Prolactin promotes normal liver growth, survival, and regeneration in rodents: effects on hepatic IL-6, suppressor of cytokine signaling-3, and angiogenesis

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[1]*Instituto de Neurobiología, Universidad Nacional Autónoma de México, Querétaro, México;* [2]*Departamento de Fisiología y Farmacología, Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Aguascalientes, México; and* [3]*Institut National de la Santé et de la Recherche Médicale, U693, Université Paris-Sud, Le Kremlin-Bicêtre, France*

Moreno-Carranza B, Goya-Arce M, Vega C, Adán N, Triebel J, López-Barrera F, Quintanar-Stéphano A, Binart N, Martínez de la Escalera G, Clapp C. Prolactin promotes normal liver growth, survival, and regeneration in rodents: effects on hepatic IL-6, suppressor of cytokine signaling-3, and angiogenesis. *Am J Physiol Regul Integr Comp Physiol* 305: R720–R726, 2013. First published August 15, 2013; doi:10.1152/ajpregu.00282.2013.—Prolactin (PRL) is a potent liver mitogen and proangiogenic hormone. Here, we used hyperprolactinemic rats and PRL receptor-null mice (PRLR$^{-/-}$) to study the effect of PRL on liver growth and angiogenesis before and after partial hepatectomy (PH). Liver-to-body weight ratio (LBW), hepatocyte and sinusoidal endothelial cell (SEC) proliferation, and hepatic expression of VEGF were measured before and after PH in hyperprolactinemic rats, generated by placing two anterior pituitary glands (AP) under the kidney capsule. Also, LBW and hepatic expression of IL-6, as well as suppressor of cytokine signaling-3 (SOCS-3), were evaluated in wild-type and PRLR$^{-/-}$ mice before and after PH. Hyperprolactinemia increased the LBW, the proliferation of hepatocytes and SECs, and VEGF hepatic expression. Also, liver regeneration was increased in AP-grafted rats and was accompanied by elevated hepatocyte and SEC proliferation, and VEGF expression compared with nongrafted controls. Lowering circulating PRL levels with CB-154, an inhibitor of AP PRL secretion, prevented AP-induced stimulation of liver growth. Relative to wild-type animals, PRLR$^{-/-}$ mice had smaller livers, and soon after PH, they displayed an approximately twofold increased mortality and elevated and reduced hepatic IL-6 and SOCS-3 expression, respectively. However, liver regeneration was improved in surviving PRLR$^{-/-}$ mice. PRL stimulates normal liver growth, promotes survival, and regulates liver regeneration by mechanisms that may include hepatic downregulation of IL-6 and upregulation of SOCS-3, increased hepatocyte proliferation, and angiogenesis. PRL contributes to physiological liver growth and has potential clinical utility for ensuring survival and regulating liver mass in diseases, injuries, or surgery of the liver.

liver growth and regeneration; prolactin; vascular endothelial growth factor; interleukin-6; suppressor of cytokine signaling-3; liver angiogenesis

HEPATOCYTES ARE LONG-LIVED and rarely divide under normal conditions except soon after birth and in association with female reproductive events. In rodents, hepatocyte replication peaks during the first 2 wk of age (18), doubles during pregnancy (15), and continues to increase throughout lactation (27). Pathological conditions such as toxic injury and infection trigger hepatocyte proliferation to replace damaged tissue (17).

Numerous growth factors and cytokines stimulate liver growth, and cross-circulation experiments (32) and liver tissue transplants into extrahepatic sites (25) indicate that triggering agents are present in the circulation. Prolactin (PRL), the hormone fundamental for lactation, may contribute to these triggering mechanisms.

PRL increases in the circulation under conditions of physiological and pathological liver growth. Its levels are high in neonates (21), in pregnant and lactating females (3), in patients with liver cirrhosis (33), and in rodents after partial hepatectomy (PH) (9). Liver is the organ that expresses the highest levels of PRL receptors (34), and injection of PRL increases hepatic DNA synthesis, the liver-to-body weight ratio (LBW) (10), and activity of transcription factors involved in hepatic cell proliferation [activator protein-1 (AP-1), Jun, STAT-3] (36). PRL triggers mitogenic signaling pathways in isolated hepatocytes (4) and may also promote liver growth by inducing angiogenesis. PRL stimulates endothelial cell proliferation in various organs through direct stimulation of endothelial cells (13) but also indirectly by inducing the synthesis of VEGF and fibroblast growth factor-2 in nonendothelial cells (13), and it promotes VEGF expression in regenerating livers (36).

Here, we tested the hypothesis that PRL contributes to physiological liver growth and regeneration using hyperprolactinemic rats and PRL receptor-null (PRLR$^{-/-}$) mice that were either intact or subjected to PH. The results support our hypothesis and identify regulation of IL-6, suppressor of cytokine signaling-3 (SOCS-3), sinusoidal endothelial cell (SEC) proliferation, and VEGF expression as mechanisms mediating the actions of PRL.

