Differential Expression of Guanine Nucleotide-binding Proteins Enhances cAMP Synthesis in Regenerating Rat Liver

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Abstract

Events leading to cAMP accumulation after partial hepatectomy (PH) and effects of cAMP on hormonal induction of DNA synthesis in hepatocytes were characterized. Hepatic cAMP peaked biphasically post-PH and paralleled changes in adenylyl cyclase activity. Fluctuations in cyclase activity were not explained by variations in glucagon receptor kinetics, but reflected altered G-protein expression. Membrane levels of the stimulatory G-protein, Gs alpha, increased early after PH and were sustained. Levels of the inhibitory G-protein, Gi_2 alpha, increased more slowly, peaked later, and quickly fell. Levels of both G-proteins correlated poorly with levels of their mRNAs, suggesting posttranscriptional factors modify their membrane concentrations. When growth factor-induced DNA synthesis was compared in hepatocyte cultures grown with or without agents that increase intracellular cAMP, DNA synthesis was inhibited by sustained high levels of cAMP but was enhanced when high cAMP levels fell. In both regenerating liver and hepatocyte cultures, the expression of a "differentiated" hepatocyte gene, phosphoenolpyruvate carboxykinase, correlated with elevated cAMP levels. These data suggest that the differential expression of G-proteins integrates signals initiated by several growth factors so that the accumulation of cAMP is tightly regulated post-PH. The ensuing variations in cAMP levels modulate both growth and differentiated functions during liver regeneration. (*J. Clin. Invest.* 1992. 89:1706–1712.) Key words: G-proteins • liver regeneration • cyclic AMP

Introduction

The role of cAMP-dependent signal transduction in regulation of hepatocellular proliferation is uncertain. Hepatic concentrations of cAMP increase dramatically in the prereplicative phase of liver regeneration after partial hepatectomy (PH)[1] (1, 2). Furthermore, hepatic sensitivity to beta-adrenergic agents and glucagon, factors that induce cAMP accumulation, is heightened in such circumstances (3). Changes in the mRNA for the cAMP-dependent kinase, protein kinase A, also occur during the prereplicative phase of liver regeneration (4, 5). These data suggest that cAMP-dependent processes may modulate hepatic regenerative growth. However, direct evidence for cAMP-mediated stimulation of liver cell proliferation is scant (6). Indeed, in several nonhepatic cell lines, cAMP clearly inhibits cellular proliferation (7, 8). To clarify the role of cAMP-dependent processes in compensatory hepatic growth, we sought to characterize the cellular events that result in hepatic cAMP accumulation after PH and to determine whether similar levels of cAMP alter hormonal induction of DNA synthesis in normal adult hepatocytes.

Methods

Materials. Unless noted below, all chemicals used in these experiments were purchased from Sigma Chemical Co. (St. Louis, MO). Reagents for determination of hepatic protein content were purchased from Bio-Rad Laboratories (Richmond, CA). Guanidine isothiocyanate, sarcosyl, and agarose were obtained from Bethesda Research Laboratories (Life Technologies, Gaithersburg, MD). Phenol was purchased from Fluka (Ronkonkoma, NY). All radioactive isotopes were obtained from Amersham Corp. (Arlington Heights, IL). Nylon membranes for Northern analysis were from ICN Biomedicals, Inc. (Irvine, CA). Membranes (Immobolin-P) for Western analysis were purchased from Millipore Corp. (Bedford, MA). Kodak XAR-5 film was used for all autoradiographs. Tissue culture media (Medium 199) and type 1 collagen (Vitrogen) were purchased from Gibco Laboratories (Grand Island, NY). 60-mm Falcon culture plates came from Becton Dickinson Labware (Lincoln Park, NJ).

Primary antisera to specific G-protein subunits were raised in rabbits against synthetic peptides corresponding to defined regions of G-protein subunits (generously supplied by Dr. Janet Robishaw, Geisinger Clinic, Danville, PA). Antiserum C584, which was raised against a synthetic carboxy-terminal peptide specific for Gs alpha, recognized the 45- and 52-kD forms of Gs alpha (9). Antiserum A54, which specifically recognizes G_1 alpha-2, was raised against a synthetic peptide unique to the middle of Gi alpha-2 protein and recognized a 40-kD protein. B_2 antiserum was raised to a specific carboxy-terminal peptide of the B_2 subunit and recognized a 35-kD protein.

cDNA probes for the mRNAs of GTP-binding proteins were provided by Dr. Levine (The Johns Hopkins University, School of Medicine, Baltimore, MD) (10). The cDNA probe for phosphoenolpyruvate carboxykinase (PEPCK) was provided by Dr. Axel Kahn (Institut Cochin de Genetique Molecularie, INSERM, Paris, France) (11). cDNA for 18S was provided by Dr. Barbara Sollner-Webb, The Johns Hopkins University (12).

