Science
AAAS

Platelet-Derived Serotonin Mediates Liver Regeneration
Mickael Lesurtel, *et al.*
Science **312**, 104 (2006);
DOI: 10.1126/science.1123842

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

11. Materials and methods are available as supporting material on *Science* Online.
12. R. W. Taylor, *Science* **201**, 979 (1978).
13. S. O. Shattuck, *Syst. Entomol.* **17**, 199 (1992).
14. A. Nel, G. Perrault, V. Perrichot, D. Néraudeau, *Geol. Acta* **2**, 23 (2004).
15. G. M. Dlussky, A. P. Rasnitsyn, *Russ. Entomol. J.* **11**, 411 (2003).
16. D. Grimaldi, M. S. Engel, *Evolution of the Insects* (Cambridge Univ. Press, New York, NY, 2005).
17. R. H. Crozier, L. S. Jermiin, M. Chiotis, *Naturwissenschaften* **84**, 22 (1997).
18. S. G. Brady, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6575 (2003).
19. P. S. Ward, S. G. Brady, *Invert. Syst.* **17**, 361 (2003).
20. G. M. Dlussky, *Paleontol. J.* **31**, 616 (1997).
21. P. R. Crane, E. M. Friis, K. R. Pedersen, *Nature* **374**, 27 (1995).
22. H. Schneider *et al.*, *Nature* **428**, 553 (2004).
23. C. D. Bell, D. E. Soltis, P. S. Soltis, *Evolution Int. J. Org. Evolution* **59**, 1245 (2005).
24. C. C. Davis, C. O. Webb, K. J. Wurdack, C. A. Jaramillo, M. J. Donoghue, *Am. Nat.* **165**, E36 (2005).
25. B. Farrell, *Science* **281**, 555 (1998).
26. P. Wilf *et al.*, *Science* **289**, 291 (2000).
27. E. O. Wilson, B. Hölldobler, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 7411 (2005).
28. J. E. Tobin, in *Nourishment and Evolution in Insect Societies*, J. H. Hunt, C. A. Nalepa, Eds. (Westview Press, Boulder, CO, 1994), pp. 279–307.
29. D. W. Davidson, S. C. Cook, R. R. Snelling, T. H. Chua, *Science* **300**, 969 (2003).

30. We thank the following for the use of specimens: G. D. Alpert, A. N. Andersen, C. J. Burwell, S. P. Cover, L. Davis, M. A. Deyrup, D. Donoso, R. Eastwood, K. Eguchi, X. Espadaler, P. R. Fernández, B. L. Fisher, D. M. General, R. A. Johnson, J. E. Lattke, D. J. Lohman, J. T. Longino, D. B. Merrill, H. G. Robertson, C. Schöning, M. A. Travassos, E. O. Wilson, and K. Yeo. E. O. Wilson, S. P. Cover, G. D. Alpert, and A. J. Berry gave useful suggestions and comments on earlier versions of the manuscript. We thank M. Cornwall, S. P. Cover, S. V. Edwards, B. D. Farrell, K. M. Horton, C. Labandeira, J. E. Moreau, B. A. Perry, J. B. Plotkin, S. Peck Quek, E. O. Wilson, and J. Zhang for assistance during the preparation of this manuscript and J. M. Girard for assistance in the laboratory. For access to computational resources, we thank D. L. Swofford (NSF Information Technology Resources Program grant EF 03-31495, Florida State University). This research was supported by a grant from the Green Fund to C.S.M. and an NSF DEB-0447242 grant to N.E.P.

Supporting Online Material

www.sciencemag.org/cgi/content/full/312/5770/101/DC1
Materials and Methods
SOM Text
Figs. S1 and S2
Tables S1 to S4
References and Notes
Appendices S1 to S2

12 January 2006; accepted 1 March 2006
10.1126/science.1124891

Platelet-Derived Serotonin Mediates Liver Regeneration

Mickael Lesurtel,[1] Rolf Graf,[1] Boris Aleil,[3] Diego J. Walther,[4] Yinghua Tian,[1] Wolfram Jochum,[2] Christian Gachet,[3] Michael Bader,[5] Pierre-Alain Clavien[1]*

The liver can regenerate its volume after major tissue loss. In a mouse model of liver regeneration, thrombocytopenia, or impaired platelet activity resulted in the failure to initiate cellular proliferation in the liver. Platelets are major carriers of serotonin in the blood. In thrombocytopenic mice, a serotonin agonist reconstituted liver proliferation. The expression of 5-HT2A and 2B subtype serotonin receptors in the liver increased after hepatectomy. Antagonists of 5-HT2A and 2B receptors inhibited liver regeneration. Liver regeneration was also blunted in mice lacking tryptophan hydroxylase 1, which is the rate-limiting enzyme for the synthesis of peripheral serotonin. This failure of regeneration was rescued by reloading serotonin-free platelets with a serotonin precursor molecule. These results suggest that platelet-derived serotonin is involved in the initiation of liver regeneration.