MATERIALS AND METHODS

Animal experimentation. Male Wistar rats (200–250 g) and PRLR$^{-/-}$ and wild-type (PRLR$^{+/+}$) mice (16-wk-old males and females of *129SvJ* background) were maintained and treated according to local institutional guidelines and in compliance with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health. The Bioethics Committee of the Institute of Neurobiology of the National University of Mexico approved all animal experiments. All surgical procedures were performed between 8:00 AM and 12:00 PM. To avoid stress-induced PRL release, animals were handled daily for 7 days before death. Seventy or 60% PH was carried out in rats and mice, respectively, according to the method of Higgins and Anderson (22). Animals were weighed before PH and at the time of death when resected liver and regenerating liver remnants were weighed, formalin-fixed, or frozen; blood was then collected for subsequent analysis. Fifteen days before PH, hyperprolactinemia was induced in rats by implanting two anterior pituitary

glands (AP) under the kidney capsule, as previously described (2). Control rats were subjected to similar surgery without implantation. Groups of AP-implanted and control rats received a daily, intraperitoneal injection of saline or CB-154 (5 mg/kg body wt) 4 or 7 days before death.

Liver-to-body weight ratio. Liver growth was assessed by the liver-to-body weight ratio (LBW), calculated as 100 times the weight of the organ divided by the body weight of the animal at death. The rate of recovery of liver mass after PH was evaluated by comparing the LBW of the remnant liver immediately after (*day 0*) and at *days 2*, *4*, and *6* after surgery.

Hepatocyte and SEC proliferation. Liver samples were fixed in 10% formalin, dehydrated in a graded alcohol series, and embedded in paraffin. Eight-micrometer paraffin sections were incubated with a 1:500 dilution of anti-PCNA antibody (clone PC10; DakoCytomation, Freiberg, Germany) overnight at 4°C. Immunoreactive cells were detected using the avidin-biotin-peroxidase kit (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). The slides were cover-slipped with Permount, scanned, and analyzed using the ScanScope Digital Scanner (Aperio Technologies, Vista, CA) and Pro-Plus software (Media Cybernetics, Silver Spring, MD). Cell proliferation was evaluated by quantifying the number of PCNA-positive nuclei in hepatocytes or SEC per square millimeter The spindle-shaped sinusoid-lining cells in the open sinusoids were considered to be SEC. Three areas per section and three different sections were evaluated per rat.

Serum PRL. Serum PRL was measured in rats by RIA using standard procedures and reagents provided by the National Hormone and Pituitary Program and by Dr. A. F. Parlow (Harbor-University of California, Los Angeles Medical Center, Los Angeles, CA).

Real-time quantitative RT-PCR. Total RNA was extracted from liver tissue using TRIzol reagent (Invitrogen, Carlsbad, CA), cDNA was synthesized using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA), and real-time quantitative reverse-transcriptase PCR was performed using the Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, Hanover, MD) with a Bio-Rad CFX96 PCR detection system (Bio-Rad Laboratories, Hercules, CA). The sequences of primers used were as follows: *VEGF*: 5'-TGCCAAGTGGTCCCAG-3' (forward) and 5'-GTGAGGTTTGATC-CGC-3' (reverse); *IL-6*: 5'-GAGGATACCACTCCCAACAGACC-3' (forward) and 5'-AAGTGCATCATCGTTGTTCATACA-3' (reverse); *SOCS-3*: 5'-CCCGCGGGCACCTTTCTTAT-3' (forward) and 5'-CAC-TGGATGCGTAGGTTCTTGGTC-3' (reverse); and *ppia* 5'-GGCG-GCAGGTCCATCTACG-3' (forward) and 5'-CTTGCCATCCAGC-CATTCAGTC-3' (reverse). All primers distinguished between genomic and cDNA amplification. Results are expressed as fold induction compared with baseline after normalization to *ppia*.

Serum IL-6. IL-6 serum levels were determined using the BD OptEIA Mouse IL-6 ELISA Set (BD Biosciences Pharmigen, San Diego, CA) following the instructions of the manufacturer.

Statistics. Results are expressed as means ± SE. Differences between two groups at a single time point (Figs. 1 and 2) were evaluated by unpaired Student's *t*-test, whereas comparisons between two and more than two groups at various time points were examined by two-way ANOVA followed by Tukey's post hoc test (Figs. 3 and 4). SigmaStat 7.0 software (Systat Software, San Jose, CA) was used. Survival of PRLR$^{+/+}$ vs. PRLR$^{-/-}$ was analyzed by Cox's *F* test using Statistica software (StatSoft, Tulsa, OK). Differences in means with $P < 0.05$ were considered statistically significant.