Animals. Adult male Sprague–Dawley rats (weight ~ 300 g) were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA).

Partial hepatectomy experiments. 64 rats were housed with a 12-h light/12-h dark cycle and permitted ad lib. access to a standard rat chow, pellet-type diet for 1 wk. At the end of this equilibration period, 12 animals underwent sham laparotomy. During this procedure, the liver was manipulated, but not resected. Sham-operated animals were killed at 1, 3, 6, or 24 h postoperatively and liver tissue was removed and saved for subsequent analysis. 52 animals underwent a 70% PH

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1. *Abbreviations used in this paper:* AC, adenylyl cyclase; IBMX, isobutylmethylxanthine; LDH, lactate dehydrogenase; PEPCK, phosphoenolpyruvate carboxykinase; PH, partial hepatectomy.

with light ether anesthesia in midmorning (8–10 a.m.) (13). The resected tissues were saved at −70°C until analysis. Oral intake was not restricted before or after surgery. Perioperative mortality was < 5%. Rats were killed at various times (1, 3, 6, 12, 24, 36, 48, and 72 h) after PH. Resected liver tissue was immediately freeze clamped in liquid nitrogen and then stored at −70°C for up to 4 wk.

Adenylyl cyclase (AC) assay and Western analysis. Liver membranes were prepared from at least four rats killed at each time point after PH. The livers were homogenized in 1 mM Tris-HCl, 1 mM EDTA, 10% sucrose (pH 7.4). The homogenates were passed through a 200-μm nylon mesh and then centrifuged for 5 min at 500 *g* to remove debris and nuclei. Supernatants were centrifuged at 170,000 *g* for 20 min and resuspended in 1 mM Tris-HCl, 1 mM EDTA (pH 7.4) (12). Aliquots were taken for protein determination (14). AC activity was assayed as previously described (11). Membranes were added at a concentration of 100 μg/ml (10 μg/assay) and assays were done in triplicate at 30°C for 20 min. AC activity was measured in the presence of 10 mM Mg^{2+} and either no activator (basal), GTP-gamma-S (10^5 M), glucagon (10^5 M), forskolin (10 μM), or $MnCl_2$ (20 mM) (15). For Western analysis, equal amounts of membrane protein (100 μg) were electrophoresed on SDS–polyacrylamide gels; electrotransferred to membranes; and probed with rabbit anti-Gs alpha, Gi_2 alpha, and beta 2 as previously described (9, 15). At least three separate experiments were performed to ensure that the findings on individual immunoblots were reproducible. The integrated optical densities of the individual bands on the autoradiograms were determined with an image analysis system (LOATS RAS-1000; Amersham Corp.) (16).

Measurement of cAMP. cAMP levels of the liver cytosols of tissues from at least three rats per time point post–sham laparotomy and post-PH were determined in triplicate by radioimmunoassay (17). Protein concentrations of these samples were determined using the technique of Bradford, as described above. Results are expressed as pmol cAMP/mg protein.

RNA isolation and Northern analysis. Total RNA was isolated from liver tissues as described by Chomczynski and Sacchi (18) and quantified by measurement of ultraviolet (UV) absorption at 260 nm. Electrophoresis on 1% agarose/6% formaldehyde gels with subsequent ethydium bromide staining was used to confirm RNA concentration and assess the degree of RNA degradation. RNA from pooled samples (three or four rats/pool) was fractionated (20 μg/lane) on 1% agarose/6% formaldehyde denaturing gels in 20 mM MOPS (3-[*N*-morpholino]propanesulfonic acid), 5 mM sodium acetate, and 1 mM EDTA buffer, pH 7.0, and transferred to nylon membranes by capillary blotting in 20× standard saline citrate (SSC; 3.0 M NaCl and 0.3 M sodium citrate, pH 7.0). After prehybridization in 50% formamide, 5× SSC, 5× Denhardt's solution, 0.1% SDS, 10 mM sodium phosphate, pH 7.3, with low molecular weight DNA at 42°C for 2 h, membranes were hybridized overnight at 42°C in the same solution containing random-primed, ^{32}P-labeled cDNA probes for Gs alpha, Gi_2 alpha, or PEPCK (sp act 10^8 cpm/μg cDNA; 10^6 cpm/ml hybridization solution). To control for slight variations in the amount of RNA applied to each lane, all blots were stripped by boiling in 0.1% × SSC, 1% SDS for 20 min and hybridized with a random-primed ^{32}P-labeled cDNA for ribosomal 18S, a message that is not affected by PH. The integrated optical densities of the individual bands on the autoradiograms were determined with a LOATS RAS-1000 Image Analysis system as described above. Autoradiograms from at least three separate Northern blots were analyzed to ensure reproducibility of the findings. For each sample, data for the specific mRNA of interest were normalized to the amount of 18S mRNA present on the blot and expressed as fold stimulation over the control value.

