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ATP release after partial hepatectomy regulates liver regeneration in the rat

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Abstract

Background & aims—Paracrine interactions are critical to liver physiology, particularly during regeneration, although physiological involvement of extracellular ATP, a crucial intercellular messenger, remains unclear. The physiological release of ATP into extracellular milieu and its impact on regeneration after partial hepatectomy were investigated in this study.

Methods—Hepatic ATP release after hepatectomy was examined in the rat and in human living donors for liver transplantation. Quinacrine was used for *in vivo* staining of ATP-enriched compartments in rat liver sections and isolated hepatocytes. Rats were treated with an antagonist for purinergic receptors (Phosphate-6-azo(benzene-2,4-disulfonic) acid, PPADS), and liver regeneration after hepatectomy was analyzed.

Results—A robust and transient ATP release due to acute portal hyperpressure was observed immediately after hepatectomy in rats and humans. Clodronate liposomal pre-treatment partly inhibited ATP release in rats. Quinacrine-stained vesicles, co-labeled with a lysosomal marker in liver sections and isolated hepatocytes, were predominantly detected in periportal areas. These vesicles significantly disappeared after hepatectomy, in parallel with a decrease in liver ATP content. PPADS treatment inhibited hepatocyte cell cycle progression after hepatectomy, as revealed by a reduction in bromodeoxyuridine incorporation, phosphorylated histone 3 immunostaining, cyclin D1 and A expression and immediate early gene induction.

Conclusion—Extracellular ATP is released immediately after hepatectomy from hepatocytes and Kupffer cells under mechanical stress and promotes liver regeneration in the rat. We suggest that in hepatocytes, ATP is released from a lysosomal compartment. Finally, observations made in living donors suggest that purinergic signalling could be critical for human liver regeneration.

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Keywords

ATP release; purinergic signalling; liver regeneration

Introduction

After two-thirds partial hepatectomy (PH), rodent liver completely restores its initial mass within seven to ten days, while hepatic functions are only slightly and transiently altered [1]. Although cytokines and growth factors are known to be involved in the multi-step process of liver regeneration [1], other endocrine, paracrine and neural factors may also play important roles especially in early stages, including extracellular adenosine triphosphate (ATP) [2] which has been less studied. Signalling via extracellular ATP and their plasma membrane purinergic receptors i.e. P2Y (G protein-coupled receptors) and P2X (ligand-gated ion channels), is vital not only in excitable but also in non-excitable cells and tissues [3]. An extracellular release of ATP from epithelial cells has been reported to occur under different stress conditions, particularly under osmotic or mechanical challenge [4]. The mechanisms involved in this release are not completely understood and include mainly vesicular exocytosis and membrane channels and transporters, that have not been precisely defined [4]. Vascular shear stress and/or organ distension are known to trigger ATP release from endothelial, epithelial and other cell types [5], suggesting that ATP release may occur in liver diseases with vascular or biliary obstruction. Moreover, each liver cell type expresses its own repertoire of purinoceptor subtypes and ectoATPases [6] and evidence for a crucial impact of purinergic signalling in liver physiology is growing, including modulation of bile secretion [7] and ischemia protection [8]. Recent studies suggested that extracellular ATP had a positive effect on proliferation of primary rat hepatocytes *in vitro* [2], and revealed that the well known depletion of liver ATP content in response to PH [9] occurred immediately after surgery [10]. However in these studies, direct data supporting a potential ATP release in this context and a complete liver regeneration analysis were lacking.

In the present work, we provide the first *in vivo* evidence for a transient and robust ATP release from the rat liver immediately after PH, resulting from an intrahepatic mechanical stress. We also show that the released ATP potentially contributes to hepatocyte cell cycle entry in response to PH. Finally, we found that ATP was released from liver after PH in living donors for transplantation and that human hepatocytes expressed purinoceptors, suggesting that purinergic signalling may as well regulate human liver regeneration.

Materials and methods

Patients

Four living donors for liver transplantation — two men and two women (mean age 39.0 ± 5.1 years) — were studied. The surgical techniques used for harvesting a right-sided liver graft from a living donor have been described elsewhere [11]. Blood samples (5 ml) were collected from portal and hepatic veins before, immediately (30 s), 20 min and one hour after right lobe clamping (portal vein and hepatic artery), mixed with a saline stop solution containing 4.15 mM EDTA and 40 mM Tricine buffer for nucleotide stabilization [12], and centrifuged twice for 2 min at 13,000 g (20°C); plasma was stored at -80°C until use. Isolated human hepatocytes were prepared as previously reported [13].

