was determined in whole cell lysates by immunoblotting with phospho-specific antibodies to CREB^{ser133}. Hepatocytes were treated with Rp-cAMP for 30 min before stimulation with cAMP for 15 min. In these studies, Rp-cAMP (500 μM) consistently inhibited both cAMP-stimulated PKA activity and CREB^{ser133} phosphorylation (Fig. 2). Collectively, these results suggest that cAMP’s antiapoptotic effect in hepatocytes is largely PKA independent.

cAMP activates Rap 1 in hepatocytes. Because emerging evidence suggests that cAMP-mediated activation of Rap 1 represents a PKA-independent method by which cAMP controls cell signaling (3, 4), the effect of cAMP on the Rap 1 signaling pathway in hepatocytes was investigated. Treatment of rat hepatocytes with 100 μM CPT-cAMP for varying periods of time resulted in sustained activation of Rap 1 (Fig. 3A). Treatment with glucagon (200 nM), which raises cAMP levels by binding to a G protein-coupled receptor, also activated Rap 1 in hepatocytes (Fig. 3, B and C).

The effect of a novel cAMP analog, CPT-2-Me-cAMP, in nonhepatic cells on cAMP-mediated events in hepatocytes was investigated. This analog contains a modification in the cAMP/PKA binding domain that substantially decreases the ability of the compound to activate PKA but does not affect the binding of cAMP to cAMP-GEFs that control Rap activation (6, 9, 22, 43). Cultured hepatocytes were treated with CPT-2-Me-cAMP for 15 min, and the amount of active GTP-bound Rap 1 was determined. The CPT-2-Me-cAMP analog activated Rap 1 to a degree comparable to that seen with cAMP (Table 1). The effect of this analog on PKA activity in rat hepatocytes was assessed by directly determining PKA activity in cytosolic extracts and by determination of the amount of CREB^{ser133} phosphorylation. Treatment with cAMP (100 μM) significantly increased PKA activity and CREB^{ser133} phosphorylation, whereas CPT-2-Me-cAMP had no effect on these PKA-mediated events (Table 1). Even the highest concentration of...
CPT-2-Me-cAMP tested (100 μM) failed to phosphorylate CREB ser133 and resulted in only a mild twofold increase in PKA activity (data not shown). Cumulatively, these results demonstrate that CPT-2-Me-cAMP activates Rap 1 in hepatocytes without appreciable effect on PKA activity or PKA-mediated phosphorylation events. Because this selectivity was somewhat diminished at higher concentrations, a 20 μM dose of CPT-2-Me-cAMP was used for further studies.

Because bile acids must enter hepatocytes to induce apoptosis, the effect of CPT-2-Me-cAMP on the uptake of a radiolabeled bile acid was investigated. Taurocholate accumulation in untreated hepatocytes and hepatocytes treated with 20 μM CPT-2-cAMP for 30 min. Some cultures were pretreated with 500 μM Rp-cAMP 30 min before incubation with cAMP. Cytosolic PKA activity was determined by using a biotinylated Kemtide substrate according to the manufacturer’s instructions. B: whole cell lysates were prepared from hepatocyte cultures treated for 15 min with 100 μM CPT-cAMP with or without 30-min pretreatment with 500 μM Rp-cAMP. The amount of PKA-dependent CREB phosphorylation was determined by Western blot analysis (50 μg) with phosphospecific CREB ser133 antibodies. The relative activity (means ± SD) represents the amount of phosphorylated protein compared with untreated control cultures after normalization for protein loading and is the result of 3 separate experiments. *Value is significantly different from that seen in control cells; #value is statistically different that that seen in the presence of cAMP.

CPT-2-Me-cAMP is antiapoptotic in hepatocytes. Cultured hepatocytes were pretreated with 20 μM CPT-2-Me-cAMP for 30 min before the addition of GCDC, Fas ligand, or TNF-α, and the amount of apoptosis was determined at sequential time points by morphological evaluation of Hoechst-stained cells. CPT-2-Me-cAMP protected hepatocytes from apoptosis mediated by each of the apoptotic stimuli (Fig. 4).