RESULTS

PRL promotes growth, hepatocyte and SEC proliferation, and VEGF expression in intact livers. To determine the effect of PRL on normal liver growth, we evaluated the LBW in rats rendered hyperprolactinemic by placing two AP under the kidney capsule for 15 days and in PRLR$^{-/-}$ mice. LBW was elevated in AP-grafted rats, confirmed to be hyperprolactine-

mic ($P = 0.006$, Fig. 1*A*). Hyperprolactinemia was responsible for the increase in LBW, because the effect was abrogated by lowering PRL levels with CB-154, a dopamine D$_2$ receptor agonist that inhibits AP PRL release (38) (Fig. 1*A*). Liver growth was then assessed by the immunohistochemical evaluation of PCNA, a marker of cell proliferation (7). PCNA-positive hepatocytes were detectable in the nongrafted rats, and their number was significantly elevated in the AP-implanted animals ($P = 0.01$, Fig. 2, *A* and *B*). PCNA-positive SEC were identified by the immunoreactivity to PCNA located in elongated nuclei of spindle-shaped sinusoid-lining cells, and their number was found to be significantly higher in the AP-grafted animals ($P = 0.04$, Fig. 2, *A* and *C*). Hyperprolactinemia-induced liver angiogenesis was further suggested by the increased hepatic expression of VEGF in AP-implanted vs. nonimplanted rats ($P < 0.01$, Fig. 2*D*). Consistent with the findings, LBW was reduced in PRLR$^{-/-}$ mice relative to wild-type mice ($P < 0.001$, Fig. 1*B*).

Effect of hyperprolactinemia on liver growth and angiogenesis after PH. Liver regeneration over time was then evaluated in hyperprolactinemic rats subjected to PH 15 days after AP implantation. Circulating PRL levels increase during the first 15 min after PH and return to control values thereafter (9). Consistent with this report, no differences in PRL serum concentrations were found in the nongrafted controls at 2, 4, and 6 days after PH (Fig. 3*A*). AP-grafted rats showed a 7- to 12-fold increase in serum PRL levels over nongrafted controls throughout the 6-day period following PH ($P \leq 0.01$, Fig. 3*A*). LBW was higher at *day 0* and continued to be elevated at *days 2*, *4*, and *6* post-PH ($P \leq 0.01$ - 0.04 vs. control, nongrafted rats, Fig. 3*B*). Because hyperprolactinemic rats start out with a higher LBW value prior to PH, recovery of liver mass was evaluated by plotting LBW levels after PH relative to estimated whole LBW values immediately after surgery (*day 0*) (Fig. 3*C*). Two days after PH, hyperprolactinemic animals showed a significantly higher recovery of liver mass that leveled out thereafter, indicating a faster recovery of liver mass in hyperprolactinemic compared with normoprolactinemic rats. To assess the causal relationship with hyperprolactinemia, AP-im-
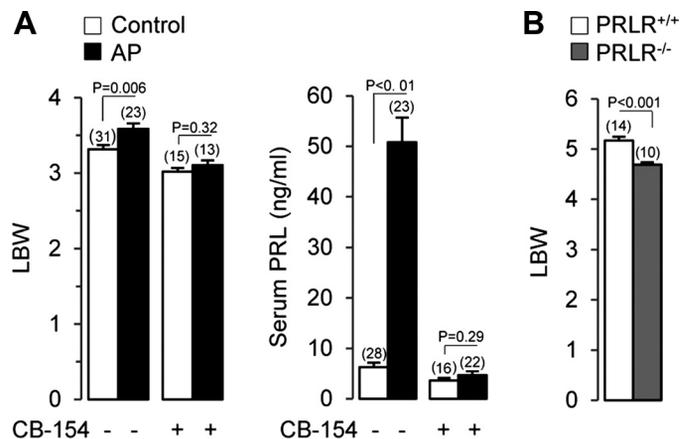

Fig. 1. Prolactin (PRL) promotes growth in intact livers. (*A*) Liver-to-body weight ratio (LBW) and serum PRL levels in rats nontransplanted (control) or transplanted with two anterior pituitary glands under the kidney capsule (AP) and injected or not with the dopamine D$_2$ receptor antagonist, CB-154. *B*: LBW in wild-type (PRLR$^{+/+}$) and PRL receptor-deficient (PRLR$^{-/-}$) mice. Bars represent means ± SE. Numbers inside parentheses indicate *n* values.
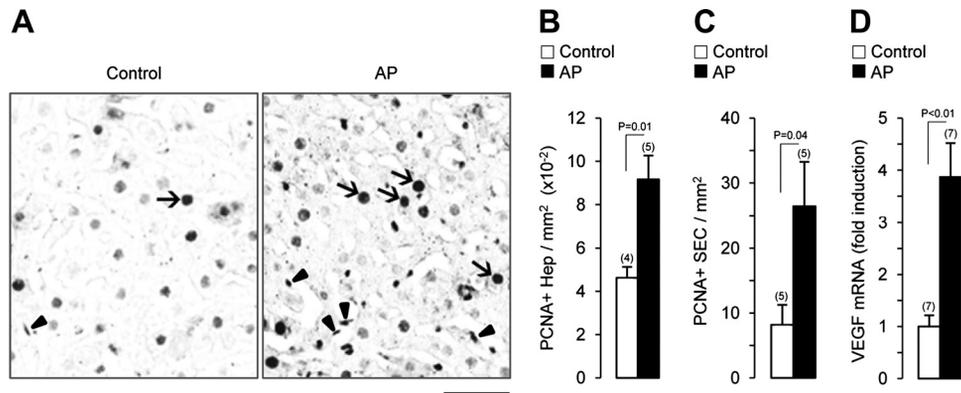
Fig. 2. PRL stimulates the proliferation of hepatocytes (Hep) and sinusoidal endothelial cells (SEC) and the expression of VEGF in intact livers. *A*: representative photographs of hepatocytes and SEC immunostained for PCNA in liver sections from rats nontransplanted (control) or transplanted with two anterior pituitary glands under the kidney capsule (AP). Arrows and arrowheads indicate some PCNA-positive Hep and SEC, respectively. Scale bar = 100 μm. Quantification of hepatocytes (*B*) and SEC (*C*) positive for PCNA/mm^2 of liver tissue in Control and AP rats. *D*: quantitative RT-PCR-based evaluation of VEGF mRNA levels in livers from control and AP-implanted rats. Bars represent means ± SE. Numbers inside parentheses indicate n values.