Animal surgery

Animal experiments were carried out according to the CEE directives for animal experimentation (decree 2001-131; 'J.O.' 06/02/01). Two-thirds PH and sham surgery was

performed on fed adult male Sprague-Dawley rats (250-270 g) as described [14,15], with 100% survival rates.

Treatments and blood sampling in rats

Pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid), PPADS (Sigma), a well known P2 purinergic receptor general antagonist [16], or its vehicle (NaCl 0.9%), was injected in the portal vein (0.3 ml, 20 mg/ml) 5 min before PH. In some experiments, clodronate liposomal (4 ml/kg) was injected intravenously 48 h before inclusion in the experiments, to eliminate Kupffer cells [17] (Supp. Fig. 1). Quinacrine (Mepacrine, Sigma, 10 mg/ml in NaCl 9%) was injected (50 mg/kg, IP) in a separate series of rats 24 hours before PH, in order to stain intracellular ATP-storing compartments [18,19], and liver samples were taken before and 5 min after PH, frozen and either cryocut or homogenized for spectrofluorimetric measurement of quinacrine fluorescence ($\lambda_{ex} = 435$ nm, $\lambda_{em} = 500$ nm).

Blood ATP concentration was measured in the anesthetized rat, after a catheter was inserted via the jugular vein in the inferior vena cava, downstream of hepatic veins. Blood samples were serially taken before and after PH, and immediately mixed with the stop solution [12]. Bile was collected through a catheter inserted in the bile duct (Biotrol, France) before and after PH, diluted in perchloric acid (0.8 M), and precipitated in K_2HPO_4 as described [20]. After centrifugation of plasma and bile samples, supernatants were frozen (-80°C) until ATP measurements.

Isolated perfused liver

Liver perfusion, bile flow ($\mu\text{l}/\text{min}/\text{g}$ liver) and portal pressure (mm Hg) measurements were performed as previously described [15]. Effluent perfusate and bile were taken and treated as described above for ATP measurements [12,20]. In a series of experiments, lobe ligation was performed while two thirds of the incoming portal perfusate flow were simultaneously derived, in order to prevent hyperpressure in the remnant liver (referred as “isobaric PH”, see Supp. Fig. 2).

Biochemical assays

ATP measurements were made using the luciferin-luciferase ATP bioluminescent assay kit (Sigma, St Louis, MO, USA) and a Lumac luminometer (M1071). Plasma alanine aminotransferases (ALT) were measured by a Synchron LX20 Clinical System (Beckman Coulter, France) analyzer.

Immunohistochemistry and cytochemistry

One hour before liver sampling, rats were injected with bromodeoxyuridine (BrdU) (50 mg/kg IP) and BrdU immunohistochemistry was performed on frozen liver sections, as described by the manufacturer (Roche Diagnostics, France) [15]. Phosphorylated histone H3 antibody (1/200, Upstate Biotech, US) was used as a mitosis marker [21].

In quinacrine-pretreated rats, liver cryosections were analyzed by epifluorescence and confocal microscopy. Fresh cryocut liver sections were incubated 30 min at 37°C with TRITC-phalloidin (1/400) to detect sub-membrane F-actin and with 100 nM LysoTracker Red ($\lambda_{Ex}: 577$ nm, $\lambda_{Em}: 590$ nm, Molecular Probes, US) to detect lysosomes.

Rat hepatocytes were isolated as previously described [15] and co-labeled with $5 \mu\text{M}$ quinacrine and with 100 nM LysoTracker Red or with 250 nM mito Tracker Red ($\lambda_{Ex}: 581$ nm, $\lambda_{Em}: 644$ nm, Molecular Probes, US) for 30 min at 37°C .

Immunoblot

Proteins were analyzed by SDS-polyacrylamide gel electrophoresis with antibodies specific for cyclin D1 and cyclin A (Santa Cruz Biotechnology, Santa Cruz, CA). The protein bands were detected by enhanced chemiluminescent reaction and signal intensity was semi-quantified by densitometry, as described previously [15].