**Fig. 3.** cAMP activates Rap 1 in rat hepatocytes. Whole cell lysates were prepared from untreated control hepatocytes and hepatocytes treated with 100 μM CPT-cAMP or 200 nM glucagon for 15 min. Some cultures were pretreated with 20 μM Ly-294002 (Ly) for 30 min before incubation with cAMP. The amount of activated GTP-bound Rap was determined by using a pull-down assay with a glutathione-agarose conjugate of Ral GDS binding domain and detected by immunoblotting with Rap 1 antibodies. A: time course of cAMP-mediated Rap 1 activation. Rat hepatocytes were treated with 100 μM CPT-cAMP, and whole cell lysates were prepared at the indicated times for determination of the amount of Rap1-GTP. B: representative immunoblot from the experiments is shown. Positive and negative controls represent the amount of activated GTP-bound Rap 1. C: graphical depiction of the amount of Rap1-GTP present in cell lysates loaded in vitro with GTPγS and GDP, respectively. *Value is significantly different from that seen in the presence of cAMP. #Value is statistically different from that seen in the presence of cAMP. *Statistically different from the value in control cells.
CREB, cAMP response element binding protein.

METHODS. CPT-2-Me-cAMP, 8-(4-chlorophenylthio)-2‘-O-methyl-cAMP was determined. PI3-kinase inhibition pre-treated with Ly-294002 on the antiapoptotic effect of the cAMP-GEF-kinase dependent (55, 58), the effect of PI3-kinase inhibition associated cytoprotection against GCDC-induced apoptosis is PI3-kinase-dependent event in hepatocytes. Treatment with Ly-294002 had no effect on cAMP-mediated inhibition of ERK phosphorylation, whereas CPT-2Me-cAMP had no effect on ERK phosphorylation (Fig. 6C). These results show that cAMP-mediated inhibition of ERK phosphorylation is a PKA-dependent event in hepatocytes.

Table 1. Effect of cAMP analogues on PKA and Rap 1 activation

| Treatment          | Rap 1-GTP, fold increase over control | CREBser133, fold increase over control | PKA Activity, pmol·min⁻¹·μg⁻¹ | Values represent the means ± SD; n = 3 rat hepatocytes. Rat hepatocytes were treated with the indicated cAMP analog for 15 min. Cell lysates were prepared, and the amount of GTP-bound Rap, PKA activity, and the degree of CREB phosphorylation were determined as indicated in MATERIALS AND METHODS. CPT-2Me-cAMP, 8-(4-chlorophenylthio)-2‘-O-methyl-cAMP; CREB, cAMP response element binding protein.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00</td>
<td>1.00</td>
<td>0.116 ± 0.07</td>
</tr>
<tr>
<td>CPT-cAMP (100 μM)</td>
<td>3.07 ± 0.36</td>
<td>6.2 ± 0.45</td>
<td>1.28 ± 0.34</td>
</tr>
<tr>
<td>CPT-2Me-cAMP (20 μM)</td>
<td>3.67 ± 0.43</td>
<td>1.10 ± 0.05</td>
<td>0.178 ± 0.04</td>
</tr>
</tbody>
</table>