Serotonin (5-hydroxytryptamine, 5-HT) is not only a neurotransmitter but also a hormone with various extraneuronal functions (*1*). It is a potent mitogen and modulates the remodeling of tissue (*2*–*5*). Platelets (thrombocytes) carry serotonin in the blood and release it at sites of tissue injury as part of their action on hemostasis (*6*–*8*). However, platelets are also involved in the inflammatory reaction after tissue injury, which is independent of coagulation (*9*). In the liver, platelets interact with leukocytes in response to cold ischemia and induce them to adhere to the endothelium of blood vessels, thereby enhancing tissue injury (*10*, *11*). Concurrent activation of liver macrophages called Kupffer cells leads to further endothelial cell damage and hepatocyte apoptosis (*12*). Depending on the extent of initial tissue injury, the liver can regenerate in a highly synchronized and organized fashion. Because platelets interact with endothelial cells in the early phase after injury, they might also have an effect on the initiation of liver regeneration.

To establish the role of platelets and their secretory products in liver regeneration, partial hepatectomy was performed in mice in which platelet function was inhibited pharmacologically or platelets were depleted. Initially, thrombocytopenia was induced by injecting busulfan, an alkylating agent that causes massive loss of platelets (*13*). Furthermore, platelets were functionally targeted by the application of clopidogrel, which selectively and irreversibly antagonizes the P2Y12 adenosine diphosphate (ADP) receptors on platelets, leading to the inhibition of platelet aggregation (*14*). After injection of these drugs in mice, a 70% hepatectomy was performed to study regeneration of the liver. Although control animals reacted with an increase in hepatic proliferation [5-bromo-2′-deoxyuridine (BrdU)–, Ki67-, and proliferating cell nuclear antigen (PCNA)–positive] 2 days after hepatectomy, busulfan-injected mice exhibited a reduced response (Fig. 1, A to C, and E). In busulfan-treated mice, the number of platelets was reduced in a dose-dependent fashion and the leukocyte count was decreased, but erythrocytes were unaffected (Fig. 1D). Thus, these mice exhibited a combined thrombocytopenia and leukopenia. The impairment of hepatocyte proliferation after hepatectomy may be attributed to a lack of each cell type alone or a combination of both.

To investigate the role of platelets more selectively, an antibody to GPIbα recognizing an epitope on platelets was injected into mice before hepatectomy (*15*). The number of platelets fell below 10% (Fig. 2A), whereas leukocyte and erythrocyte counts were not affected (Fig. 2, B and C), indicating a specific thrombocytopenia. After 70% hepatectomy, all markers of hepatocellular proliferation were reduced (Fig. 2, D to F) in thrombocytopenic mice.

We also tested whether the inhibition of platelet activity, without affecting the number of platelets, was sufficient to block liver regeneration. Clopidogrel, which inhibits the aggregation response to ADP without affecting platelet stability, reduced hepatocyte proliferation in partially hepatectomized livers, but this effect was less pronounced than in busulfan-treated mice. In mice treated with an enantiomer of clopidogrel, which lacks antiaggregation properties, proliferation was not different from controls (Fig. 1, A to C).

Platelets store and release serotonin. About 95% of all serotonin found in blood is stored in platelets. In vitro, serotonin is a potent mitogen and stimulates hepatocyte mitosis (*3*, *16*). The 5-HT2A and 1C receptors appear to mediate mitogenic effects in fibroblasts (*17*, *18*), and the 5-HT2B receptor is involved in the development of the heart (*19*) and the enteric nervous system (*20*). To test whether serotonin induces hepatocyte proliferation in vivo, thrombocytopenic mice were treated with the serotonin receptor 5-HT(2A/2C) agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI-hydrochloride). The application of this drug had no effect on the extent of thrombocytopenia (Fig. 2G) induced by concurrent treatment with the antibody to GPIbα. In the presence of the serotonin agonist, proliferation was completely restored (Fig. 2, D to F).

[1]Department of Visceral and Transplantation Surgery and [2]Department of Pathology, University Hospital of Zurich, Switzerland. [3]Institut National de la Santé et de la Recherche Médicale 311, Etablissement Français du Sang-Alsace, Strasbourg, France. [4]Max Planck Institute for Molecular Genetics, Berlin, Germany. [5]Max Delbrück Center for Molecular Medicine, Berlin, Germany.