Primary hepatocyte culture experiments. Liver cells were isolated from adult male rats by collagenase perfusion as described by Mezey et al. (19). Rats used in these experiments were of similar age and weight and had been maintained under conditions identical to those used for the PH experiments. Hepatocytes were purified from liver cell suspensions by differential centrifugation (19). Hepatocyte viability was assessed by trypan blue staining. All preparations used in these studies had viabilities > 90%. Cells suspended in 3 ml of serum-free media were plated on collagen-coated plastic dishes at a density of 1.5×10^6 cells/60-mm plate in the presence or absence of growth factors (dexamethasone [1 μg/ml], glucagon [10^{-9} M], epidermal growth factor [10 ng/ml], and insulin [500 U/liter]) (20) and in the presence or absence of agents (10 μM 8-Br-cAMP or 100 μM isobutylmethylxanthine [IBMX] plus 1 μM forskolin) known to induce cAMP accumulation (21). 52 plates/experiment were used to test each condition. Three separate perfusion experiments were performed. In each experiment, eight plates were harvested at 24, 42, 48, 66, and 72 h and immediately homogenized in RNA lysis buffer (18) so that RNA could be isolated for subsequent Northern analysis as described above. An additional four plates for each condition were harvested after 24, 48, and 72 h of culture. 1 h before harvesting, 5 μCi (5 μl) of [^{3}H]thymidine (sp act 20 Ci/mmol) was added to three of these four plates. The remaining plate received 5 μl of buffer. At the time of harvest, media was poured from the plates and saved for subsequent analysis of lactate dehydrogenase (LDH) activity (19). Plates were rinsed twice with 3 ml of cold Hanks' buffer and then scraped from the plate in 2 ml of TE buffer. DNA was subsequently isolated from the cell pellet of the three radioactive plates and quantitated by its absorbance at 260/280 nm (22). The tritium radioactivity of an aliquot of this isolated DNA was determined so that [^{3}H]thymidine incorporation could be expressed/μg of DNA. cAMP and protein concentrations were measured in the cells scraped from the nonradioactive plates.

Statistical analysis. Data are expressed as the means±SEM and have been analyzed by analysis of variance. $P < 0.05$ is considered significant.

Results

As shown in Fig. 1, cAMP levels peak biphasically after PH. cAMP rapidly accumulates in the liver during the first 6 h after PH. Levels then fall toward pre-PH values by 24 h before gradually increasing again during the next 48 h post-PH. These variations in hepatic cAMP require hepatic resection, since hepatic cAMP concentrations did not increase during the initial 24 h after sham laparotomy (data not shown).

Changes in hepatic cAMP concentration post-PH closely parallel changes in liver AC activity. This is particularly true for glucagon-stimulated AC activity but is also true for AC activity after GTP-gamma-s stimulation (Fig. 2, *A* and *B*). Similar changes in AC activity occur in response to manganese or forskolin (data not shown).

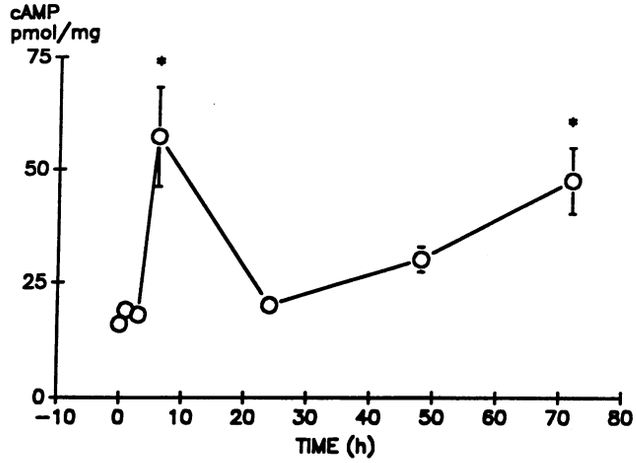

Figure 1. Hepatic cAMP concentration after PH. cAMP concentrations were determined in liver homogenates from at least three rats before (time 0) and at each of various time points after PH. *P < 0.005 vs. time zero cAMP concentration.