planted and nonimplanted rats were injected with CB-154 before and after being subjected to PH and killed 2 days after surgery. Treatment with CB-154 reduced circulating PRL to basal levels and blunted the increase in LBW in AP-implanted rats (Fig. 3, *A* and *B*), indicating that hyperprolactinemia is responsible for this increase. Liver growth and angiogenesis were then evaluated in hyperprolactinemic rats by measuring the proliferation of hepatocytes and SEC after PH. Immuno-histochemical analysis of PCNA staining in regenerating livers of control rats showed higher numbers of proliferating hepatocytes and SEC that peaked at 2 and 4 days after surgery (Fig. 3, *D–F*). Hepatocyte proliferation was significantly increased on postoperative *days 0, 2,* and *4* in hyperprolactinemic vs. control rats ($P \leq 0.01$–0.04, Fig. 3*E*). Also, hyperprolactinemic animals showed elevated SEC proliferation on *days 0* and *4* post-PH ($P \leq 0.01$, Fig. 3*F*) and enhanced *VEGF* mRNA levels on *days 0* and *2* after surgery ($P \leq 0.01$–0.05, Fig. 3*G*). These results confirm the mitogenic effect of PRL on hepatocytes and suggest that enhanced angiogenesis contributes to its stimulatory effect on liver regeneration.

PRLR$^{-/-}$ mice show increased mortality, elevated hepatic expression, and serum levels of IL-6, reduced hepatic SOCS-3 expression, and reduced liver growth after PH. Mortality after PH was higher [Cox's *F* test: $F(22,42) = 1.87$, $P = 0.038$] in PRLR$^{-/-}$ mice (21 of 53, 40%) compared with wild-type animals (11 of 47, 23%). PRLR-null mice died between 1 and 2 days post-PH, suggesting that mortality was not related to the surgical procedure. IL-6 is a key cytokine produced and released into the circulation mainly by Kupffer cells during the first hours of liver regeneration that promotes hepatoprotection and mitogenesis (43). However, overproduction of IL-6 can also inhibit hepatocyte growth and cause liver injury (47) and mortality (28). Because PRL reduces LPS-induced IL-6 synthesis in Kupffer cells (49), we tested whether PH-induced production of IL-6 is altered in PRLR$^{-/-}$ mice and may, thus, contribute to enhanced mortality. As expected (44), expression and circulating levels of IL-6 were elevated at 3 and 6 h after PH in wild-type mice ($P < 0.01$–0.001; Fig. 4, *A* and *B*). In PRLR$^{-/-}$ mice, hepatic *IL-6* mRNA levels were 3- to 15-fold higher throughout the first 24 h after PH ($P < 0.01$–0.001, Fig. 4*A*) and were associated with significantly higher levels of IL-6

in the circulation at 3 and 6 h post-PH ($P = 0.02$–0.047, Fig. 4*B*). Because SOCS-3 is a negative regulator of IL-6 synthesis and signaling that is transiently expressed during liver regeneration (11) and is upregulated by PRL in the liver (37), we examined whether deficiency of PRLR affected hepatic SOCS-3 expression in the early phases of PH. PRLR-null mice showed significantly lower levels of *SOCS-3* mRNA at 3 and 6 h after PH compared with wild-type mice ($P < 0.001$–0.006, Fig. 4*C*).