*To whom correspondence should be addressed. E-mail: clavien@chir.unizh.ch

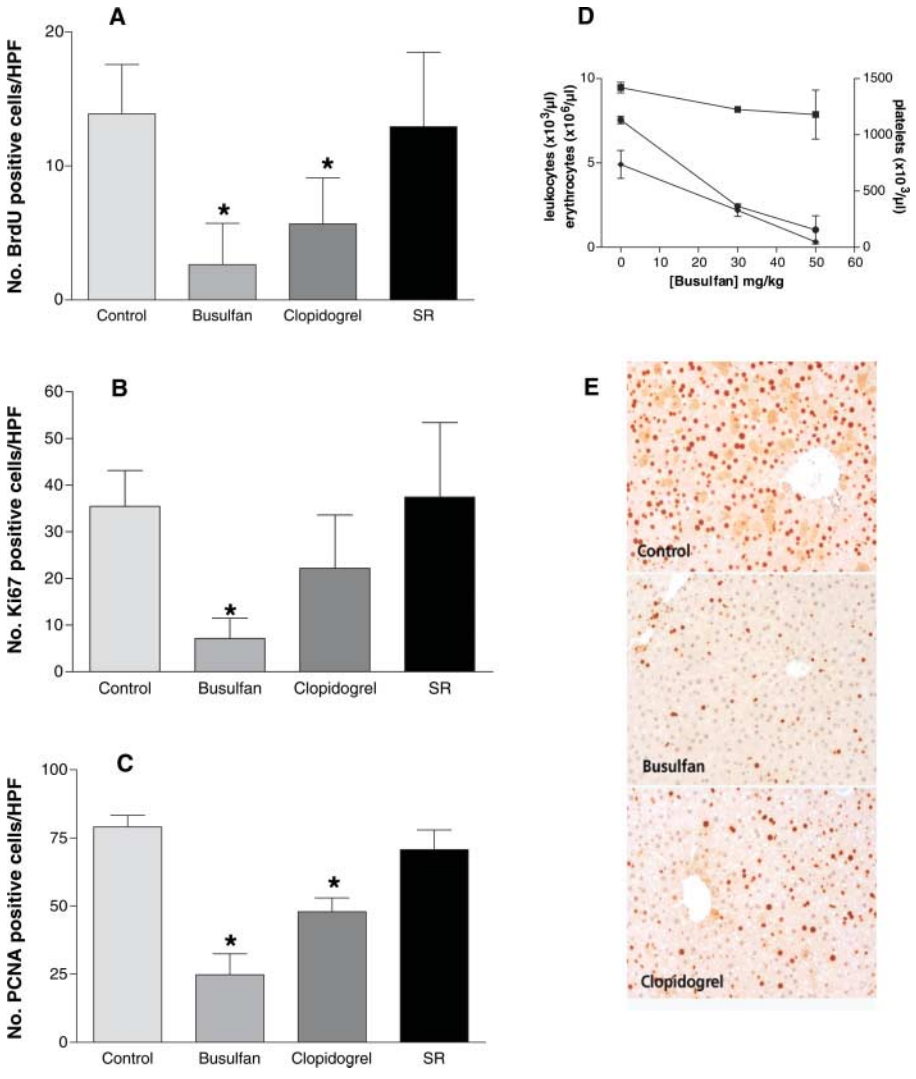

Fig. 1. Effect of drugs targeting platelets on liver regeneration. (**A**) Number of BrdU-positive hepatocytes 2 days after hepatectomy in the remnant liver of control animals and animals treated with either busulfan (thrombocytolytic), clopidogrel (ADP receptor antagonist), or SR25989 (SR) (clopidogrel enantiomer). One-way analysis of variance (ANOVA) was significantly different from 1 ($P = 0.0002$) in the number of BrdU-positive cells in busulfan- and clopidogrel-treated animals. A post-test using Bonferroni comparison exhibited statistical differences between control and busulfan ($P < 0.001$), and between control and clopidogrel ($P < 0.05$) (indicated by asterisk). (**B**) Number of Ki67-positive hepatocytes in control, busulfan-, clopidogrel-, or SR-treated animals. The same remnant livers were used for this analysis. One-way ANOVA indicated a statistical difference ($P = 0.003$) and the Bonferroni post-test indicated a significant difference between the controls and busulfan-treated animals (asterisk). (**C**) Number of PCNA-positive cells in the same remnant liver lobes. One-way ANOVA indicated a difference with $P = 0.0001$, whereas both busulfan ($P < 0.001$) and clopidogrel ($P < 0.01$) were different from controls (asterisk). (**D**) Determination of blood cell counts in animals treated with busulfan. Fifteen days after a single intraperitoneal (ip) injection of busulfan, blood was drawn to determine the number of platelets (circles), leukocytes (diamonds), and red blood cells (squares). Initially, time curves and dose-response curves were evaluated to determine the optimal time and concentration of busulfan. In a second set of experiments, the number of blood cells was evaluated by using two concentrations of busulfan [30 mg busulfan per kg mouse mass (mg/kg) and 50 mg/kg] compared with the vehicle control. (**E**) Remnant liver sections immunohistochemically stained for PCNA 2 days after hepatectomy. Representative sections of controls (top panel), busulfan-injected (center panel), and clopidogrel-treated mice (bottom panel) are shown. Error bars indicate standard deviation.