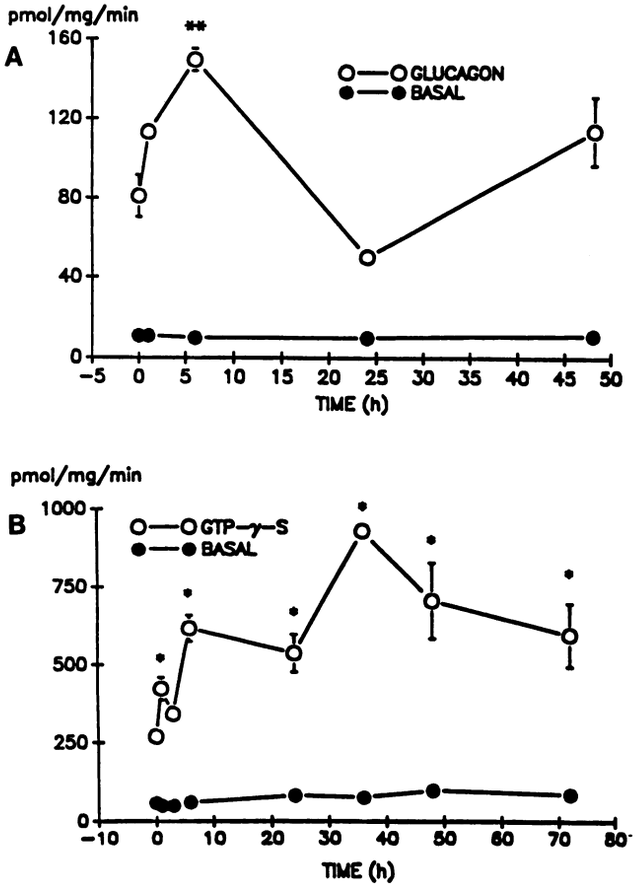

Figure 2. Basal and stimulated AC activity after PH. AC activity was measured in rat liver membranes isolated at various times after PH. AC activity was determined in vitro in the absence of any stimulatory substance (basal activity) or in the presence of glucagon (*A*) or GTP-gamma-S (*B*) to stimulate cyclase activity. $^{**}P < 0.005$ vs. activity at time 0; $^{*}P < 0.02$ vs. activity at time 0.

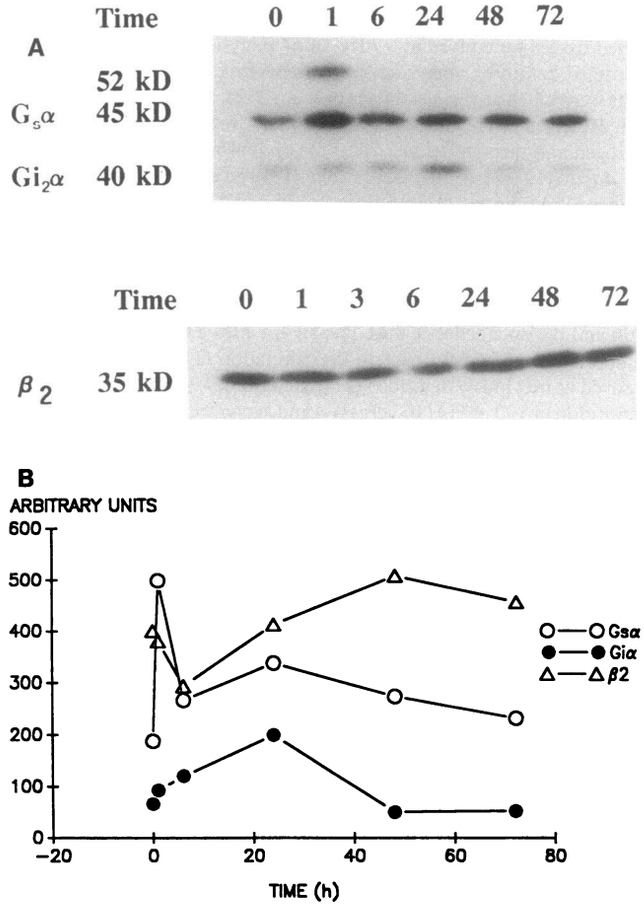

Figure 3. Altered expression of GTP-binding proteins after PH. (*A*) A representative immunoblot illustrates changes in the membrane content of the stimulatory (Gs alpha) and inhibitory (Gi_2 alpha) GTP-binding proteins and B_2 subunits. (*B*) The integrated optical densities of individual bands on autoradiograms from three separate immunoblots were determined with LOATS RAS-1000 Image Analysis System and plotted against time.

The marked increase in stimulated AC activity during the initial 6 h post-PH cannot be explained by an increase in glucagon receptors. The number and affinity of hepatic glucagon receptors is similar before and 6 h after PH, the time when in vitro stimulation with glucagon is maximal (data not shown). This suggests that postreceptor events may mediate enhanced cyclase activity during the prereplicative phase of liver regeneration.

To assess this possibility, we measured liver levels of the protein and mRNA for the stimulatory (Gs alpha) and inhibitory (Gi_2 alpha) subunits of the GTP-binding proteins, which couple plasma membrane receptors to AC. As shown in Fig. 3, *A* and *B*, the antibody to Gs alpha recognized the expected 52- and 45-kD isomers of that protein. As anticipated, anti-Gi_2 alpha bound to a 40-kD protein and anti-B_2 recognized a 35-kD band. Antibodies to the B_2 subunit were selected because liver is particularly enriched in that isoform.

