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Parathyroid Hormone-Related Protein Is Induced in the Adult Liver during Endotoxemia and Stimulates the Hepatic Acute Phase Response

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ABSTRACT

Previously, we reported that PTH-related protein (PTHrP) gene expression is induced in vital organs, including the liver, during endotoxemia. The liver plays a central role in the acute phase response (APR), a cytokine-mediated host defense against infection and inflammation that includes increased production of acute phase proteins and lipids by hepatocytes. Because PTHrP is thought to act locally at its site of production, *in vivo* studies were carried out to determine whether PTHrP could contribute to the induction of the hepatic APR. Hepatic PTHrP messenger RNA (mRNA) levels were induced acutely in rat liver in response to a near lethal dose of endotoxin. PTHrP protein, which was located by immunohistochemical staining in hepatocytes from both control and LPS-treated rats, was markedly induced in periportal hepatocytes in response to LPS treatment. Co-incident with this transient increase in PTHrP gene expression, PTH/PTHrP receptor mRNA levels were down-regulated. Administration of PTHrP(1–34), a PTH/PTHrP receptor agonist, to mice increased hepatic serum amyloid A (SAA) mRNA levels as well as circulating levels of SAA. In addition, PTHrP(1–34) increased serum triglyceride (TG) levels in rats and mice in a dose-dependent fashion. The hypertriglyceridemic effect of PTHrP(1–34) was accompanied by an increase in hepatic fatty acid synthesis. In contrast, PTHrP(7–34) amide, a receptor antagonist, had no effect on serum SAA or TG levels. These results, which provide evidence for the regulated expression of PTHrP in adult liver, suggest that PTHrP may be one additional member of the cytokine cascade produced locally in liver that can act to stimulate the hepatic acute phase response. (*Endocrinology* **138:** 2665–2673, 1997)

PTH-RELATED PROTEIN (PTHrP) has been identified and defined by its relation to PTH, a hormone that follows the normal endocrine paradigm of localized glandular production and distant action. However, accumulating evidence suggests that PTHrP may in fact act more like a cytokine than a classic hormone, being widely expressed in normal tissues by a variety of cell types, where it is thought to have local autocrine, paracrine, or intracrine effects (see Ref. 1 for comprehensive review).

Previous work by our laboratory has added a new dimension to this view of PTHrP as a cytokine-like peptide, showing that PTHrP is a member of the cascade of proinflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), which is induced during the host response to bacterial endotoxin (LPS, or lipopolysaccharide) (2–5). Specifically, we have shown that PTHrP gene expression is induced in the liver, spleen, heart, lung, and kidney in rats in response to injection of a lethal dose of LPS (5). Using sublethal doses of LPS, we have shown that TNF, a key proinflammatory cytokine that is induced during infection, is a major mediator of LPS-induced PTHrP gene expression in spleen (2). The proinflammatory nature of PTHrP is underscored by our studies in mice showing that the administration of antibody directed against PTHrP prevents LPS-induced mortality (5). The similarity of these results to other studies using TNF and IL-1 antagonists therefore suggests that PTHrP is one additional component of the cascade of proinflammatory cytokines mediating the toxic effects of LPS (6–8).

Despite their potential for toxicity during overwhelming infections, proinflammatory cytokines also mediate many beneficial responses to infection and inflammation (9). In particular, TNF and IL-1 are known to be major mediators of the hepatic acute phase response, an important protective arm of the host defense against infection (10). Given our previously reported finding that PTHrP gene expression is induced in the liver during endotoxemia (5), we therefore hypothesized that locally produced PTHrP may contribute to the induction of the hepatic acute phase response that accompanies endotoxemia. This hypothesis is particularly intriguing in light of previous reports that, unlike other normal adult tissues where PTHrP is constitutively expressed, the liver stood apart as one site where PTHrP appeared to be constitutively expressed during fetal development, but not in adulthood (1, 11–13). However, our data suggest that PTHrP expression is indeed inducible in adult liver, and raises the possibility that this peptide may therefore mediate an important hepatic function that is only activated during inflammation, namely the hepatic acute phase response.

The hepatic acute phase response is characterized by profound alterations in the hepatic synthesis both of lipids and a number of plasma proteins, the acute phase proteins (14–17). Hypertriglyceridemia accompanies infection and is inducible in animal models by LPS, as well as by the proin-

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flammatory cytokines, TNF and IL-1 (14, 15). Because TG-rich lipoproteins bind and detoxify endotoxin as well as viruses (18–21), this elevation in triglyceride (TG) levels is thought to play a protective role during infection (15). Similarly, the cytokine-mediated increased hepatic production of positive acute phase proteins, such as serum amyloid A (SAA) or C-reactive protein, that accompanies infection and endotoxemia, is also thought to be protective (16, 17). For example, SAA, a high density lipoprotein (HDL)-associated apolipoprotein that is a major acute phase protein in humans, as well as in mice, is thought to alter cholesterol metabolism during infection in ways that may be beneficial to the host (16, 17, 22–24).

To obtain evidence to support the hypothesis that the induction of PTHrP expression in hepatocytes during endotoxemia could be contributing to the stimulation of the hepatic acute phase response, further studies were conducted to delineate the effect of endotoxin on hepatic expression of both PTHrP and the PTH/PTHrP receptor, to localize the site of LPS-inducible PTHrP protein in the liver, and to determine the effect of administration of PTHrP(1–34), a peptide that binds to and activates the PTH/PTHrP receptor, on serum levels of triglyceride and SAA as well as the hepatic synthesis of fatty acids and hepatic SAA gene expression.

Materials and Methods

Materials

Escherichia coli strain O55:B5 endotoxin was obtained from Difco Laboratories (Detroit, MI). Human PTHrP(1–34) and PTHrP(7–34) amide were obtained from Bachem Bioscience (King of Prussia, PA). The murine PTHrP complementary DNA (cDNA) probe, which includes the region encoding amino acids 1–114 of the mature peptide, was kindly provided by Dr. Arthur E. Broadus (Yale University School of Medicine, New Haven, CT) (13). The full-length rat PTH/PTHrP receptor cDNA was kindly provided by Dr. Gino V. Segre (Massachusetts General Hospital/Harvard Medical School, Boston, MA) (25). The cDNA probe for rat cyclophilin was kindly provided by Dr. Gordon Strewler (West Roxbury VA Medical Center, Brockton, MA). The cDNA probe for human SAA1 was obtained from the American Type Culture Collection (Rockville, MD).