DISCUSSION

The major aim of the present study was to determine whether the antiapoptotic effect of cAMP is mediated via PKA and/or cAMP-GEF. Results show that activation of cAMP-GEF and/or cAMP-GEF activation role of cAMP-GEF in modulation of ERK and PI3-kinase/Akt. Our previous studies (58) have implicated a role for PI3-kinase-dependent activation of Akt in cAMP-mediated survival from bile acid-induced apoptosis. We and others (10, 21, 27, 34, 58) have shown that cAMP can activate PI3-kinase and Akt in hepatocytes. To determine whether this is a PKA-mediated event, hepatocytes were pretreated with Rp-cAMP before exposure to cAMP. In cell lysates pretreated with Rp-cAMP, cAMP still increased Akt phosphorylation (Fig. 6A). In addition, treatment with CPT-2Me-cAMP resulted in phosphorylation of Akt in hepatocytes and this phosphorylation was prevented by pretreatment with Ly-29402 (Fig. 6B). Treatment with Ly-29402 had no effect on cAMP-mediated Rap activation, indicating that Rap activation by cAMP occurs upstream of PI3-kinase (Fig. 3, B and C). These results show that cAMP-mediated activation of Akt is PI3-kinase independent in hepatocytes.

Because there is conflicting evidence in the literature on the role of cAMP activation of PKA and cAMP-GEFs on ERK modulation, hepatocytes were treated with Rp-cAMP or the CPT-2Me-cAMP analog, and the effect on ERK phosphorylation was observed. RP-cAMP reversed cAMP-mediated inhibition of ERK, whereas CPT-2Me-cAMP had no effect on ERK phosphorylation (Fig. 6C). These results show that cAMP-mediated inhibition of ERK phosphorylation is a PKA-dependent event in hepatocytes.

Because we have previously shown that CPT-cAMP-mediated cytoprotection against GCDC-induced apoptosis is PI3-kinase dependent (55, 58), the effect of PI3-kinase inhibition with Ly-294002 on the antiapoptotic effect of the cAMP-GEF-specific analog was determined. PI3-kinase inhibition prevented the protective effect of CPT-2Me-cAMP in bile acid-induced apoptosis (Fig. 5A). The protective effect of CPT-2Me-cAMP was accompanied by inhibition of caspase 3 processing, which was abolished in the presence of PI3-kinase inhibition (Fig. 5B).

As with GCDC-induced apoptosis, CPT-2Me-cAMP protection from Fas ligand-induced apoptosis was dependent on PI3-kinase activation (Fig. 5, C and D). Pretreatment with Ly-294002 augmented TNF-α-induced apoptosis but had no effect on the ability of the CPT-2Me-cAMP to protect against TNF-α-mediated apoptosis (Fig. 5E and F). Similar to the results seen with the CPT-2Me-cAMP analog, PI3-kinase inhibition with Ly-2954002 blocked cAMP protection in Fas ligand-mediated apoptosis but had no effect on the protective effect of cAMP in TNF-α-mediated apoptosis (data not shown).

Role of CPT-2Me-cAMP in modulation of ERK and PI3-kinase/Akt. Our previous studies (58) have implicated a role for PI3-kinase-dependent activation of Akt in cAMP-mediated survival from bile acid-induced apoptosis. We and others (10, 21, 27, 34, 58) have shown that cAMP can activate PI3-kinase and Akt in hepatocytes. To determine whether this is a PKA-mediated event, hepatocytes were pretreated with Rp-cAMP before exposure to cAMP. In cell lysates pretreated with Rp-cAMP, cAMP still increased Akt phosphorylation (Fig. 6A). In addition, treatment with CPT-2Me-cAMP resulted in phosphorylation of Akt in hepatocytes and this phosphorylation was prevented by pretreatment with Ly-294002 (Fig. 6B). Treatment with Ly-294002 had no effect on cAMP-mediated Rap activation, indicating that Rap activation by cAMP occurs upstream of PI3-kinase (Fig. 3, B and C). These results show that cAMP-mediated activation of Akt is PI3-kinase independent in hepatocytes.
GEFs confers PKA-independent protection against hepatocyte apoptosis and results in PI3-kinase-dependent activation of Akt. In addition, the antiapoptotic effect associated with cAMP-GEF activation is PI3-kinase dependent for apoptosis induced by hydrophobic bile acids and Fas ligand but PI3-kinase independent for TNF-α-mediated apoptosis.