Membrane levels of Gs alpha protein more than double within 1 h of PH. Gs alpha levels then fall to ~ 1.5 times pre-PH values and remain elevated within this range until 72 h post-PH. Membrane levels of Gi_2 alpha also increase post-PH. However, increase in the inhibitory GTP-binding protein is more gradual, with Gi_2 alpha levels only achieving twice the pre-PH values by 24 h post-PH. Gi_2 alpha levels then abruptly fall to below pre-PH values at 48 and 72 h post-PH. Membrane levels of the beta 2 protein subunit vary little early after PH and only slightly exceed pre-PH values 1 d post-PH. The doubling of the stimulatory (Gs alpha) protein levels within the initial 6 h post-PH is associated with a doubling of AC activity and cAMP accumulation during this same period. Cyclase activity and cAMP concentrations fall from 6–24 h post-PH. This is associated with a fall in levels of the stimulatory G protein subunit but peak levels of the inhibitory subunit, Gi_2 alpha. The subsequent, late (24–72 h) rise in cyclase activity and cAMP concentration parallels a marked fall in levels of Gi_2 alpha, the inhibitory protein, during steady expression of the stimulatory subunit, Gs alpha.

As shown in Fig. 4, *A* and *B*, fluctuations in the levels of the G-protein subunits correlate poorly with the steady-state levels of their respective mRNAs. Indeed, the two generally appear to be reciprocally related. For example, although Gs alpha protein levels are greater than pre-PH values for the first 48 h post-PH, steady-state levels of Gs alpha mRNA steadily decrease during the first 24 h post-PH and approach baseline levels only by 72 h

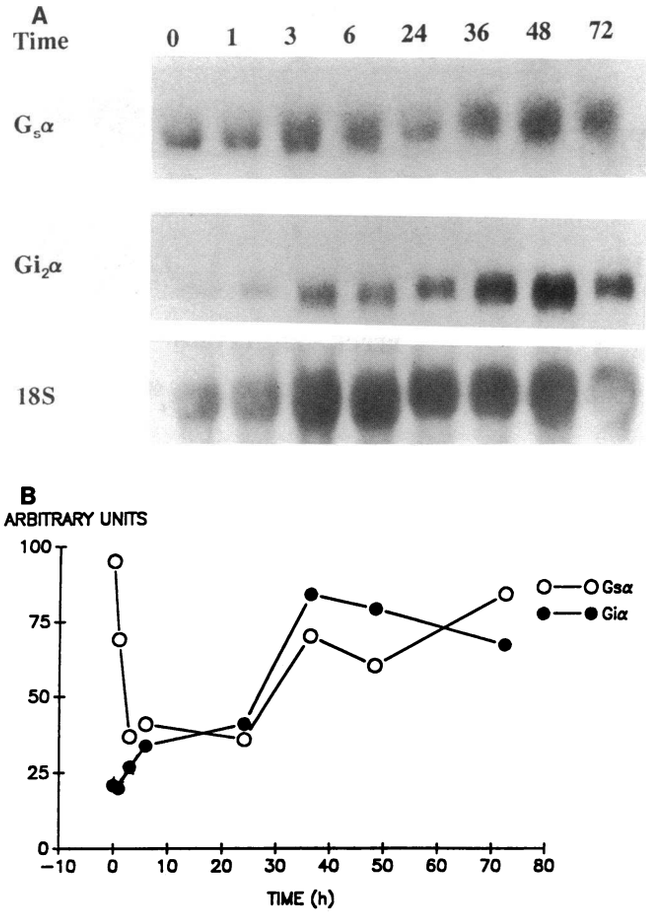

Figure 4. Changes in GTP-binding protein mRNAs after PH. (*A*) Northern blot analysis of the expression of the stimulatory (Gs alpha) and inhibitory (Gi_2 alpha) G-protein mRNAs at various times after PH. 20 μg of total RNA was applied to each lane. Membranes were hybridized with a cDNA to Gs alpha, yielding a 1.8-kb band, and to Gi_2 alpha, yielding a 2.4-kb hybridizing band. To normalize for disparities in the amount of RNA in each lane, membranes were stripped and rehybridized with a cDNA to 18S RNA, the expression of which is unaffected by PH. (*B*) The integrated optical densities of individual bands on autoradiograms from three separate Northern blots were determined with a LOATS RAS-1000 Image Analysis System. For each sample, data for the specific mRNA of interest were normalized to the amount of 18S control RNA present and expressed as fold stimulation over the control value.

post-PH. Similarly, falling Gi_2 alpha protein levels from 24–72 h post-PH are associated with increasing levels of Gi_2 alpha transcripts during that period. In contrast, increasing Gi_2 alpha protein levels from 0 to 24 h post-PH parallel increasing Gi_2 alpha transcripts during that interval.

To more directly evaluate the effect of elevated cAMP levels on hepatocellular proliferation, cAMP levels were increased in isolated hepatocytes by exposing them to either IBMX plus forskolin or 8-Br-cAMP. DNA synthesis, as assessed by the incorporation of [^{3}H]thymidine into hepatocyte DNA, was then triggered by exposing the hepatocytes to growth factors.