Affinity purified rabbit polyclonal antibody generated against human PTHrP[34–53] and the PTHrP[34–53] peptide were obtained from Oncogene Science (Cambridge, MA). Goat sera, biotinylated goat antirabbit IgG antibody and diaminobenzidine were obtained from Vector Laboratories (Burlingame, CA). Rabbit IgG and trypsin were obtained from Sigma Chemical Co. (St. Louis, MO).

Animal procedures

Male Sprague Dawley rats, 200–220 g in weight, were purchased from Simonsen (Gilroy, CA). C57BL/6 mice (4- to 5-week-old males) were purchased from The Jackson Laboratory (Bar Harbor, ME). On the morning of study, after the removal of food, animals were divided into groups and injected with endotoxin (LPS, or lipopolysaccharide), PTHrP(1–34), PTHrP(7–34) amide or the appropriate vehicle alone (controls). LPS, diluted in apyrogenic 0.9% saline (Kendall McGraw Laboratories, Irvine, CA), was injected iv (via tail vein) into rats. The PTHrP peptides were administered iv to rats or ip to mice after dilution in a solution of 0.1% human serum albumin that contained undetectable levels of LPS (<10 pg/ml).

At the indicated times, livers were harvested and/or blood was obtained from the inferior vena cava of anesthetized rats or mice. Livers were processed as indicated below. Serum samples were either assayed immediately for serum triglycerides and serum amyloid A or stored at −70 C before assay.

Endotoxin assay

All PTHrP peptide solutions used were assayed for LPS contamination using a standard chromogenic Limulus assay as previously described (3). Endotoxin content of PTHrP peptide stock solutions ranged from undetectable (<200 pg/mg peptide) to 400 pg/mg peptide. Endotoxin content of injected peptide solutions was less than 25 pg/animal in all reported experiments and was similar for comparable doses of PTHrP(1–34) and PTHrP(7–34) amide. These amounts of endotoxin are 1,000-fold lower than the doses required to increase serum TG levels in rodents, stimulate hepatic fatty acid synthesis in mice, or increase serum SAA levels in mice (26–29).

Northern blot analysis

Messenger RNA (mRNA) was isolated from rat or mouse livers, fractionated in 1% agarose gels containing 2.2 M formaldehyde, transferred to nylon membranes electrophoretically, and hybridized to random prime-labeled [^{32}P]cDNA probes, as previously described (2). Blots were exposed to film at −70 C using Cronex intensifying screens (DuPont, Wilmington, DE) for the time indicated in figure legends, and autoradiographic intensity was quantitated as previously described (3).

Immunohistochemistry

Whole rat livers were removed 4.5 h after treatment with 5 mg/250 g body weight LPS (LD_{50}=10 mg/250 g at t = 14 h for the lot of LPS used) or with vehicle alone, fixed in 4% paraformaldehyde/1% glutaraldehyde, and embedded in JB-4 plastic (Polysciences Inc., Warrington, PA). Three-micrometer sections were digested with 0.05% trypsin in normal saline, pH 7.2, for 10 min at 37 C before quenching endogenous peroxidase activity with 1% hydrogen peroxide. Sections were blocked with 50% goat serum in PBS containing 0.05% Tween (Solution A) before incubation with 2.5–3.3 μg/ml PTHrP(34–53) antibody at 4 C overnight. Incubation with biotinylated goat antirabbit antibody (6 μg/ml), diluted with 10% goat serum in Solution A, was carried out at room temperature for 60 min, followed by serial incubation with avidin-biotin-peroxidase complex (ABC Elite Kit, Vector Laboratories, Burlingame, CA) and diaminobenzidine. Nuclei were stained with methyl green. Specificity of staining for PTHrP was confirmed by the absence of staining that resulted when PTHrP antibody was replaced with an equivalent concentration of rabbit IgG or, more importantly, with PTHrP(34–53) antibody that had been preincubated overnight at 4 C with a 30- to 40-fold excess, by weight, of PTHrP(34–53) peptide.

Serum triglyceride levels

Serum triglycerides were measured in rats using a colorimetric assay that measures triglycerides after their enzymatic conversion by lipoprotein lipase to glycerol (Diagnostic Kit no. 339, Sigma, St. Louis, MO). Because murine glycerol levels are high relative to triglyceride levels, triglyceride levels in mice were similarly measured and then corrected for measurement of true serum TG levels by subtraction of endogenous glycerol levels (Diagnostic kit no. 337, Sigma).

Hepatic fatty acid synthesis

Thirty minutes after the ip administration of PTHrP(1–34) (1 μg/mouse) or vehicle alone, livers were obtained from anesthetized C57BL/6 mice. As previously described (30), 0.5-mm liver slices were prepared (McIlwain Tissue Slicer, Brinkmann, Westbury, NY), weighed, and then incubated *ex vivo*, in duplicate for each animal (n = 5 animals/condition), with 1 μCi [^{14}C]sodium acetate (NEN, Boston, MA) at 37 C for 1.5 h. After addition of an internal standard ([^{3}H]oleic acid), tissue slices were saponified and fatty acids were extracted. Incorporation of [^{14}C]acetate into fatty acids, corrected for fatty acid yield by use of the internal standard, was then determined by scintillation counting and reported as mean ± SEM of ^{14}C-acetate (μmole) incorporated/g tissue/1.5 h.

Serum amyloid A protein levels

Murine SAA serum levels were measured using a commercially available solid phase sandwich ELISA (BioSource International, Camarillo,

CA). This assay, which utilizes two monoclonal antibodies directed against murine SAA, detects ≥ 200 ng/ml SAA.

Statistics

Values are presented as the mean ± SEM. Statistical significance was determined by analysis of variance and use of Student's *t* test using InStat 2.01 statistical software (GraphPad Software, San Diego, CA).