To further evaluate the involvement of PRL in the control of liver regeneration, we compared the LBW values after PH between control and PRLR$^{-/-}$ mice (Fig. 4*D*). Consistent with the reduced LBW found in intact PRLR$^{-/-}$ mice (Fig. 1*B*), LBW was significantly lower immediately after PH (*day 0*) ($P = 0.029$ PRLR$^{+/+}$ vs. PRLR$^{-/-}$, Fig. 4*D*). The LBW continued to be reduced in PRLR$^{-/-}$ mice 2 days after PH ($P = 0.043$ PRLR$^{+/+}$ vs. PRLR$^{-/-}$) but was similar to the LBW of control mice at *days 4* and *6* after PH (Fig. 4*D*). Because PRLR$^{-/-}$ mice start out with a lower LBW prior to PH, recovery of liver mass was evaluated by plotting LBW levels after PH relative to estimated whole LBW values immediately after surgery (*day 0*) (Fig. 4*E*). A similar recovery of liver mass was found between PRLR$^{-/-}$ and PRLR$^{+/+}$ mice 2 days after PH ($P = 0.47$ PRLR$^{+/+}$ vs. PRLR$^{-/-}$, Fig. 4*E*), indicating that the reduction in LBW found in PRLR$^{-/-}$ mice at this time (Fig. 4*D*) is due not to decreased liver regeneration but to the fact that these mice have lower LBW values prior to PH. Moreover, PRLR$^{-/-}$ mice showed a higher liver mass recovery 4 and 6 days after PH ($P < 0.02$–0.03, Fig. 4*E*), indicating that the absence of PRL signaling enhances the final phases of liver regeneration.

DISCUSSION

The central role of endocrine factors in physiological liver growth and hepatic regeneration has been known for decades (6, 25, 27, 32), but the contribution of specific hormones is still unclear. The present study demonstrates that PRL can act both on the normal and regenerating liver to stimulate their growth and angiogenesis. Moreover, we show that PRL promotes animal survival after PH by mechanisms that may involve the
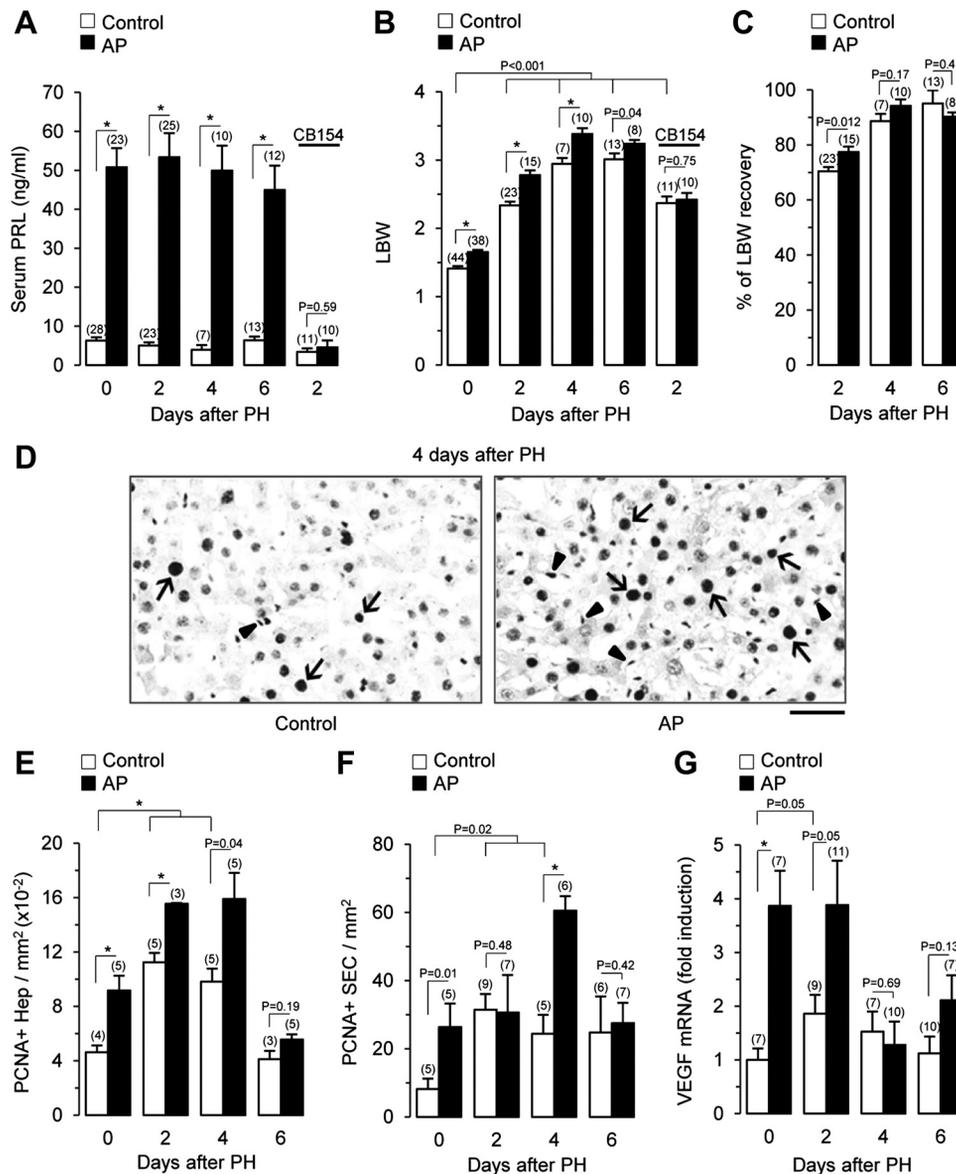
Fig. 3. PRL stimulates liver growth, hepatocyte (Hep), and SEC proliferation, and expression of VEGF after PH. Serum PRL levels (*A*) and LBW (*B*) in rats nontransplanted (control) or transplanted with two anterior pituitary glands under the kidney capsule (AP) immediately (*day 0*), and 2, 4, and 6 days after partial hepatectomy (PH). Some control and AP-transplanted animals were injected with CB-154 and evaluated 2 days after PH. *C*: percentage of recovery of LBW in control and AP rats 2, 4, and 6 days after PH. *D*: representative photographs of Hep and SEC immunostained for PCNA in liver sections from rats nontransplanted (control) or transplanted with two anterior pituitary glands under the kidney capsule (AP) and evaluated 4 days after PH. Arrows and arrowheads indicate some PCNA-positive Hep and SEC, respectively. Scale bar = 100 μm. Quantification of hepatocytes (*E*) and SEC (*F*) positive for PCNA/mm^2 of liver tissue in control and AP rats immediately (*day 0*), and 2, 4, and 6 days after PH. *G*: qRT-PCR-based evaluation of VEGF mRNA levels in livers from control and AP rats on *days 0, 2, 4,* and *6* after PH. Bars represent means ± SE. Numbers inside parentheses indicate *n* values. *$P \leq 0.01$.