Viability, as assessed by LDH release, was poor in both groups of cells grown in the absence of growth factors. Hence, these groups were eliminated from further analysis. Treatment with IBMX and forskolin led to significant and sustained increases in hepatocyte cAMP (Fig. 5). cAMP concentrations in IBMX/forskolin-treated cells were similar to those noted in the regenerating liver 6 h after PH. Media concentrations of LDH were similar in control and IBMX/forskolin-treated plates (data not shown). Hence, this degree of cAMP accumulation did not compromise hepatocyte viability. Growth factors produced a significant increase in hepatocyte DNA synthesis, which peaked 72 h after plating. Treatment with IBMX/forskolin significantly blunted growth factor induction of DNA synthesis at both 48 and 72 h (Fig. 6). Similar levels of cAMP and inhibition of DNA synthesis were noted when cultures were maintained continuously in the presence of 10 μM 8-Br-cAMP (149±22 vs. 1,482±36 cpm/μg DNA in 8-Br-cAMP–treated and control cultures, respectively, $P < 0.001$). However, when cells were exposed transiently (e.g., 24 h) to 10 μM 8-Br-cAMP and then returned to media that lacked 8-Br cAMP, within 24 h cAMP levels fell and DNA synthesis was greater than that of control cultures that had never been exposed to 8-Br-cAMP (2,518±294 after 8-Br-cAMP withdrawal vs. 1,482±36 cpm/μg DNA in control plates, $P < 0.05$). Thus, sustained high levels of cAMP inhibited hormonal induction of DNA synthesis by isolated hepatocytes. However, transient surges of cAMP to similar levels stimulated DNA synthesis in these cells. These data suggest that alterations in the concentration of hepatocyte cAMP may serve to regulate the growth of the regenerating liver.

In adult liver, cAMP modulates intermediary metabolism (21). Hence, regeneration-associated fluctuations in hepatic cAMP may alter these "differentiated" hepatocyte functions. To explore the latter possibility, expression of a cAMP-regulated gene involved in gluconeogenesis, PEPCK, was assessed at various times after PH. A close temporal correlation between increased hepatic cAMP levels and increased steady-state levels of mRNAs for PEPCK was noted (Fig. 7 *A*). To further clarify

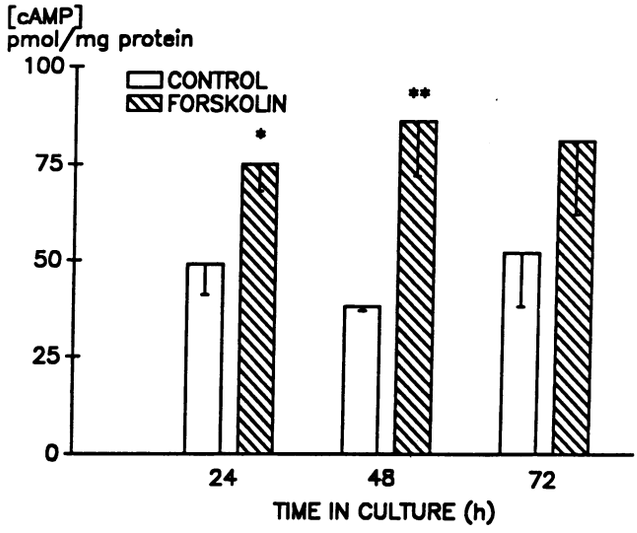

Figure 5. cAMP concentration in primary hepatocyte cultures treated with growth factors. Hepatocytes (1.5×10^6 cells/60-mm plate) were cultured in M-199 with growth factors (1 μg/ml dexamethasone, 10^{-9} M glucagon, 10 ng/ml EGF, 500 U/liter insulin). IBMX (100 mM) and forskolin (1 mM) were added to one-half of the cultures. The cAMP concentration of hepatocytes harvested 24, 48, and 72 h after plating was determined. *$P < 0.05$ vs. control plates at 24 h. **$P < 0.025$ vs. control plates at 48 h.