Results

Effect of a near-lethal dose of LPS on hepatic PTHrP and PTH/PTHrP receptor gene expression in the rat.

While constitutive levels of PTHrP mRNA are at or below the limits of detection in rat liver, administration of a near lethal dose of LPS (5 mg/250 g) caused a marked induction of hepatic PTHrP mRNA levels, with increases occurring as early as 45 min after injection (Fig. 1A). Hepatic PTHrP mRNA levels, which increased over 13-fold, peaked at 4 h and returned to baseline by 6–8 h (Fig. 1B). Coincident with this up-regulation of PTHrP gene expression, mRNA levels for the PTH/PTHrP receptor, which are readily detectable in control animals, rapidly and profoundly decreased when rats were treated with a near lethal dose of LPS (Fig. 1A). However, after normalization of hepatic PTHrP mRNA levels at 6 h, PTH/PTHrP receptor mRNA levels also began to return towards baseline (Fig. 1B).

Immunohistochemical localization of hepatic PTHrP.

To identify the site of PTHrP production within the liver during the host response to endotoxin, immunohistochemical studies utilizing antibody directed against PTHrP(34–53) were performed on livers obtained from rats treated with a near-lethal dose of LPS (or vehicle alone). Livers were obtained from the animals 4.5 h after LPS administration, a time that follows peak induction of hepatic PTHrP mRNA levels by 30 min. Hepatocytes stained specifically for PTHrP in livers obtained from both control (Fig. 2, A and C) and LPS-treated animals (Fig. 2, B and D). However, LPS-treatment resulted in a marked increase in the intensity of PTHrP staining in hepatocytes. In contrast, no definitive staining of sinusoidal lining cells was appreciated in either control or LPS-treated livers. In hepatocytes from control and LPS-treated animals (Fig. 2, A and B), PTHrP immunostaining was present predominantly in the cytoplasm, but in a smaller number of cells (*e.g.* approximately 30% of periportal cells), was also seen in discrete areas of the nucleus. However, LPS treatment, in addition to increasing the intensity of PTHrP staining in hepatocytes, also caused a slight change in the distribution of cytoplasmic PTHrP. Cytoplasmic staining in hepatocytes from LPS-treated animals appeared more punctate and occurred in all areas of the cytoplasm, including the perinuclear area, whereas in control hepatocytes, there was some sparing of the perinuclear area. In addition, a marked gradient of PTHrP staining in the liver lobule was noted in LPS-treated animals; PTHrP staining was strongest in periportal hepatocytes (Fig. 3B), while hepatocytes in the central areas were PTHrP-negative (Fig. 3D). In contrast, in the livers from control animals, while a slight gradient may also have been present, a low level of specific PTHrP staining was noted diffusely throughout the lobule in many, but not all, hepatocytes, including those in the central area (Fig. 3, A and C).

Effect of PTHrP(1–34) on serum triglyceride levels in rodents.

In rodents, as in humans, hypertriglyceridemia is one component of the acute phase response (14, 15). Therefore, the ability of PTHrP(1–34), a peptide that binds to and activates the PTH/PTHrP receptor, to cause an increase in serum TG levels was investigated. Intravenous administration of PTHrP(1–34) to rats (10 μg/250 g) caused a transient increase in serum triglyceride levels at 2 h (Fig. 4A). This effect was dose dependent (Fig. 4B), occurring with doses of PTHrP(1–

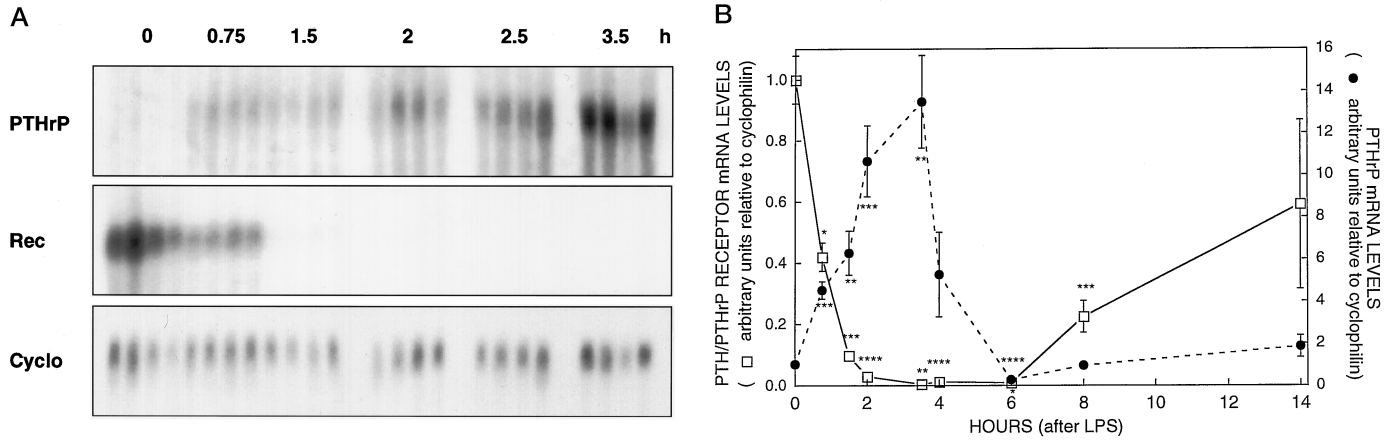

FIG. 1. Acute (A) and prolonged (B) time courses of LPS induction of hepatic PTHrP and PTH/PTHrP receptor mRNA levels in rats. Male Sprague Dawley rats were injected via tail vein with 5 mg/250 g body weight LPS (LD_{50} = 10 mg/250 g). At the indicated times, livers were removed and processed for Northern analysis of polyadenylated mRNA. The membranes were sequentially hybridized with murine PTHrP, rat PTH/PTHrP receptor, and rat cyclophilin cDNA probes. (A) Northern blot showing acute (0–3.5 h) effects of LPS on hepatic PTHrP (*upper panel*) and PTH/PTHrP receptor 2.4 kb (*middle panel*) mRNA levels (n = 4/time point). Cyclophilin mRNA levels are shown in bottom panel. B, Prolonged time course (0–14 h) showing effect of LPS on hepatic PTHrP and PTH/PTHrP mRNA levels. Results are reported as mean ± SEM (n = 3–7/time point) in arbitrary scanning densitometry units normalized to cyclophilin with *P* values determined by two-tailed Student's *t* test. *, $P < 0.05$ *vs.* t = 0; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. Results are statistically significant by ANOVA.