downregulation and upregulation of hepatic IL-6 and SOCS-3, respectively.

PRL is a versatile hormone that acts on a wide variety of target tissues to regulate reproduction, osmoregulation, energy metabolism, brain function, immune response, growth, and angiogenesis (3, 6, 13). The liver is the organ with the largest number of PRL receptors (34), and PRL regulates liver function and growth, yet these actions are poorly understood. PRL acts on fetal liver to promote the differentiation of red blood progenitor cells (40) and stimulate lipid metabolism (30). It induces adult liver to produce Hageman factor (20) and may contribute to bile secretion by promoting the proliferation of cholangiocytes under normal and diseased conditions (41).

PRL was reported to be a mitogen for normal liver nearly 30 years ago, when repeated injections of pharmacological doses of the hormone stimulated hepatic DNA synthesis and the mitotic index, caused hepatomegaly, and promoted the expression of preneoplastic lesions when given after a hepatocarcinogen (10).

Here, we have used mice carrying a homozygous deletion of the *PRLR* gene to investigate the role of PRL in normal liver growth. We found that absence of the PRLR confers reduced liver mass. It is possible that nonspecific effects of PRL during development contribute to this phenotype. Placental lactogens and pituitary PRL lead to metabolic adaptations in the mother, such as increased food intake, β-cell expansion, and insulin
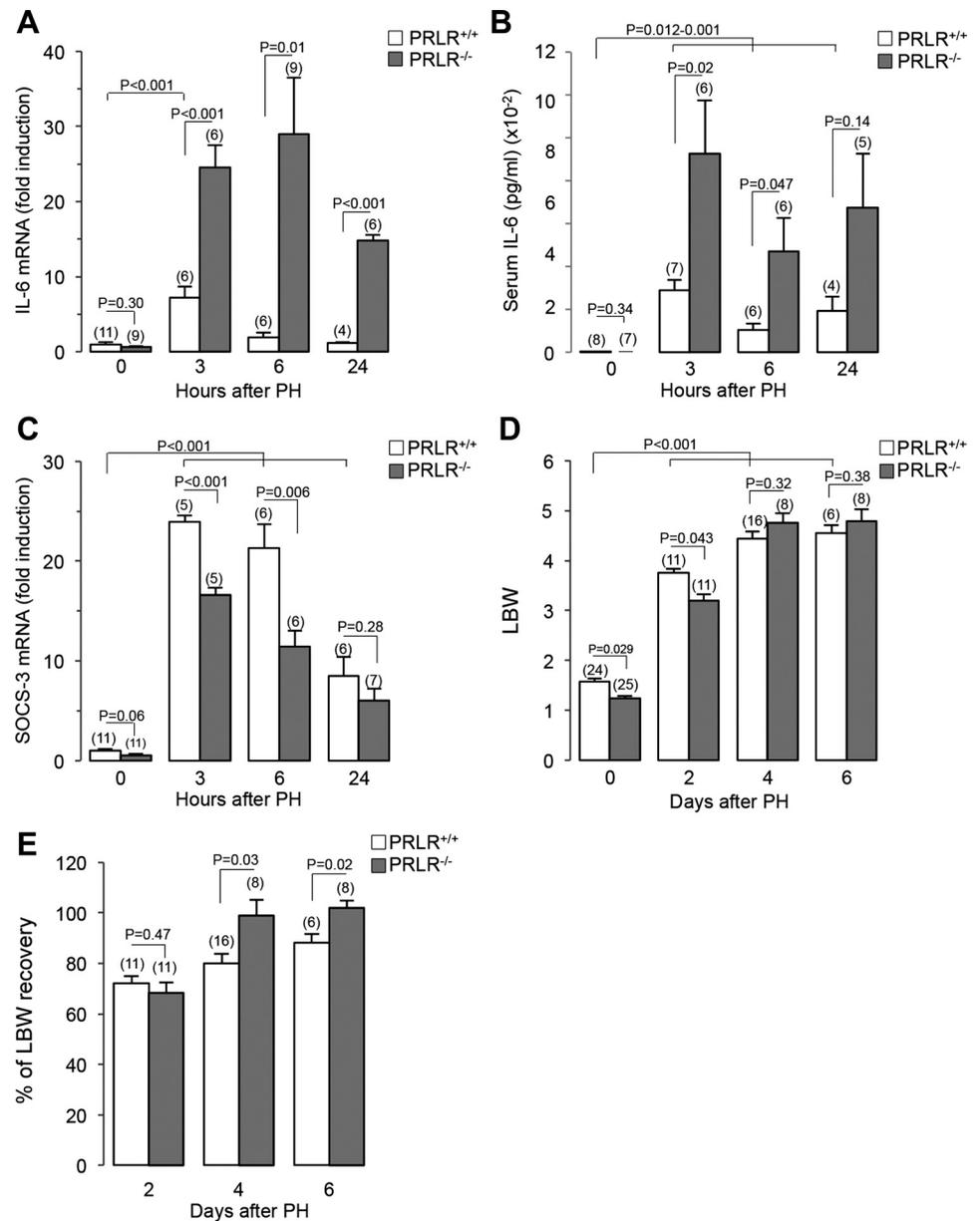
Fig. 4. PRL receptor-deficient (PRLR$^{-/-}$) mice show increased hepatic expression and circulating levels of IL-6, lower hepatic expression of SOCS-3, and reduced liver growth. PRLR$^{-/-}$ and wild-type (PRLR$^{+/+}$) mice were evaluated at 0, 3, 6, and 24 h post-PH for the hepatic expression of IL-6 mRNA (*A*), circulating IL-6 (*B*), hepatic SOCS-3 mRNA (*C*), and LBW (*D*). mRNA was quantified by quantitative RT-PCR. *E*: percentage of recovery of LBW in PRLR$^{+/+}$ and PRLR$^{-/-}$ mice 2, 4, and 6 days after PH. Bars represent means ± SE. Numbers inside parentheses indicate *n* values.