To identify the putative 5-HT receptors involved in liver regeneration, we analyzed the expression of the various 5-HT receptor types in the resting liver using real-time polymerase chain reaction with probes recognizing transcripts coding for 1A, 1B, 1D, 1F, 2A, 2B, 2C, 3A, and 3B serotonin receptor types. In the naïve liver, RNA encoding of all receptor types was detectable, except for 1F and 2C. Two days after hepatectomy, a three- to fourfold up-regulation of 2A and 2B receptor expression was observed, suggesting that type 2A and 2B receptors contribute to liver regeneration (Fig. 2H). Mice were treated with specific 5-HT receptor antagonists and submitted to partial hepatectomy. When a 5-HT2A receptor antagonist was used, hepatocyte proliferation measured by Ki67 and PCNA (Fig.2I) staining was reduced, compared with vehicle-treated controls. The 5-HT2B receptor antagonist caused a reduced Ki67 staining, whereas PCNA was not different. Neither 5-HT2C nor 5-HT3 receptor antagonists reduced the labeling indexes (Fig. 2, I and J). These experiments suggest that 5-HT2A and 2B receptor subtypes mediate serotonin-dependent regeneration.

The reconstitution of hepatocyte proliferation by a serotonin agonist suggests that serotonin might be a comitogen in liver regeneration. To further test this hypothesis, we used knock-out mice that lack peripheral serotonin but retain neural serotonin action (*21*). In wild-type animals, tryptophan is converted to 5-hydroxytryptophan (5-HTP) by tryptophan-hydroxylase 1 (TPH1) in the small intestine (fig. S1). In a further step, 5-HTP is converted to 5-HT by a ubiquitous aromatic L–amino acid decarboxylase (*22*). Serotonin is then transferred to platelets by a transporter system. In TPH1$^{-/-}$ mice, platelets lack serotonin (*22*), which is confirmed in platelet-rich plasma (Fig. 3A). To test directly the function of serotonin in liver regeneration, we performed partial hepatectomy on TPH1$^{-/-}$ and wild-type control mice. In hepatectomized TPH1$^{-/-}$ mice, all markers of hepatocyte proliferation were reduced (Fig. 3, B to D) 2 days after hepatectomy. To exclude the possibility that liver regeneration is delayed and not impaired in TPH1$^{-/-}$ mice, hepatocyte proliferation was also analyzed 1 and 4 days after hepatectomy. Neither wild-type nor TPH1$^{-/-}$ mice exhibited proliferative activity at those time points, supporting the idea that the peak of regeneration is at 2 days and that the reduction of hepatocyte proliferation was not caused by a temporal shift (Fig. 3F). This result suggests that a molecular action of serotonin was involved in the induction of hepatoctye proliferation after a major loss of hepatic tissue.

To further substantiate the mitogenic activity of serotonin, TPH1$^{-/-}$ mice were injected with the serotonin precursor 5-HTP to reload their platelets. In these mice, platelets carried completely reconstituted levels of serotonin (Fig. 3A), and all markers of proliferation were restored after partial hepatectomy (Fig. 3, B to E). In addition, livers of TPH$^{-/-}$ mice showed a reduction of mitotic figures, whereas after reloading with 5-HTP, the number reached those of wild-type animals. Similarly, the 5-HT2A antagonist treatment reduced mitotic figures (fig. S2). Thus, serotonin is pivotal for hepatic proliferation after a major tissue loss.