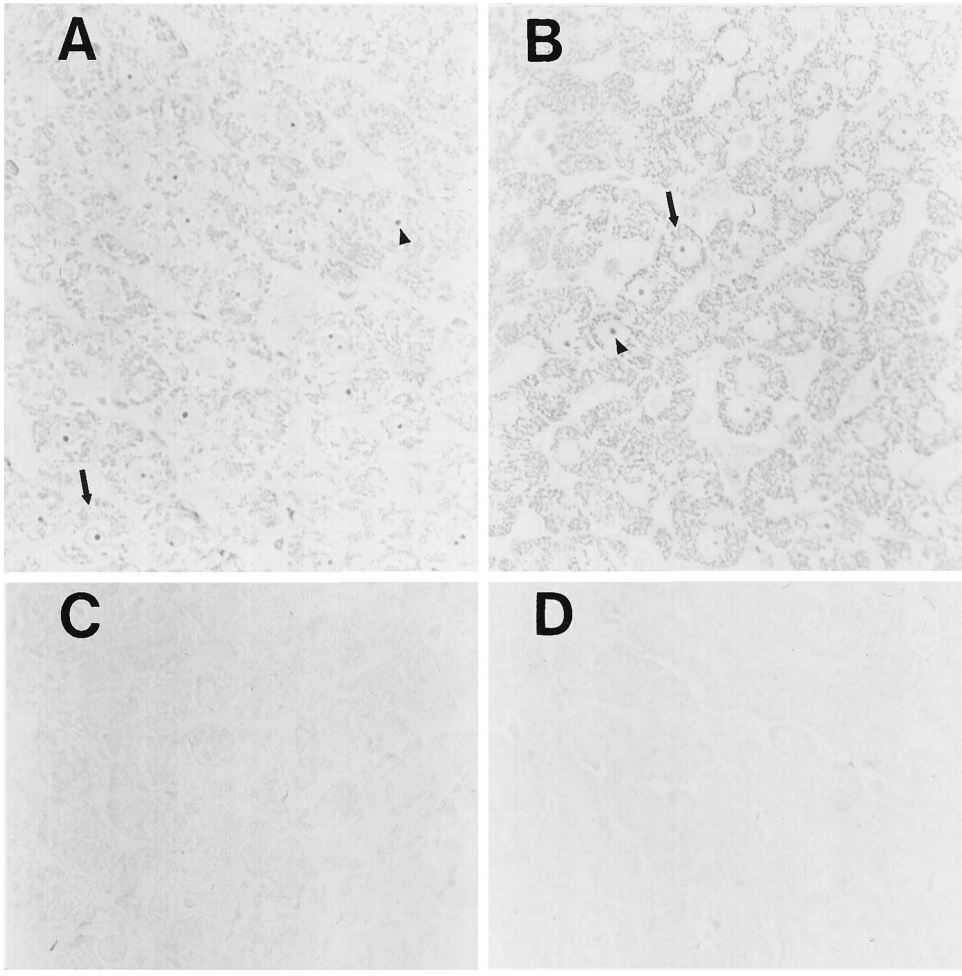

FIG. 2. Immunohistochemical localization of PTHrP in rat liver hepatocytes 4.5 h after treatment with vehicle alone (A and C) or LPS (5 mg/250 g) (B and D). Sections of liver from control (A) or LPS-treated (B) animals were stained with antibody directed against PTHrP(34–53). Specificity of staining was verified by comparison with sections from control (C) and LPS-treated (D) animals that were treated with PTHrP(34–53) antibody that had been preincubated with a 30-fold excess by weight of peptide antigen. All sections were stained with methyl green to visualize nuclei. Examples of specific nuclear (*arrowheads*) or cytoplasmic (*arrows*) staining of PTHrP in hepatocytes are noted.

34) $\geq$ 10 μg/250 g (40 μg/kg) (Fig. 4A/B). PTHrP(1–34) also caused a transient increase in serum TG levels in mice (Fig. 4C), a species that also constitutively expresses hepatic PTH/PTHrP receptor mRNA (data not shown). As was seen in rats, PTHrP(1–34) stimulation of serum TG levels in mice was dose dependent (Fig. 4D). However, in contrast to rats, mice responded to much lower doses of PTHrP(1–34) on a per weight basis, with increases occurring at doses of PTHrP(1–34) as low as 0.01 μg/mouse (0.5 μg/kg) (Fig. 4D). Hence, further lipid studies were conducted with mice.

Effect of PTHrP(1–34) on hepatic lipid synthesis in mice

The increase in serum triglycerides (TG) that accompanies infection is due in part to a cytokine-mediated increase in hepatic fatty acid synthesis (15, 26–28, 30, 31). Therefore, the effect of PTHrP(1–34) on fatty acid synthesis was determined. Incorporation of ^{14}C-acetate into fatty acids was measured in liver slices obtained from mice treated with PTHrP(1–34) (or with vehicle alone). PTHrP(1–34) increased hepatic fatty acid synthesis 2-fold during a 1.5-h *ex vivo* incubation of liver slices obtained 30 min after PTHrP(1–34) administration (control 5.13 $\pm$ 0.56 μmol ^{14}C-acetate incorporated/g tissue/1.5 h *vs.* PTHrP(1–34)-treated 11.05 $\pm$ 1.00 μmole ^{14}C-acetate incorporated/g tissue/1.5 h; $P \leq 0.001$), a time span corresponding with peak induction of serum TG *in vivo*.