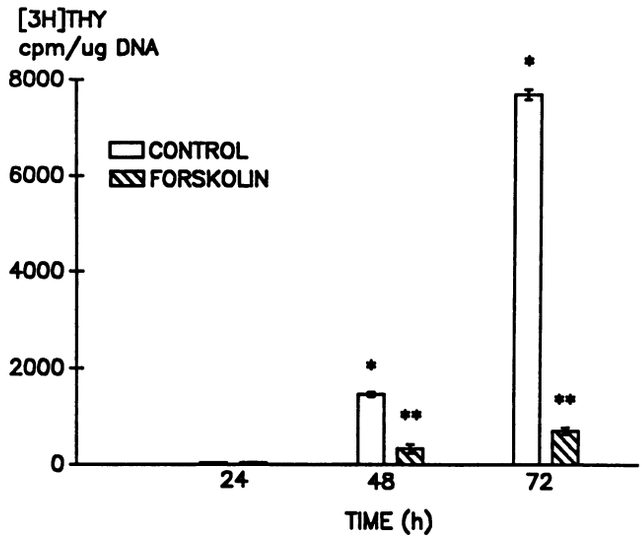

Figure 6. DNA synthesis in primary hepatocyte cultures treated with growth factors. Hepatocytes were cultured as described in the legend to Fig. 5. 2 h before harvesting, 5 μCi [^{3}H]thymidine was added to each plate. Plates were harvested at 24, 48, or 72 h. DNA was subsequently isolated and the specific activity of [^{3}H]thymidine/μg DNA determined. *$P < 0.001$ vs. [^{3}H]thymidine incorporation of control plates at 24 h. **$P < 0.001$ vs. [^{3}H]thymidine incorporation of control plates at either 48 or 72 h.

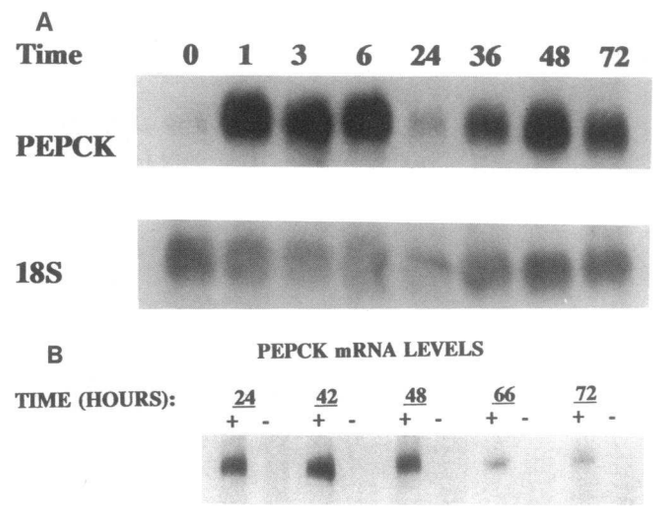

Figure 7. Changes in PEPCK mRNA after PH or in vitro manipulation of hepatocyte cAMP concentration. (*A*) Northern blot analysis of PEPCK expression at various times after PH. 20 μg of total RNA was applied to each lane. Membranes were hybridized with a cDNA to PEPCK, yielding a 2.8-kb hybridizing band. To normalize for slight disparities in the amount of RNA in each lane, membranes were stripped and rehybridized with a cDNA to 18S RNA. (*B*) Changes in PEPCK mRNA in primary hepatocyte cultures grown in serum-free, growth factor–enriched media either with (+) or without (−) IBMX plus forskolin. Northern analysis was performed as described above. Ethidium bromide staining of the gel and methylene blue staining of the membrane confirmed that equal amounts of RNA had been loaded and transferred. PEPCK expression was increased in the cells cultured in the presence of IBMX plus forskolin, agents that elevated cAMP concentrations.

the role of cAMP in regulation of PEPCK expression during growth stimulation, the expression of PEPCK was compared in cells cultured in growth factor–enriched media with or without IBMX plus forskolin. As shown in Fig. 7 *B*, PEPCK expression was consistently enhanced in the cultures where cAMP had been increased by IBMX plus forskolin.

Discussion

Although cAMP has been recognized as a second messenger for decades (21), the role of cAMP-dependent signal transduction in the regulation of compensatory hepatic growth remains uncertain. The present experiments confirm the results of others (1, 2) and demonstrate that cAMP peaks biphasically in the liver during the prereplicative phase of regeneration that follows PH. Furthermore, our data suggest that these fluctuations in cAMP levels result from variations in the rate of synthesis of cAMP by AC, since changes in cAMP concentration correlate well with changes in the activity of this enzyme after PH.

Others have noted that hepatic sensitivity to a number of discrete beta-adrenergic agents and glucagon is enhanced after PH (1, 23, 24). Plasma levels of catecholamines, glucagon, and insulin increase post-PH (1, 23–26). Gradual changes in the kinetics of glucagon and alpha- and beta-adrenergic receptors after PH have been reported (24–26). However, the maximal increases in these receptors occur relatively late in the prereplicative period (24–26). We were unable to explain the regenerating liver's heightened sensitivity to glucagon early post-PH on the basis of changes in glucagon receptor kinetics. Hence, although all of the adrenergic receptors were not studied in our experiments, it seems unlikely that changes in the number and/or affinity of these receptors totally account for the fluctuations in AC activity post-PH. Rather, our data suggest that differential expression of stimulatory and inhibitory GTP-binding proteins serves to integrate signals initiated by various receptor–ligand interactions in order to tightly regulate AC activity during compensatory hepatic growth. Predominant regulation of AC activity by postreceptor events could explain previous observations (1, 23, 24) that the regenerating liver exhibits a generalized increase in sensitivity to various ligands that induce cAMP synthesis.