We demonstrated a block of hepatocyte proliferation in thrombocytopenic mice and after clopidogrel treatment, which inhibits platelet func-

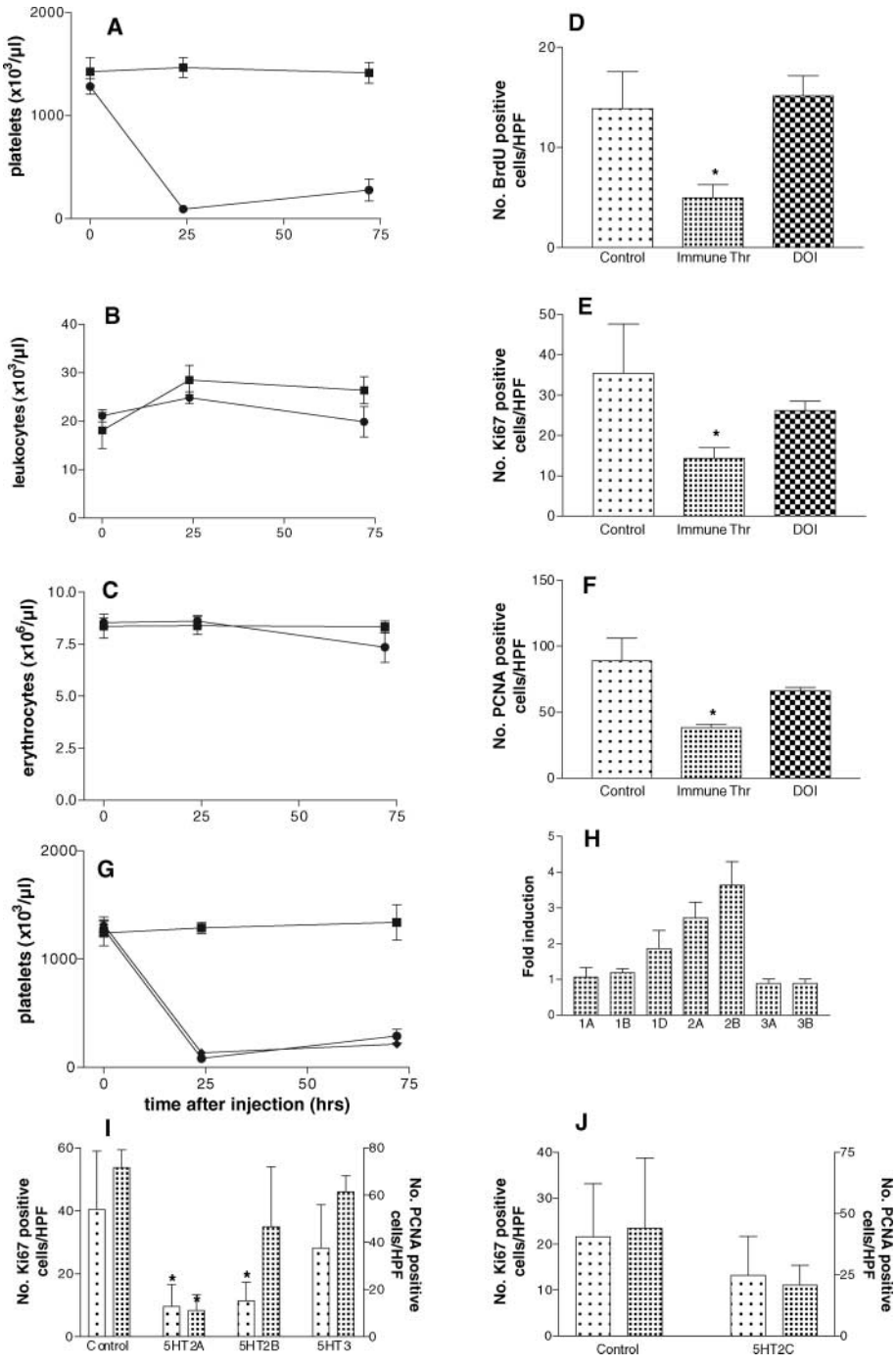

Fig. 2. Effect of thrombocytopenia on liver regeneration. Mice were injected ip with an antibody directed against a platelet epitope, GPIbα, or an immunoglobulin G2 (IgG2) control at 0 and 24 hours. To monitor cell counts, blood was drawn at 0, 24, and 72 hours after the initial injection. (**A**) Number of platelets in anti-GPIbα–treated (circles) and control animals (squares). (**B** and **C**) Number of leukocytes and erythrocytes in the same animals. (**D**) Effect of 70% hepatectomy on proliferation of hepatocytes in the remnant liver 2 days later. BrdU-positive cells were counted in controls, thrombocytopenic (anti-GPIbα), and thrombocytopenic mice treated with the serotonin agonist DOI. (**E**) Number of Ki67 positive cells in the same specimen. (**F**) Detection of PCNA in controls, thrombocytopenic, and DOI-treated animals. In (D) to (F), one-way ANOVA indicated a statistical difference ($P < 0.003$) and the Bonferroni post-test indicated that the controls and the DOI-treated animals were significantly different from the anti-GPIbα–treated animals (asterisk). (**G**) To determine whether the serotonin agonist had any effect on platelet number, thrombocytopenic animals were injected with DOI and bled according to the scheme depicted above. Thrombocytopenic (circles), control animals (squares), DOI (diamonds). (**H**) Induction of transcripts coding for 5-HT receptor subtypes 1A, 1B, 1D, 1F, 2A, 2B, 2C, 3A, and 3B. Subtypes 1F and 2C were below the limit of detection and are not included. The levels of mRNA expression in resected tissue was used as the baseline to calculate the fold induction of transcripts after regeneration. (**I** and **J**) Effect of serotonin antagonists on liver regeneration. Antagonists were injected before and during the period of regeneration. Subtype-specific antagonists are: ketanserin (5-HT2A), SB 206553 (5-HT2B/2C), SB 242084 (5-HT2C), and odansetron (5-HT3). Controls included saline injections, and for 5-HT2C, a separate series with the solvent for SB 242084. Error bars indicate standard deviation.