Effect of PTHrP(1–34) on serum amyloid A levels in mice

Increased hepatic synthesis of acute phase proteins is a hallmark of the host response to infection or inflammation (16, 17). The pattern of induction of acute phase proteins is species specific. In mice, as in humans, serum amyloid A (SAA) is one of the major acute phase response proteins produced by the liver (17). Therefore the effect of PTHrP(1–34) (*vs.* vehicle alone) on serum SAA levels was studied in mice. Serum SAA increased as early as 4 h after PTHrP(1–34) administration, with peak elevations occurring at 16–24 h (Fig. 5). The effect of PTHrP(1–34) on SAA levels appeared to be dose dependent (Fig. 5).

Effect of PTHrP (1–34) on hepatic SAA gene expression in mice

The liver is believed to be the primary site of synthesis of acute phase response proteins (16, 24). To determine whether PTHrP stimulation of serum SAA levels was accompanied by an increase in hepatic SAA gene expression, the effect of PTHrP(1–34) on hepatic SAA mRNA levels was determined by Northern analysis of polyadenylated mRNA. SAA mRNA

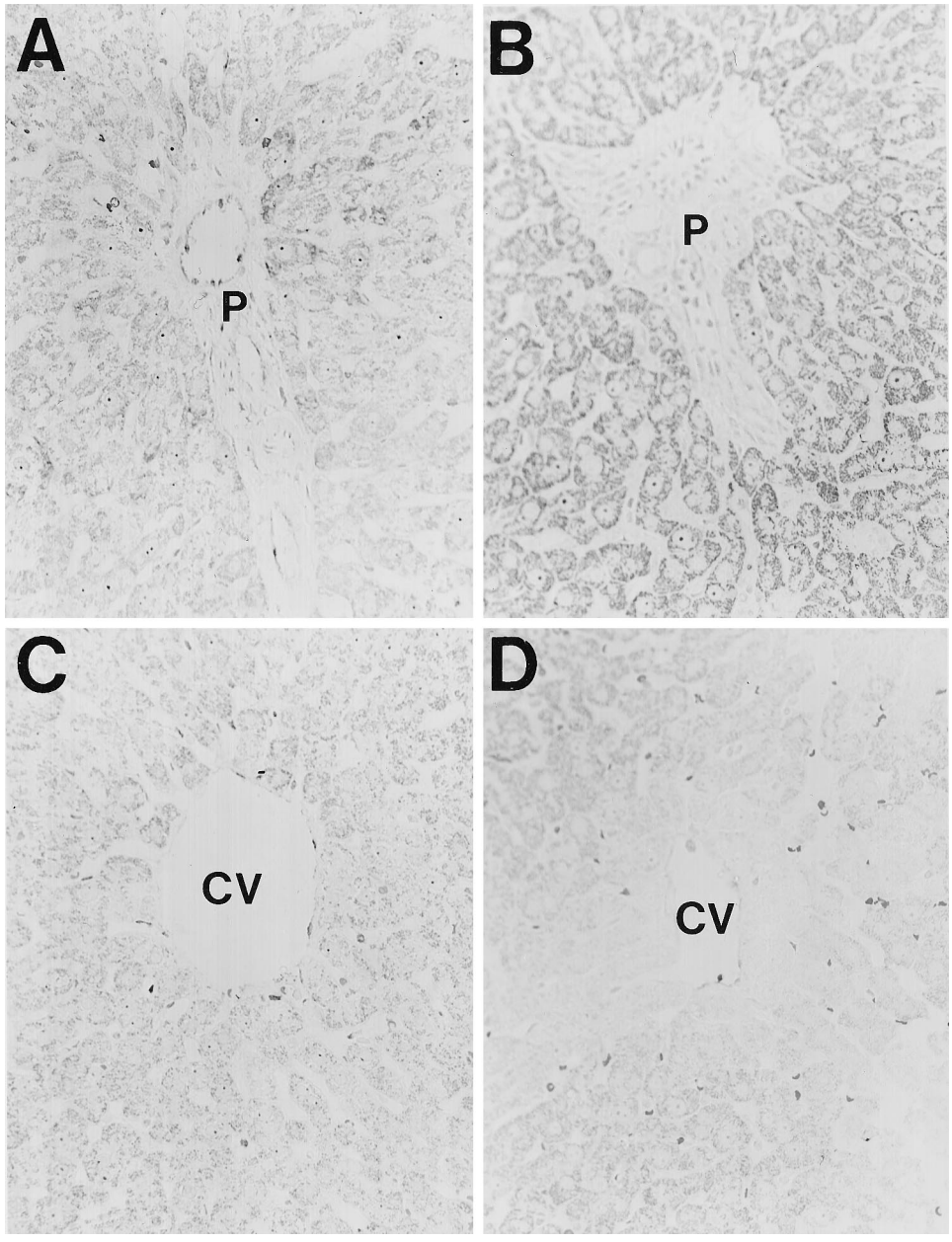

FIG. 3. Distribution of immunoreactive PTHrP within the liver lobules of control (A and C) and LPS-treated (B and D) rats 4.5 h after treatment. Staining for PTHrP was induced in the periportal hepatocytes (*P* = portal triad) after treatment with LPS (B) as compared with control animals (A). In contrast, the minimal staining for PTHrP that was present in control animals (C) in hepatocytes in the area of the central vein (CV = central vein) was completely eliminated with LPS treatment (D). All sections were stained with methyl green to visualize nuclei. Nonspecific staining of erythrocytes present in hepatic sinusoids can be seen in some views.

levels were barely detectable in the livers of control animals (Fig. 6, t = 0). In contrast, PTHrP(1–34) stimulated SAA gene expression as much as 20-fold, with increases in SAA mRNA levels occurring as early as 2 h after administration of 50 μg PTHrP(1–34) (Fig. 6).

Effect of PTHrP(7–34) antagonist on serum TG and SAA levels in mice

Further experiments were conducted to determine whether activation of the hepatic acute phase response by PTHrP was a specific PTH/PTHrP receptor-mediated effect rather than a nonspecific immune response to peptide injection or to LPS contamination of peptide solutions. The effect of PTHrP(1–34) on TG and SAA levels in mice was compared with the effect of PTHrP(7–34) amide, a peptide receptor antagonist that contained the same low level of endotoxin contamination. As can be seen in Table 1, while 50 μg/mouse of PTHrP(1–34) elevated TG levels by 34%, administration of the same dose of PTHrP(7–34) amide did not affect serum TG levels. Similarly, while PTHrP(1–34) increased SAA levels almost 2-fold, PTHrP(7–34) amide did not affect serum levels of SAA (Table 1).