Rt-Pcr

Total RNA was isolated from total human liver or isolated hepatocytes, and from total rat liver, with TRI Reagent (Sigma, France) according to the manufacturer's recommendations. cDNA was amplified by PCR in the presence of specific primers detailed in supporting material.

Statistical analysis

Student's t test was used to compare sample means with paired or unpaired controls, as appropriate. Results are expressed as means $\pm$ SEM. P values ≤ 0.05 were considered statistically significant.

Results

ATP is released by the liver immediately after PH in response to mechanical stress

The mechanical stress occurring following PH [22] has been suggested as an initial trigger for liver regeneration [23]. We observed that ATP output downstream hepatic veins increased immediately after lobe ligation and was sustained during 4 to 5 minutes (Fig. 1A). An important increase in ATP release in bile was also observed after PH (Fig. 1A, insert). As a control, ATP did not increase in blood from the femoral vein at the same time points after PH (data not shown). As observed *in vivo*, in the isolated perfused liver, ATP output immediately increased after lobe ligation and was sustained for 4 to 5 minutes (Fig. 1B). An elevation of ATP release was also documented in bile in the isolated perfused liver (Fig. 1C). The robust increase observed in ATP output after PH could not be due to cell lysis, because K^+ and protein output was not increased after PH (data not shown). Portal vein pressure (PVP) rose immediately after PH, *in vivo* (Fig. 2A), as well as in the isolated perfused liver (Fig. 2B). When this hyperpressure was prevented in the isolated perfused liver, by performing perfusate derivation during PH (Fig. 2C, "isobaric PH", see methods and Supp. Fig. 2), ATP release did not occur (Fig. 2D). Moreover, in the same experiments, ATP release was further demonstrated to be causally linked with hyperpressure, since it was observed when full rate perfusion was restored (Fig. 2D). The source of ATP release in the liver could potentially be parenchymal as well as non-parenchymal cells, which are all subjected to the PH-induced mechanical stress. As a first approach to address this question, selective elimination of Kupffer cells by administration of clodronate liposomal [17] was performed and resulted in a significant decrease of ATP output in the hepatic venous effluent (Fig. 1B) but not in biliary ATP output (Fig. 1C) as compared with controls. Thus, ATP is abruptly released in the liver after PH under mechanical stress, because of the immediate intrahepatic hyperpressure.

ATP is zonally distributed and stored in lysosomes in the rat liver

We used quinacrine, a fluorescent dye that belongs to the quinoline-acridine class to detect releasable ATP stores [18,19]. One day after rats were injected with quinacrine, livers were sampled before and 5 min after PH. In pre-PH liver sections, quinacrine staining was predominantly detectable in the periportal (PP) areas whereas perivenous (PV) zones were weakly stained (Fig. 3A-C). Confocal microscopic analysis of sections stained with quinacrine and phalloidin (used to identify the sub-plasmalemmal cytoskeleton) revealed

that hepatocytes, as well as sinusoidal cells, contained vesicular ATP-enriched compartments (Fig. 3G). Vesicles were distributed throughout hepatocyte cytoplasm but were particularly found beneath the plasma membrane, including at the canalicular poles, as delineated by their tubular-shaped actin reinforcements (see arrows in Fig. 3G). Further analysis is required to fully identify extra-hepatocytic quinacrine labeled cells, but at least endothelial cells, which have been already reported to release ATP under shear stress stimulation [5], and Kupffer cells (see above and Fig. 1B), are likely candidate cells. Furthermore, as shown on rat isolated hepatocytes (Fig. 4A-C) and liver sections (Fig. 4E-G), hepatocyte ATP-containing vesicles were co-labeled with lysotracker, suggesting that a lysosomal ATP storage compartment exists in hepatocytes and may be releasable upon stimulation. In isolated rat hepatocytes, quinacrine staining was distinct from mitotracker labeling (Fig. 4D), and was clearly reinforced in the pericanalicular area in hepatocyte doublets (Fig. 4H). Thus, our data suggest that, at least in hepatocytes, lysosomal exocytosis could occur immediately after PH, releasing ATP in the extracellular spaces.

ATP distribution in the liver is altered after PH in the rat

The lobular pattern of ATP-containing vesicles was significantly altered five minutes after PH, i.e., almost the entire lobule was uniformly stained, the vesicular feature of the staining being markedly lost. Vesicular-shaped staining was still only visible very close around the central veins (Fig. 3D-F). These data suggested that ATP was released after PH from the vesicular storage compartment described above, mainly from the PP area.