Results of the present study raise the possibility for the first time that the antiapoptotic effect of cAMP may be due to activation of cAMP-GEFs instead of PKA in hepatocytes. This conclusion is supported by results that show that the activation of cAMP-GEFs results in cytoprotection against apoptosis induced by bile acid, Fas, and TNF-α but is not associated with
mediated protection is partially PKA dependent in both TNF-
(27, 55) in primary hepatocytes have shown that cAMP-
CPT-cAMP is largely independent of PKA. Previous studies
activation of PKA. Moreover, the cytoprotective effect of
CPT-cAMP is largely independent of PKA. Previous studies
were separated by SDS-PAGE, transferred to nitrocellulose,
and immunoblotted with phospho-specific antibodies for
Aktser473 (A and B) or p42/p44 ERKthr202/tyr204 (C). Membranes
were reprobed with phosphorylation state-independent antibod-
ies to Akt and p42/p44 ERK. The amount of phosphorylated
kinase was quanti-
ed by using computer software, normalized
for equal protein loading, and expressed as the relative activity.
Results represent the means ± SD from 3 separate experiments.
*Statistically different from that in control cells; #statistically
different from that in cells treated with cAMP.

Fig. 6. Role of PI3-kinase and PKA in cAMP modulation of
Akt and ERK. Whole cell lysates were prepared from control
hepatocytes and hepatocytes treated for 15 min with CPT-
cAMP (100 µM) (A and C) or CPT-2-Me-cAMP (10, 20 or 50
µM) (B and C) alone or after pretreatment for 30 min with
Ly-294002 (20 nM) or Rp-cAMP (500 µM). Proteins (100 µg)
were separated by SDS-PAGE, transferred to nitrocellulose,
and immuno-

activation of PKA. Moreover, the cytoprotective effect of
CPT-cAMP is largely independent of PKA. Previous studies
(27, 55) in primary hepatocytes have shown that cAMP-
mediated protection is partially PKA dependent in both TNF-α
and bile acid-induced apoptosis. Results of these studies must
be evaluated in light of the fact that a fairly nonspeci-
fic cAMP analog protects against hepatocyte apoptosis.

In previous studies (58), we have shown that cAMP’s
antiapoptotic action in bile acid-induced apoptosis is linked to
PI3-kinase-dependent activation of Akt. Results of the present
study clearly indicate that cAMP-mediated activation of Akt is
a cAMP-GEF-dependent but PKA-independent event in hepa-
tocytes. Specific PKA inhibition in hepatocytes fails to block
cAMP-mediated Akt phosphorylation, and CPT-2-Me-cAMP
mediates PI3-kinase-dependent activation of Akt without acti-
vating PKA. cAMP Rap 1 activation via cAMP-GEFs modu-
lates Akt activity in nonhepatic cells. In some cells (WRT
thyroid cells, C6 glioma cells, HEK-293 cells), Rap activation
increases Akt phosphorylation, whereas in other cells (B lym-
phocytes, PCCl3 thyroid cells) it inhibits Akt (5, 29, 33, 38,
54). Such cell-type-specific response to cAMP is not unusual
(25). The biological basis for divergent cellular responses to
cAMP are incompletely understood but may be associated with
the relative abundance of cAMP binding proteins such as PKA
and EPAC, their subcellular distribution, or the presence of
distinct populations of downstream effectors in cAMP signal-
ing pathways.