Discussion

While progress has been made in recent years in delineating the role and regulation of PTHrP in many tissues throughout the body, little attention has been afforded to the liver as a site of PTHrP expression and action because adult liver did not appear to be an important site of constitutive PTHrP production (1, 11–13). In contrast, a larger body of literature, predating the discovery and identification of PTHrP, described possible hepatic effects of PTH (32–40).

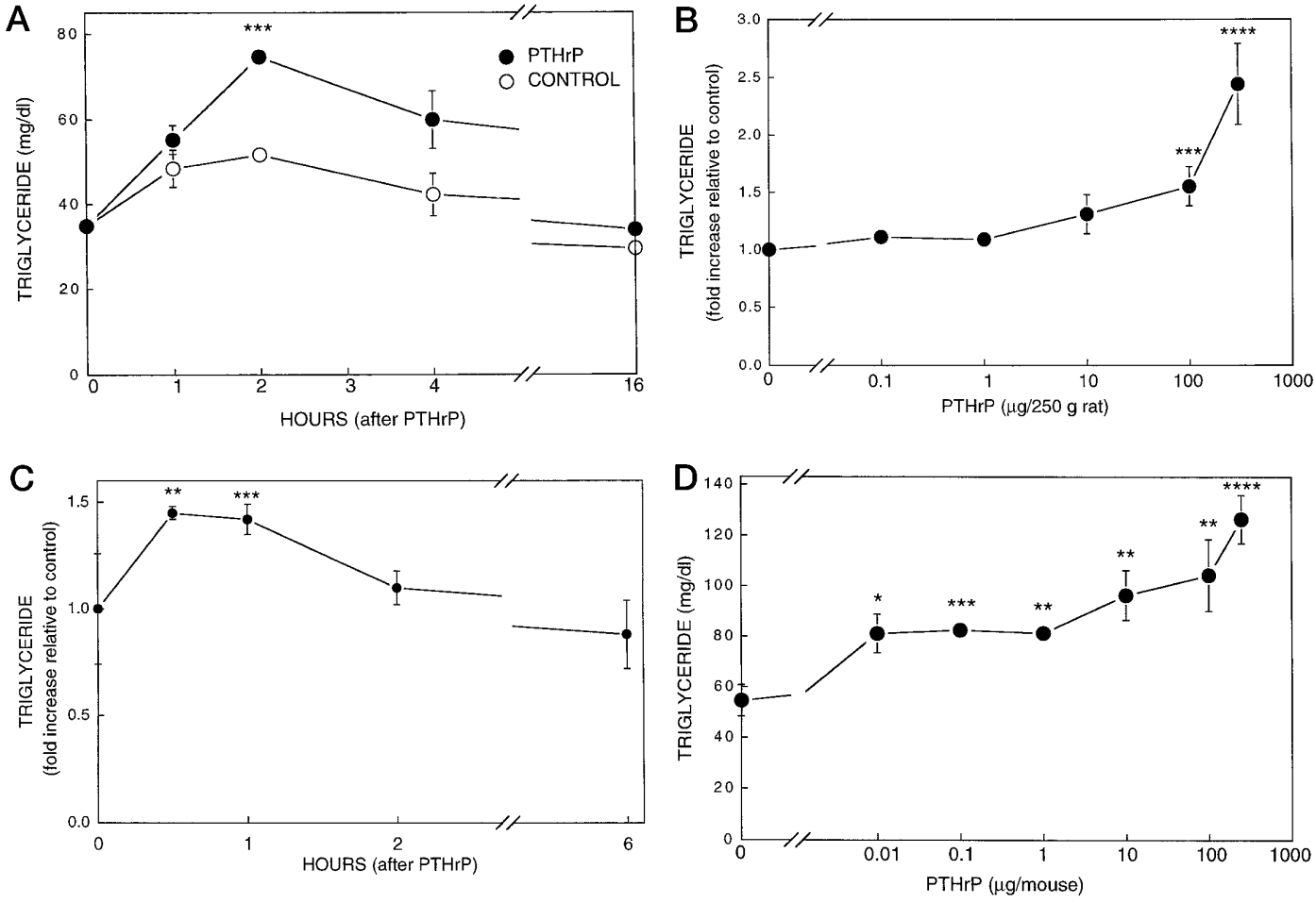

FIG. 4. Effect of PTHrP(1–34) on serum TG levels in rats (A and B) and mice (C and D). A, Sprague Dawley rats were injected with 10 μg/250 g PTHrP(1–34) (*closed circles*) or vehicle alone (*open circles*). At the indicated times, blood was obtained from the inferior vena cava of anesthetized animals. B, In separate experiments, blood was also obtained from rats 2 h after injection of the indicated doses of PTHrP(1–34). C, C57BL/6 mice were injected with 50 μg/mouse PTHrP(1–34) or vehicle alone and blood was obtained from the inferior vena cava of anesthetized animals. D, In separate experiments, blood was also obtained from mice 1 h after injection of the indicated doses of PTHrP(1–34). TG values, measured as described in *Materials and Methods*, are reported as mean ± SEM (n = 3–4/condition) with P values determined by two-tailed Student's *t* test for treated *vs.* control. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$; ****, $P < 0.001$; *****, $P < 0.0001$. Differences are statistically significant by ANOVA.

Indeed, the liver has been identified as an important site of PTH/PTHrP receptor expression, being second only to the kidney in terms of the relative abundance of receptor RNA levels in parenchymal organs (41, 42).

The studies presented here provide evidence for regulated expression of PTHrP in adult liver. Induction of PTHrP mRNA levels by LPS in the liver exhibited a more prolonged time course than found previously in other vital organs, increasing as early as 45 min after LPS administration and reaching maximal levels at 4 h, a time when mRNA levels in other organs and serum PTHrP levels have already returned to baseline (5). This prolonged induction of hepatic PTHrP gene expression suggests both that hepatic regulation of PTHrP gene expression may be differentially regulated when compared with other vital organs and that the liver may not be a major source of increased circulating PTHrP during endotoxemia.