In line with these data, a significant reduction in quinacrine fluorescence on liver homogenates was observed 5 min after PH (Fig. 3H), consistent with the reported fall in hepatic ATP content in response to PH [9,10].

ATP contributes to liver regeneration after PH in the rat

ATP is thus strongly released by the liver after PH, and it can regulate hepatocyte proliferation *in vitro* [2]. We thus treated rats with either PPADS, a broad P2 purinergic receptor antagonist [16], or its vehicle, and examined liver regeneration after PH. As shown in figure 5, a significant inhibition of hepatocyte cell proliferation after PH was observed in treated as compared to control rats. The DNA synthesis peak (Fig. 5A) was significantly dampened in PPADS rats, as evaluated by BrdU incorporation and Ki67 immunostaining (Fig. 5A,B). Hepatocyte mitosis monitored by PH3 immunolabeling was also significantly reduced in PPADS treated rats as compared to control at 72h after PH (Fig. 5C). Moreover, cyclin A and D1 expression at H24, and cfos and cjun induction at 0.5, 1 and 2 hours after PH were inhibited by PPADS treatment (Fig. 6).

ATP release after PH in humans

Our observations in the rat led us to search for an ATP release in human livers after PH in the context of living donors for transplantation. Blood sampling was performed before and at different times after right lobe exclusion, in the portal and hepatic veins. As shown in Fig. 7A, although ATP was taken up by the liver before PH, a net ATP release was observed after PH. This ATP release was not due to cell lysis, as suggested by the lack of increase in ALT in the same plasma samples (Fig. 7B). We also documented an immediate portal hyperpressure after PH in living donors (Fig. 7C). Thus, similarly to rats, ATP is released from human liver after PH, concomitantly with an acute portal hyperpressure, with unknown functional impact. We found that human liver and isolated hepatocytes express P2Y₂, P2X₄ and P2X₇ receptor mRNAs (Fig. 7D), further suggesting that purinergic signalling machinery may be operational in human liver after PH.

Discussion

In this study we addressed the physiological role of ATP purinergic signalling during liver regeneration, with special emphasis on the mechanically-induced ATP release after PH in rats and humans. We examined ATP storage compartments in the liver and searched for the functional impact of ATP release on liver regeneration.

We show that ATP is abruptly released from the liver after PH, and demonstrate in the rat that this release is triggered by an immediate intrahepatic hyperpressure. Our results are consistent with recent data [10] showing that the known depletion in liver ATP content after PH [9] occurred within seconds to minutes and was not due to an increase in energy demand. Each liver cell type may be involved in this release. It is well established that cultured liver cells, including human hepatocytes [24], can release ATP upon shear stress or osmotic challenge [25,26]. Herein we provide evidence that Kupffer cells contribute to the ATP release after PH. The mechanisms involved, direct release in response to mechanical stimulation, or indirect enhancement of hepatocyte ATP release remains to be assessed. Hepatocytes are probably the main cell type involved. Using quinacrine as a marker of releasable ATP stores [18,19], we determined that ATP, predominantly localized in hepatocytes of PP areas, is rapidly lost from these regions, probably because PP areas are more directly exposed to the acute portal hypertension after PH. At the intracellular level, ATP-enriched compartments were found to be partly lysosomes. Consistent with this, lysosome fusion with plasma membrane constitutes a form of regulated exocytosis [27] that can allow ATP release [28]. Other pathways for ATP release, involving different membrane transporters, channels or pores, may also contribute to the observed ATP release after PH [4]. Thus our data provide the first direct evidence that ATP is immediately released after PH under mechanical stress, concomitantly with a rapid fall in hepatic ATP content. However, mechanotransduction pathways involved in PH-induced ATP release remain to be identified [29]. ATP has been shown to be released in the wall of vessels and hollow organs under mechanical stress [6]. Interestingly, distension-induced ATP release mediated by exocytosis has been already reported in the urinary tract [30], but not in the liver. Post-PH portal hyperpressure, already proposed as an early trigger of liver regeneration [23], may thus contribute to compensatory liver growth via ATP release. Hepatocyte growth factor (HGF), reported to be induced by portal hyperpressure after PH [31], was not differently expressed in PPADS and control rats (Supp. Fig. 3A), suggesting that P2 signalling was not involved in HGF induction.