Regeneration-associated changes in G-proteins do not appear to reflect nonspecific generalized membrane alterations. Indeed, membrane levels of stimulatory and inhibitory G-protein subunits are typically inversely related. During the initial 6 h post-PH, levels of the stimulatory subunit, Gs alpha, double. This is associated with an approximately twofold increase in stimulated AC activity and doubling of hepatic cAMP concentration. From 6 to 24 h, levels of the stimulatory G-protein fall, whereas levels of the inhibitory G-protein subunit, Gi_2 alpha, peak. Relatively greater levels of the inhibitory G-protein are associated with decreased AC activity and a reduction in cAMP concentration. From 24 to 72 h post-PH, levels of the inhibitory protein, Gi_2 alpha, fall dramatically, whereas levels of the stimulatory Gs alpha subunit remain greater than before PH. This "unopposed" stimulatory tone is associated with a second peak in AC activity and cAMP levels. Increased expression of the beta GTP-binding protein may also contribute to this late peak in cyclase activity.

Such rapid alterations in the concentration of membrane proteins are somewhat surprising. Northern analysis does not

suggest that these changes in G-protein levels are driven by rapid fluctuations in the rate of transcription of the respective G-protein genes. Indeed, changes in the steady-state levels of the mRNA for Gs alpha and Gi_2 alpha typically lag behind changes in the G-protein levels by several hours. This suggests that posttranscriptional events regulate membrane concentration of the G-protein subunits in the regenerating liver. Possible posttranscriptional processes that may contribute to these fluctuations include variations in the efficiency of translating G-protein mRNAs, fluctuating rates of G-protein degradation, and recycling of G-protein subunits between membrane and cytosolic pools (27, 28).

Although synthesis of cAMP is clearly tightly regulated post-PH, its role in regulation of liver regeneration is uncertain in that model. However, when levels of cAMP are manipulated in primary hepatocyte cultures, induction of DNA synthesis by epidermal growth factor (EGF), a mitogen that activates tyrosine kinase signals (29), is inhibited. Transient elevations of cAMP enhance EGF induction of DNA synthesis, whereas sustained elevations of cAMP inhibit this process. Although such diametrically opposed consequences of a single mediator may seem incongruous, similar results have been noted in many other systems. cAMP levels normally rise at the beginning of G_1 in 3T3, BHK, CHO, and HeLA cells. In these cell lines, preventing the G_1 surge from subsiding blocks subsequent DNA synthesis (2). If cAMP can both stimulate and inhibit hepatocellular proliferation, then its precisely orchestrated accumulation post-PH suggests that it may play an important role in controlling hepatic growth.

The growth-stimulatory effect of cAMP may involve induction of growth-related gene expression. cAMP-responsive elements have been identified "upstream" of several protooncogenes (30), and the catalytic unit of a cAMP-dependent kinase positively regulates c-*fos* expression (31). With reference to a potential growth-inhibitory effect of cAMP, Bang et al. (32) have recently suggested that cAMP enhances the expression of TGF-B_2 in human prostate carcinoma cells and have correlated cAMP-enhanced TGF-B_2 expression with growth inhibition in that system. Both TGF-B_1 and TGF-B_2 have been shown to reversibly inhibit the proliferative response of liver to PH (33). TGF-B expression increases within 12 h of PH (34), shortly after the initial post-PH peak in hepatic cAMP levels. At present, the role of cAMP in inducing TGF-B expression by the regenerating liver is uncertain. However, TGF-B is not expressed by hepatocytes (34, 35). Hence, it is unlikely that altered TGF-B expression can explain the cAMP-associated inhibition of DNA synthesis noted in primary hepatocyte cultures.

As a vital organ, the liver must continue to perform liver-specific function while it regenerates. This implies that the residual liver must increase its metabolic efficiency in order to preserve a tolerable level of differentiated liver-specific function post-PH. cAMP is an important regulator of many metabolic pathways (21). Indeed, cAMP has been shown to regulate the expression of PEPCK, a gene involved in glucose homeostasis in mature hepatocytes (35). Mohn et al. (36) recently reported that transcripts of PEPCK increase significantly within the first 3 h of PH. Our data indicate that liver cAMP concentration increases significantly during the first 6 h after PH. The current experiments demonstrate that steady-state levels of PEPCK mRNA also increase during this interval. Furthermore, expression of PEPCK generally correlates with cAMP levels measured at various times during the first 3 d post-PH. Steady-state levels of PEPCK mRNA are also increased in hepatocyte cultures treated with IBMX plus forskolin, agents that induce sustained elevations in intracellular cAMP concentrations. Taken together, these data suggest that the tightly regulated accumulation of cAMP post-PH may be necessary to regulate both liver growth and differentiated, liver-specific functions during liver regeneration.

Acknowledgments

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