PTH/PTHrP receptor mRNA, which is constitutively expressed in liver, exhibited reciprocal regulation with respect to PTHrP mRNA expression during endotoxemia, decreasing coincident with an increase in hepatic PTHrP mRNA levels and returning towards baseline after normalization of PTHrP mRNA levels. This reciprocal regulation of mRNA for PTHrP and its receptor, which also occurs in the spleen in response to endotoxin (4), has been reported previously in a number of classic and nonclassic PTH/PTHrP target tissues and cell types (43–47). Given the well documented PTH/PTHrP-mediated desensitization of the PTH/PTHrP receptor that occurs in cells from bone and kidney (46, 47), classic sites of PTH/PTHrP action, these data suggest that PTHrP may also be acting to feedback inhibit the hepatic PTH/PTHrP receptor during endotoxemia. In support of this hypothesis, hepatic PTH/PTHrP receptor mRNA levels have also been shown to decrease during chronic renal failure in rats, but normalize after parathyroidectomy (48).

Immunohistochemical studies demonstrated a marked increase in PTHrP protein in periportal hepatocytes immediately after peak induction of hepatic mRNA levels by LPS,

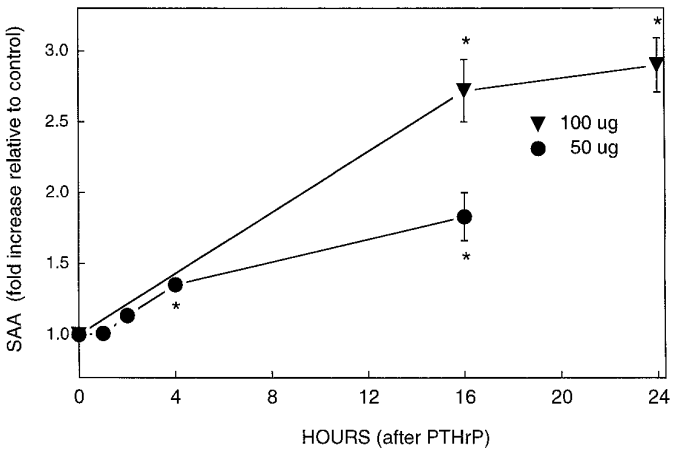

FIG. 5. Effect of PTHrP(1–34) on serum SAA levels in mice. C57BL/6 mice were injected with 50 μg (*circles*) or 100 μg (*triangles*) of PTHrP(1–34) or vehicle alone. At the indicated times after injection, blood was obtained from anesthetized animals for measurement of serum SAA levels. SAA values, measured as described in *Materials and Methods*, are reported as mean ± SEM (n = 4–8/condition). Maximal levels of SAA obtained after LPS treatment were 85.2 ± 5.4 μg/ml. P values determined by two-tailed Student's *t* test. *, $P < 0.0006$ *vs.* control. Differences are statistically significant by ANOVA.

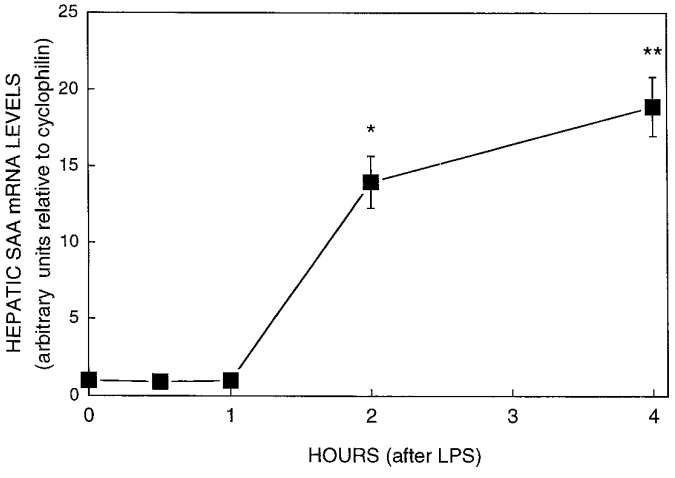

FIG. 6. Effect of PTHrP(1–34) on hepatic SAA mRNA levels in mice. C57BL/6 mice were injected with 50 μg of PTHrP(1–34). At the indicated times after injection, livers were removed for Northern analysis of polyadenylated mRNA using a human SAA1 cDNA probe. Results are reported as mean ± SEM (n = 4/condition) in arbitrary scanning densitometry units normalized to cyclophilin, with P values determined by two-tailed Student's *t* test. *, $P < 0.0002$ *vs.* t = 0; **, $P < 0.0001$. Results are statistically significant by ANOVA.

suggesting that hepatocytes may be the site of PTHrP production within the liver. Interestingly, the gradient of LPS-induced PTHrP protein within the liver lobule mirrors both the pattern of LPS uptake within the liver (which is greatest in the cytokine-producing periportal Kupffer and endothelial cells) and the pattern of hepatic production of several acute phase proteins (49–52). While it is possible that the hepatocellular PTHrP protein detected by antibody directed against PTHrP(34–53) could represent endocytosed rather than newly synthesized protein, the localization of PTHrP in discreet areas of the nucleus in a subpopulation of hepatocytes makes it unlikely that this peptide is simply being cleared by the liver. Nucleolar localization of PTHrP, which appears to require the presence of residues 87–107 of the intact peptide, has previously been reported in chondrocytes where it is thought to inhibit apoptosis (53). Additionally, in support of our hypothesis that hepatocytes are the site of hepatic PTHrP production, it has recently been reported that HepG2 cells, a human hepatocyte cell line, also produce PTHrP (54).