Only few and recent studies indirectly pointed out ATP as a potential early regulator of liver regeneration in rodents [2,10,32]. Using a broad non-selective P2 antagonist, we provide *in vivo* evidence that the ATP abruptly released after PH has a global positive impact on the physiological process of liver regeneration via P2 purinoceptor activation. The P2 receptor subtype(s) involved are currently unknown, although P2Y₂ has been proposed as a potential candidate [32]. ATP release after PH may also have specific impact on non-hepatocyte cell types. Indeed each liver cell type expresses its own repertoire of purinoceptors [6]. Sinusoidal endothelial cells have been reported to control extracellular nucleotide balance, through CD39 ectonucleotidase expression, and thereby may regulate liver regeneration [32]. Kupffer cells express purinoceptors [33] in which stimulation by ATP [34] may contribute to cytokine release after PH [1]. In the present study, induction of cytokine genes (IL6 and TNF α) in the livers from control and PPADS rats after PH was similar, suggesting that the effect observed on liver regeneration after P2 purinergic receptor antagonism does not result from an inhibition of Kupffer cell cytokine production (Supp. Fig. 3B). Thus liver ATP release and purinergic signalling may be among the earliest signals after PH, triggering in particular, growth and metabolic effects on the different liver cell types.

Data obtained in humans closely parallel those from rats in terms of PH-induced acute portal hypertension, immediate ATP release, and purinoceptor expression in hepatocytes [35,36], suggesting that purinergic signalling may regulate liver regeneration after PH in patients. More generally, ATP could be released in the human liver in other medical or surgical situations involving vascular shear stress with unknown pathophysiological consequences.

All together (Fig. 8), our results demonstrate that ATP is transiently released from the liver in response to immediate hyperpressure following PH. At least both hepatocytes and Kupffer cells contribute to this release, which may be one of the earliest signals triggered by PH. We provide evidence suggesting that hepatocytes possess vesicular ATP stores which coincide with lysosomes. During the process of liver regeneration, extracellular ATP contributes to immediate early gene induction in hepatocytes, activating the hepatocyte cell cycle progression. Finally, we show that ATP release also occurs in humans after PH and could modulate regeneration via hepatocyte purinoceptors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ATP	adenosine triphosphate
PH	partial hepatectomy
PPADS	Pyridoxal Phosphate-6-azo(benzene-2,4-disulfonic) acid
ALT	alanine aminotransferase
BrdU	Bromodeoxyuridine
PH3	phosphorylated histone 3

Figure 1. ATP release from the liver after PH in the rat

(A) ATP release in the blood (hepatic veins) and in bile (insert) *in vivo* in the anesthetized rat before and after PH (n = 4). (B) ATP output in the isolated perfused liver in control (n = 8) and clodronate liposomal (Cl2MDP)-treated rats (n = 4) before and after PH. Insert: area under curves of ATP output in Cl2MDP-treated and control rats. (C) Biliary ATP output in the isolated perfused liver before and after PH. ATP was measured in bile collected over 10 min after PH.

***: $p < 0.01$.

Figure 2. Portal hyperpressure triggers ATP release from the liver after PH

(A) *In vivo* PVP in rats before and immediately (1 min) after PH (n = 5). (B) PVP in the isolated perfused liver before and after ligation of left lateral (LL) and median (ML) lobes (n = 5). (C) PVP in the isolated perfused liver before and after “isobaric PH” (see methods). (D) Suppression of PH-induced ATP release in isobaric conditions (n = 8). Restoration of ATP release by full rate perfusion re-establishment (n = 8). ****: $p < 0.001$. Data are the means $\pm$ sem of n experiments (number in parentheses). PVP: portal vein pressure.