tion (*23*). These results suggest that platelets play an essential role in liver regeneration. In addition to the function of platelets in coagulation, reperfusion injury in a number of organs (*24*–*28*) [including the liver (*10*, *11*)], and in inflammatory processes (*9*) [such as asthma (*29*), atherosclerosis (*30*), and sepsis (*31*)], the function of platelets in liver regeneration appears to be mediated by serotonin, because mice lacking platelet serotonin displayed lack of liver regeneration. Moreover, serotonin agonists indicate that serotonin may act downstream of a potential interaction of platelets and leukocytes with endothelial cells or hepatocytes. Serotonin has been shown to exert mitogenic actions on smooth muscle cells and fibroblasts in pulmonary hypertension (*32*–*34*) and to modulate the plasticity of the nervous system (*1*, *4*) and the mammary gland (*5*).

The presence of serotonin receptor subtypes 5-HT2A and 2B in the liver, combined with the observation that 5-HT antagonists inhibit liver regeneration, suggests that serotonin acts directly in the liver and not through a remote systemic pathway. The presence of 5-HT2A and 2B in hepatocytes (*35*), which form the parenchymal mass in the liver, is in line with our observation of impaired hepatocyte proliferation after depletion of serotonin or after antagonist treatment.

Both platelets and leukocytes derived from circulating blood, as well as the presence of Kupffer cells, are required for a full hepatic response (*36*). Because hepatic regeneration may occur under different situations, different mechanisms may come into action to rescue liver failure. It has been demonstrated that Kupffer cell–derived cytokines may play a key role in initiating proliferation (*37*). Kupffer cells are predominantly activated during an ischemic insult. The release of the proinflammatory tumor necrosis factor α (TNFα) (*38*) and interleukin-6 (IL-6) (*39*) have been studied in circumstances when tissue damage by apoptosis and necrosis are observed.