TABLE 1. Comparison of the effects of PTHrP(1–34) and PTHrP(7–34) amide on serum TG and SAA in mice

Treatment	TG	SAA
Control	1.00 ± 0.03	1.00 ± 0.03
PTHrP(1–34)	1.34 ± 0.06[a]	1.83 ± 0.13[b]
PTHrP(7–34)amide	1.08 ± 0.14	1.24 ± 0.17

C57BL/6 mice were injected with 50 μg of PTHrP(1–34), 50 μg of PTHrP(7–34) amide receptor antagonist, or with vehicle alone. One hour after injection, blood was obtained from anesthetized animals for measurement of serum TG levels. In a separate set of experiments, blood was obtained from anesthetized animals 16 h after injections for measurement of serum SAA levels. TG and SAA values, measured as described in *Materials and Methods* and presented as fold increase relative to control, are reported as mean ± SEM (n = 4/condition). *P* values were determined by two-tailed Student's *t* test.

[a] $P < 0.007$ *vs.* control; [b] $P < 0.0006$ *vs.* control. Differences are statistically significant by ANOVA.

Having shown that PTHrP protein levels are increased in hepatocytes immediately after peak induction of hepatic PTHrP mRNA levels during endotoxemia, we next conducted a series of *in vivo* studies to determine what effects PTHrP may have on two important arms of the hepatic acute phase response that are activated during endotoxemia, induction of the hepatic synthesis of lipids and acute phase proteins (14–17). With the exception of one report of equivocal effects of chronic PTH injections on hepatic TG secretion in rats (55), the effect of PTH or PTHrP on hepatic lipid synthesis has not been previously studied. Similarly, we are not aware of any studies examining the effect of PTH or PTHrP on hepatic synthesis of acute phase proteins. However, in support of these hypotheses, previous *in vitro* studies have demonstrated that PTH and/or PTHrP bind specifically to hepatocytes and stimulate increases in cAMP and intracellular calcium, consistent with activation of the two signaling pathways of the classic PTH/PTHrP receptor (32–36, 54). In addition, *in vivo* studies have shown that PTH or PTHrP can stimulate both hepatic glucose production and hepatic production of IGF (35, 37–40).[1]

In the experiments reported here, serum TG levels increased in both rats and mice in a dose-dependent fashion in response to the administration of PTHrP(1–34), attaining levels that are seen during endotoxemia (26, 27). However, while TG levels rapidly rise and remain elevated for up to 16 h in response to LPS (27), the bolus administration of PTHrP(1–34) caused only a transient increase in serum TG levels. Because the hypertriglyceridemia that accompanies infection and inflammation is due, in part, induction of hepatic lipid synthesis (14–15), the effect of PTHrP(1–34) on hepatic fatty acid synthesis was also determined. PTHrP(1–34) stimulated hepatic fatty acid synthesis 2-fold, a response

[1] In our studies, we found no evidence of changes in systemic glucose levels after administration of PTHrP(1–34) (data not shown).

that is similar in magnitude to that seen with LPS, TNF, or IL-1 administration (26–28, 30, 31). In contrast, the receptor antagonist, PTHrP(7–34) amide, had no effect on TG levels, thus verifying that the effects of the PTHrP(1–34) peptide could not be explained by nonspecific, non-PTH/PTHrP receptor mediated effects such as LPS contamination of the peptide solution, which was the same for both peptides, or a nonspecific immune response to injection of a foreign peptide. These data therefore suggest that PTHrP(1–34) induction of serum TG levels is a specific, receptor-mediated effect that is due, at least in part, to stimulation of fatty acid synthesis by hepatocytes.

Because cytokine-mediated changes in TG clearance and/or lipolysis also contribute to the hypertriglyceridemia that accompanies infection (15), it is possible that PTHrP(1–34), in addition to its demonstrated effect on hepatic lipid synthesis, may also have direct effects on adipose tissue that contribute to the hypertriglyceridemia. Indeed, there is evidence that PTH can stimulate lipolysis and cause reductions in adipose lipoprotein lipase activity (55–57).

Given the fact that serum amyloid A (SAA) is a major acute phase protein in mice as well as in humans (17), we tested the effect of PTHrP(1–34) on SAA gene expression in mice. PTHrP(1–34) caused a prolonged increase in SAA levels that is similar to the time course of SAA induction seen with LPS administration, although the levels attained were approximately 10-fold lower than those reported in response to LPS (29). As has also been shown for LPS (29, 58), the PTHrP-mediated increase in serum SAA levels was preceded by a marked increased hepatic SAA mRNA levels. In contrast, as was reported for the TG studies, administration of the receptor antagonist PTHrP(7–34) amide had no effect on serum SAA levels, thus demonstrating that this effect PTHrP(1–34) administration could also not be explained by nonspecific, nonreceptor mediated effects of peptide injection. These data therefore suggest that PTHrP acts on hepatocytes via the PTH/PTHrP receptor to increase SAA gene expression and circulating SAA levels.

In summary, the studies reported here present the first evidence to support the postulate that locally induced PTHrP can act within the adult liver via PTH/PTHrP receptors to mediate an important physiological function, the induction of the hepatic acute phase response. More specifically, the data suggest that PTHrP gene expression is induced in periportal hepatocytes during endotoxemia and that locally induced PTHrP may be acting in an autocrine or intracrine fashion to enhance the production of fatty acids and acute phase proteins by hepatocytes during the host response to endotoxin.

Thus, PTH-related protein is an additional member of the cascade of proinflammatory cytokines produced locally within the liver that can stimulate the acute phase response. Cytokines classically have pleotrophic effects, and the host uses redundant cytokine pathways to ensure adequate response. For example, although IL-6 is thought to be a major inducer of Type II acute phase proteins (*e.g.* fibrinogen), IL-6 knockout mice still exhibit increases in these proteins in response to LPS, presumably due to the stimulatory effects of other proinflammatory cytokines (59). In a similar fashion, it is likely that PTHrP is but one of several mediators of the hepatic acute phase response that is unleashed during infection and inflammation. However, in certain situations, such as the clinical use of intermittent dosing with PTH(1–34), PTHrP(1–36), or PTHrP analogs for the treatment of postmenopausal osteoporosis (60–62), the possible role of PTHrP, or PTH, as a single mediator of the acute phase response may certainly be of critical importance. Further studies to determine any potential effects of intermittent stimulation of PTH/PTHrP receptors at sites other than bone will obviously be of utmost importance as these treatment trials progress.

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