production (35), which may promote fetal and neonatal liver development. However, the facts that fetal liver expresses the PRLR (19) and that PRL stimulates the proliferation of cultured hepatocytes isolated from 20-day-old rats (45) suggest that PRL can signal directly to stimulate liver growth during development and that this action is required to achieve normal liver size. Consistent with this finding, we also showed that inducing persistent hyperprolactinemia, to levels similar to those (50–250 ng/ml) circulating in pregnancy and lactation (3), stimulates liver mass, and that this effect disappears when PRL levels are lowered to basal with CB-154.

Hyperprolactinemia-induced liver growth entailed the enhanced proliferation of hepatocytes. Adult hepatocytes express the PRLR (39), and PRL acts on cultured hepatocytes to stimulate expression of the growth-related genes *Fos, Jun,* and *Src* (4) and to promote proliferation (45). In addition, hyperprolactinemia stimulated SEC proliferation and hepatic VEGF expression, indicating that PRL promotes angiogenesis in in-

tact livers. Because liver growth requires angiogenesis (5, 42) and SEC proliferation strongly depends on VEGF (48), angiogenesis may be an important component of the PRL effect on liver growth. While PRL may be acting through VEGF to stimulate SEC proliferation, a direct effect of PRL on SEC is suggested by the detection of the PRLR on hepatic sinusoidal membranes (39), and by the mitogenic action of PRL on cultured endothelial cells (12).

The facts that deletion of the PRL receptor reduces liver mass and that hyperprolactinemia makes normally quiescent hepatocytes and SEC become proliferating cells, suggest that PRL has an effect on physiological liver growth. Indeed, studies in rats show that LBW is high in nursing pups during lactation (18) when they are being exposed to high levels of PRL in milk (46). Moreover, the PRLR is upregulated in the pregnant liver (23), and hyperprolactinemia associates with liver enlargement during pregnancy and lactation (3, 15, 27).

PRL also influences early events of liver regeneration leading to animal survival and hepatic growth. Circulating PRL levels increase shortly after PH (9), and PRL administration stimulates the binding activity of AP-1, JUN, and STAT-3 within the first 5 to 12 h following PH (36). Activation of these transcriptional factors is part of the signaling cascade elicited by important cytokines, including IL-6, to promote hepatoprotection and mitogenesis (14, 43). Here, we found that the elimination of PRL signaling in PRLR$^{-/-}$ mice results in increased mortality, indicating that PRL stimulates the adaptive response to PH required for animal survival.