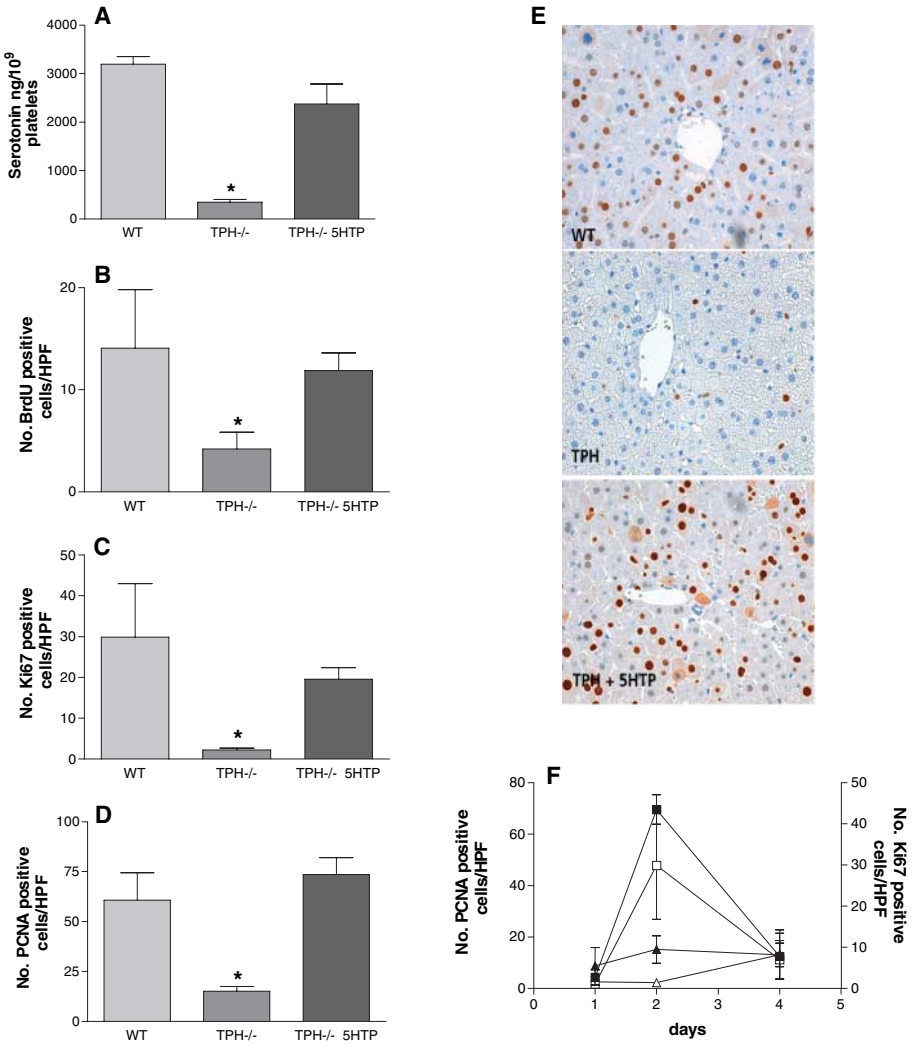

Fig. 3. Effect of serotonin on liver regeneration. (**A**) Serotonin levels in thrombocytes of wild type (WT) mice, TPH1$^{-/-}$ mice, and TPH1$^{-/-}$ mice after supplementing with 5-HTP (TPH1$^{-/-}$ 5-HTP). Platelet-rich plasma was prepared and, after counting platelet concentration, serotonin was assessed by an enzyme-linked immunosorbent assay. One-way ANOVA indicated a statistical difference ($P < 0.001$) and the Bonferroni post test indicated that WT animals ($P < 0.001$) and the TPH1$^{-/-}$ 5-HTP animals ($P = 0.001$) were significantly different from the TPH1$^{-/-}$ animals (asterisk). (**B**) Effect of serotonin depletion in platelets on hepatocyte proliferation 2 days after hepatectomy. The number of BrdU-positive cells was counted in WT, TPH1$^{-/-}$, and TPH1$^{-/-}$ mice supplemented with 5-HTP (TPH1$^{-/-}$ 5-HTP). (**C** and **D**) Number of Ki67- and PCNA-positive cells in the same liver remnants. In [(B) to (D)], one-way ANOVA indicated a statistical difference ($P < 0.01$) and the Bonferroni post-test indicated that the controls and the TPH1$^{-/-}$ 5-HTP animals were significantly different from the TPH1$^{-/-}$ animals (asterisk). (**E**) Histological examples of PCNA-stained sections from remnant livers. (**F**) Time course of labeling indexes for PCNA (solid square and solid triangles) and Ki67 (open squares and open triangles) in hepatectomized wild-type (solid and open squares) and TPH$^{-/-}$ livers (solid and open triangles). Error bars indicate standard deviation.

These cytokines determine the initial induction of proliferation. However, another factor—hepatocyte proliferation factor—appears crucial to the completion of regeneration, whereas maintenance of proliferation is not dependent on these factors. Thus, several factors appear to be involved in proliferation that are not necessarily all required to be present at the same time. Platelet-derived serotonin may influence the proliferation of hepatocytes (*16*) or may be involved in the release of growth factors, such as IL-6, at the site of liver injury (*40*, *41*). These findings have direct clinical implications. In liver transplantation, most patients have reduced platelet counts related to portal hypertension and hypersplenism and, thus, serotonin agonists may be a therapeutic option to improve the outcome.