Figure 3. ATP distribution in the liver is altered after PH

(A-G): liver sections from quinacrine-injected rats. **A.** Before PH, intense periportal (PP) quinacrine staining (X100). **(B and C)** Dense vesicular staining in PP areas and weakly stained PV zones, before PH (B: X400, C: X160). **(D)** Five minutes after PH, lobules were uniformly stained (X100). **(E and F)** Vesicular staining was lost, except near CV, 5 minutes after PH (X400). **(G)** Staining with quinacrine (green) and phalloidin (red, $\lambda_{ex} = 543$ nm, $\lambda_{em} = 560-610$ nm), before PH. A stack of 10 confocal sections is shown. Vesicular ATP-enriched compartments in hepatocytes (1) and cells from the sinusoid (presumably endothelial and Kupffer cells, see arrow heads) (2). Arrows: actin reinforcement at a canalicular pole (X630). **(H)** Spectrofluorimetric analysis of liver homogenates from quinacrine-injected rats ($\lambda_{ex} = 435$ nm, $\lambda_{em} = 500$ nm), before and 5 min after PH (n = 6, ***: $p < 0.01$).

PS: portal space; CV: central vein. Scale bars: 75 μ m in A, C and D; 20 μ m in B, E, F and G. Photographs representative of 6 rats are shown.

Figure 4. Hepatocyte ATP-enriched vesicles are lysosomes. Confocal imaging analysis in isolated hepatocytes and liver sections

(A-D): Isolated rat hepatocytes stained with quinacrine, lysotracker or both, or mitotracker (X180). Quinacrine-labeled vesicular structures (diameter: 0.5-1.5 μm) (A) also stained with lysotracker (B and C) but not with mitotracker (D). (E-G): Liver sections costained with quinacrine and lysotracker. (X120). (H): Quinacrine staining in an hepatocyte doublet. Arrow: reinforced labeling around the bile canaliculus. Scale bars: 20 μm . Photographs representative of 4 experiments are shown.

Figure 5. ATP contributes to hepatocyte cell cycle progression after PH

(**A, B upper panels**) BrdU incorporation after PH in control and PPADS-treated rats (***, $p < 0.01$, $n = 6$). BrdU incorporation was similar in pre-PH livers from the two groups of rats (0.2 % of hepatocytes) ($n = 4$). (**B lower panels**) Ki67 immunostaining in control and PPADS rats, 24 hours after PH. Representative photographs of 4 experiments in each group are shown. **C.** Phosphorylated histone H3 (PH3) immunostaining after PH in control and PPADS-treated rats (***, $p < 0.01$, $n = 6$).

Figure 6. ATP contributes to cyclin activation and immediate early gene induction after PH
(A) Cyclin activation after PH. Western blot analysis of cyclin A and cyclin D1 in control and PPADS rat livers before and after PH. A semi-quantitative analysis of western blots is shown in the lower panel ($n = 3$ experiments in each group). The Ponceau red was taken as a loading control. **(B and C)** Quantitative RT-PCR analysis of c-fos **(B)** and c-jun **(C)** mRNAs in control and PPADS rat livers before and after PH. $n = 4$ to 6 rats in each group (*: $p < 0.05$; **: $p = 0.01$).

Figure 7. ATP release from the liver after PH in human

(A) Plasma ATP before and after PH in living donors for transplantation. Before PH, ATP was uptaken by the liver (out/in ratio: 0.6 ± 0.05 , $n = 4$). A net ATP release was observed after PH (out/in ratio: 1.4 ± 0.1 , $n = 4$) (**: $p = 0.01$). (B) Alanine aminotransferase (ALT) in the same plasma samples before and after PH. Basal ALT were elevated above normal because of the surgical context. (C) PVP in 4 living donors before and immediately (5 min) after PH. (**: $p = 0.01$). (D) P2X₄, P2X₇, P2Y₂ and β -actin mRNA expression in human liver (L) and isolated human hepatocytes (H). One representative PCR experiment ($n = 4$ preparations) is shown.

Figure 8. Schematic representation of ATP release and its functional impact on liver regeneration

Immediately after PH, ATP is released from the liver, in the blood and in the bile, in response to the elevation of intrahepatic pressure. ATP is released mainly from the PP area, likely exposed to the highest mechanical stress. Vesicular ATP stores are localized both in hepatocytes and non-parenchymal cells, and Kupffer cells contribute significantly to the ATP release observed after PH. Endothelial cells may also contribute to the ATP release in the blood. Biliary ATP could come either from hepatocytes (bile canaliculus) or cholangiocytes, or both. Finally, we provided *in vivo* evidence that released ATP contributes to hepatocyte entry in the cell cycle after PH. For clarity, stellate cells have not been represented.