IL-6 is a key cytokine mediating homeostatic adaptations involved in animal survival and liver growth after resection. IL-6 deficiency increases mortality and reduces hepatocyte proliferation after PH (14, 43). However, the levels of IL-6 must be carefully adjusted since exposure to higher levels of IL-6 causes liver failure, increased mortality, and reduced regenerative capacity (24, 28, 47). Because, the hepatic expression and serum levels of IL-6 are elevated in PRLR$^{-/-}$ mice throughout the first 24 h of liver regeneration, PRL may signal to adjust IL-6 levels after PH. Although a nonhepatic source of serum IL-6 in PRLR$^{-/-}$ mice cannot be excluded, hepatic Kupffer cells are the primary source of serum IL-6 after liver resection (1), and PRL may, thus, reduce IL-6 levels by upregulating the production of SOCS-3 in the remnant liver. SOCS-3 is an important negative regulator of IL-6 production and signaling, and it acts by preventing the activity of NF-κB (8) and the tyrosine phosphorylation/activation of STAT-3 (11). PRL induces SOCS-3 expression in liver (37), and, thus, deletion of the PRLR reduces mRNA levels of *SOCS-3* during the first 24 h after PH (present study).

Consistent with PRL playing a role in liver regeneration, LBW after PH was increased in hyperprolactinemic rats. These findings agree with previous reports showing that the injection of PRL before PH stimulates the early proliferation of hepatocytes and the activity of factors promoting growth, differentiation, and energy metabolism in the liver (36). Contrary to our observations, an earlier study failed to show enhanced LBW in hyperprolactinemic, AP-grafted rats 2 days after PH (26). The reason for this discrepancy is unclear, but the lack of a significant difference may relate to a small number of animals.

The stimulatory effect of hyperprolactinemia on liver regeneration correlates with enhanced hepatocyte proliferation and angiogenesis. As expected from the proliferation dynamics of hepatic cells following PH (43), the mitogenic effect of PRL occurred earlier in hepatocytes than in SEC, and this effect could be functionally linked to VEGF. VEGF stimulates the proliferation of hepatocytes and SEC during regeneration (5, 29, 42), PRL treatment induces the early expression of VEGF in regenerating livers (36), and VEGF expression is elevated in hyperprolactinemic animals 2 days after PH (present study). However, in contrast to the stimulatory effects of hyperprolactinemia, deletion of the PRLR did not modify liver regeneration 2 days after PH but enhanced it thereafter. The reason for this discrepancy is unclear, but it is possible that compensatory mechanisms able to subserve the stimulatory role of PRL are activated in PRLR$^{-/-}$ mice and result not only in no reduction, but in enhancement of liver regeneration. Because this enhancement occurred late in the regeneration process, it may involve the overactivation of compensatory mechanisms

promoting angiogenesis, which would warrant further investigation. Thus, in spite of PRLR$^{-/-}$ mice having a nearly twofold higher mortality after PH relative to wild-type mice, the surviving animals display an improved liver regeneration. Recovery after higher (4-fold) mortality occurs in surviving IL-6$^{-/-}$ mice (14), illustrating the functional redundancy of the liver regeneration process.

Perspectives and Significance

The present study shows that PRL is required for normal liver growth and that it regulates animal survival and liver regeneration after resection. It provides insights into the action of this hormone during early events leading to body homeostasis, hepatocyte proliferation, and liver angiogenesis. However, further investigation is needed to help establish causative links between these events and hormone action and to evaluate the cellular and molecular mechanisms activated by PRL that result in hepatoprotection, liver growth, function, and regeneration both in the absence and presence of the PRLR. Interestingly, hyperprolactinemia accompanies liver growth under normal and pathological conditions. Liver cirrhosis is commonly associated with elevated levels of PRL and with gynecomastia (16, 33). Because, hyperprolactinemia may help preserve hepatic cell function and growth in the advanced disease, efforts to correct PRL levels may be disadvantageous. Moreover, the use of current medications known to increase prolactinemia (31) constitutes potential therapeutic options in liver diseases, liver injuries, or after liver surgery and warrants further investigation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: B.M.-C., M.G.-A., C.V., and C.C. conception and design of research; B.M.-C., M.G.-A., C.V., N.A., F.L.-B., and A.Q.-S. performed experiments; B.M.-C., M.G.-A., C.V., N.A., and C.C. analyzed data; B.M.-C., M.G.-A., C.V., J.T., G.M.d.l.E., and C.C. interpreted results of experiments; B.M.-C. and M.G.-A. prepared figures; B.M.-C. and C.C. drafted manuscript; B.M.-C., M.G.-A., J.T., N.B., G.M.d.l.E., and C.C. edited and revised manuscript; B.M.-C., M.G.-A., C.V., N.A., J.T., F.L.-B., A.Q.-S., N.B., G.M.d.l.E., and C.C. approved final version of manuscript.

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