References and Notes

1. J. Veenstra-VanderWeele, G. M. Anderson, E. H. Cook Jr., *Eur. J. Pharmacol.* **410**, 165 (2000).
2. K. Seuwen, J. Pouyssegur, *Biochem. Pharmacol.* **39**, 985 (1990).
3. B. L. Fanburg, S. L. Lee, *Am. J. Physiol.* **272**, L795 (1997).
4. T. Vitalis, J. G. Parnavelas, *Dev. Neurosci.* **25**, 245 (2003).
5. M. Matsuda *et al.*, *Dev. Cell* **6**, 193 (2004).
6. J. N. George, *Lancet* **355**, 1531 (2000).
7. M. Gawaz, *Blood Platelets: Physiology, Physiopathology, Membrane Receptors, Antiplatelet Principles, and Therapy for Atherothrombotic Diseases.* (Thieme, Stuttgart, Germany, 2001).
8. D. J. Walther *et al.*, *Cell* **115**, 851 (2003).
9. P. F. Mannaioni, M. G. Di Bello, E. Masini, *Inflamm. Res.* **46**, 4 (1997).
10. D. Sindram, R. J. Porte, M. R. Hoffman, R. C. Bentley, P. A. Clavien, *Gastroenterology* **118**, 183 (2000).
11. D. Sindram, R. J. Porte, M. R. Hoffman, R. C. Bentley, P. A. Clavien, *FASEB J.* **15**, 1230 (2001).
12. V. Kohli, M. Selzner, J. F. Madden, R. C. Bentley, P. A. Clavien, *Transplantation* **67**, 1099 (1999).
13. S. A. Evensen, M. Jeremic, P. F. Hjort, *Thromb. Diath. Haemorrh.* **19**, 570 (1968).
14. C. Gachet, *Thromb. Haemost.* **86**, 222 (2001).
15. J. A. Lopez *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5615 (1987).
16. S. Balasubramanian, C. S. Paulose, *Hepatology* **27**, 62 (1998).
17. D. Julius, K. N. Huang, T. J. Livelli, R. Axel, T. M. Jessell, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 928 (1990).
18. D. Julius, T. J. Livelli, T. M. Jessell, R. Axel, *Science* **244**, 1057 (1989).
19. C. G. Nebigil, J. M. Launay, P. Hickel, C. Tournois, L. Maroteaux, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 2591 (2000).
20. E. Fiorica-Howells, L. Maroteaux, M. D. Gershon, *J. Neurosci.* **20**, 294 (2000).
21. D. J. Walther *et al.*, *Science* **299**, 76 (2003).
22. D. J. Walther, M. Bader, *Biochem. Pharmacol.* **66**, 1673 (2003).
23. K. Selleng *et al.*, *Am. J. Hematol.* **78**, 188 (2005).
24. K. M. Mullane, J. C. McGiff, *J. Cardiovasc. Pharmacol.* **7**, 733 (1985).
25. R. Rosen, W. Dausch, E. Beck, W. Klaus, *Cardiovasc. Res.* **21**, 293 (1987).
26. G. Rousseau *et al.*, *Am. Heart J.* **125**, 1553 (1993).
27. R. Cywes *et al.*, *Hepatology* **18**, 635 (1993).
28. Y. Okada *et al.*, *Transplantation* **64**, 801 (1997).
29. C. Moritani, S. Ishioka, Y. Haruta, M. Kambe, M. Yamakido, *Chest* **113**, 452 (1998).
30. J. M. Munro, R. S. Cotran, *Lab. Invest.* **58**, 249 (1988).
31. M. R. Yeaman, *Clin. Infect. Dis.* **25**, 951 (1997).
32. S. Eddahibi *et al.*, *Am. J. Physiol.* **272**, H1173 (1997).
33. D. J. Welsh, M. Harnett, M. MacLean, A. J. Peacock, *Am. J. Respir. Crit. Care Med.* **170**, 252 (2004).
34. E. Marcos *et al.*, *Circ. Res.* **94**, 1263 (2004).
35. D. W. Bonhaus *et al.*, *Br. J. Pharmacol.* **115**, 622 (1995).
36. R. Taub, *Nat. Rev. Mol. Cell Biol.* **5**, 836 (2004).
37. Y. Yamada, I. Kirillova, J. J. Peschon, N. Fausto, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1441 (1997).
38. H. A. Rudiger, P. A. Clavien, *Gastroenterology* **122**, 202 (2002).
39. M. Selzner, C. A. Camargo, P. A. Clavien, *Hepatology* **30**, 469 (1999).
40. D. E. Cressman *et al.*, *Science* **274**, 1379 (1996).
41. T. Durk *et al.*, *Int. Immunol.* **17**, 599 (2005).

42. We thank P. Savi from the Sanofi-Synthelabo-Recherche in Toulouse, France, for providing us with clopidogrel (Sigma) and F. Lanza from Etablissement Français du Sang, Strasbourg, France, for the antibodies to GPIbα. We thank B. Stieger from the University Hospital of Zurich, Switzerland, for the preparation of mouse hepatocytes. M.L. received the 2003 Warren fellowship from the International Hepato-Pancreato-Biliary Association and the 2002 Lavoisier grant from the French Ministry of Foreign Affairs. This project was supported in part by the Swiss National Science Foundation, the NIH, and the Gebert Ruf Foundation.

Supporting Online Material

www.sciencemag.org/cgi/content/full/312/5770/104/DC1
Materials and Methods
Figs. S1 and S2

14 December 2005; accepted 21 February 2006
10.1126/science.1123842