The mechanism by which cAMP-GEF/Rap modulates PI3-
kinase/Akt activity is unknown. The related GTPase, Ras,
binds to and activates the p110α- and p110γ-catalytic subunits
of PI3-kinase (39, 46). Because Ras and Rap 1 have identical
effector binding regions (3, 4), it has been hypothesized that
Rap may bind Ras effectors such as PI3-kinase. In a recent
study (5), Rap 1 binding to the regulatory p85α subunit of
PI3-kinase was associated with inhibition of PI3-kinase/Akt
signaling. Alternatively, Rap could modulate PI3-kinase/Akt
activity by facilitating the movement of these kinases to bio-
logical membranes or act on a PI3-kinase/Akt lipid or serine/
threonine phosphatase. The mechanism by which cAMP-GEFs
activate PI3-kinase/Akt is presently the focus of attention in
our laboratory.
The PI3-kinase/Akt signaling pathway mediates hepatocyte survival from diverse stimuli (16, 37, 45, 50, 51, 55, 58). In the present study, PI3-kinase inhibition prevents cAMP-GEF’s protective effect in bile acid and Fas-mediated apoptosis. Because apoptosis induced by hydrophobic bile acids proceeds at least in part by ligand-independent activation of the Fas receptor (17), it is not surprising that the response to cAMP is similar in these models of apoptosis. These results suggest that a cAMP-GEF/Rap 1/PI3-kinase/Akt signaling cascade may be important in cAMP survival effect in Fas receptor-mediated apoptosis. It is presently unknown how PI3-kinase/Akt signaling pathways protect against Fas/bile acid-mediated apoptosis, but previous studies (51, 53, 58) suggest that the PI3-kinase-dependent survival effect may occur at or above the level of the mitochondrial amplification cascade seen in death receptor-mediated apoptosis.

Unlike the situation in Fas-mediated apoptosis, cAMP-GEF-mediated protection from TNF-α apoptosis appears to be PI3-kinase independent (27). Our results, like that of others, do support a role for PI3-kinase in TNF-α apoptosis, because we see that PI3-kinase inhibition potentiates TNF-α-mediated cell death (37). The finding of divergent cAMP responses in different models of apoptosis is not unprecedented as the PKA and PI3-kinase dependence of cAMP’s antiapoptotic action in fibroblast and pancreatic beta cell apoptosis is stimulus dependent (8, 20, 59, 60). Thus it appears that additional PKA-independent cAMP-GEF-mediated events are involved in cAMP’s antiapoptotic actions in apoptosis mediated by TNF-α.

There is conflicting information on the role of ERK signaling in hepatocyte survival. In some studies, ERK activation is antiapoptotic (41, 45), whereas other studies suggest ERK modulation has no effect on hepatocyte apoptosis (14, 15). In the present study, cAMP inhibition of ERK is a PKA-dependent event. This observation, coupled with the finding that cAMP’s antiapoptotic effect is largely independent of PKA activation, implies that ERK inhibition does not play a large role in cAMP’s antiapoptotic effect. Previously, we have shown that ERK inhibition with PD-9059 or U-0126 results in a mild protective effect against bile acid-induced apoptosis (55, 57). It is possible that this modest protection reflects the small amount of apoptosis not reversed by PKA inhibition in this study (~10%).

In summary, the present study shows for the first time that a PKA-independent cAMP/cAMP-GEF/Rap 1 pathway exists in hepatocytes. In addition, activation of this pathway with a novel cAMP analog, CPT-2-Me-cAMP, promotes hepatocyte survival as well as PI3-kinase/Akt activation. cAMP is an important second messenger in hepatic stress hormone signaling, and increases in intracellular cAMP may represent a general means by which hepatocytes upregulate survival mechanisms during times of metabolic stress. Promotion of cell survival appears to be a common property of the cAMP-coupled glucagon receptor family as glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 promote pancreatic beta cell survival, whereas vasoactive intestinal polypeptide and pituitary adenylate cyclase activating polypeptide are antiapoptotic in baby hamster kidney cells and neurons, respectively (32). Elucidation of prosurvival cAMP signaling pathways could thus lead to the identification of potential therapeutic targets to ameliorate cell death in a diverse array of disease processes.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-02721 and DK-061950 (to C. R. L. Webster) and DK-333436 (to M. S. Anwer).

REFERENCES


