Am J Physiol Gastrointest Liver Physiol 296: G963–G968, 2009.
First published February 26, 2009; doi:10.1152/ajpgi.90709.2008.

Deletion of the serotonin transporter in rats disturbs serotonin homeostasis without impairing liver regeneration

Ramadhan B. Matondo,[1] Carine Punt,[1] Judith Homberg,[2] Mathilda J. M. Toussaint,[1] Ronald Kisjes,[1] Suzanne J. A. Korporaal,[3] Jan Willem N. Akkerman,[3] Edwin Cuppen,[2] and Alain de Bruin[1]

[1]*Department of Pathobiology, Faculty of Veterinary Medicine,* [2]*Hubrecht Institute, Royal Academy of Arts and Sciences and University Medical Center, and* [3]*Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands*

Submitted 19 December 2008; accepted in final form 16 February 2009

Matondo RB, Punt C, Homberg J, Toussaint MJ, Kisjes R, Korporaal SJ, Akkerman JW, Cuppen E, de Bruin A. Deletion of the serotonin transporter in rats disturbs serotonin homeostasis without impairing liver regeneration. *Am J Physiol Gastrointest Liver Physiol* 296: G963–G968, 2009. First published February 26, 2009; doi:10.1152/ajpgi.90709.2008.—The serotonin transporter is implicated in the uptake of the vasoconstrictor serotonin from the circulation into the platelets, where 95% of all blood serotonin is stored and released in response to vascular injury. In vivo studies indicated that platelet-derived serotonin mediates liver regeneration after partial hepatectomy. We have recently generated serotonin transporter knockout rats and demonstrated that their platelets were almost completely depleted of serotonin. Here we show that these rats exhibit impaired hemostasis and contain about 1–6% of wild-type serotonin levels in the blood. Despite the marked reduction of serotonin levels in blood and platelets, efficient liver regeneration and collagen-induced platelet aggregation occur in rats lacking the serotonin transporter. These results provide evidence that liver regeneration is not dependent on the release of serotonin from platelets. Our findings indicate that very low levels of serotonin in blood are sufficient for liver regeneration.

platelet; aggregation; bleeding; hepatectomy; knockout

THE MAMMALIAN LIVER IS ONE of the few adult organs capable of completely regenerating itself in response to injury through the release of growth factors that stimulate reentry of terminally differentiated hepatocytes into the cell cycle (for review, see Refs. 4, 16, 25). Amputation/resection (hepatectomy) of up to 70% of liver mass results in synchronous induction of sharp peaks in hepatocyte DNA replication (S phase) and mitosis (M phase), and the original size of the organ will be restored within 7–10 days in rats and 3–6 mo in humans.

Several recent studies highlight the importance of serotonin in rodent liver regeneration. Serotonin, also known as 5-hydroxytryptamine (5-HT), functions as a ligand for the family of 14 5-HT receptor subtypes and is synthesized mainly in enterochromaffin cells of the gastrointestinal tract (28, 29). Outside the gastrointestinal tract, 5-HT is also synthesized in neurones and plays a major role in neurotransmission within the central nervous system (12). Peripherally, 5-HT is able to mediate vascular contraction and relaxation, gastrointestinal motility, cell proliferation, apoptosis, and platelet aggregation (21). In vitro studies with 5-HT caused a dose-dependent increase in [^{3}H]thymidine incorporation into hepatic DNA in the presence of insulin and epidermal growth factor (1). In vivo studies demonstrated a mobilization of intestinal 5-HT and accumulation in the liver following partial hepatectomy (14). Subsequent work showed enhanced hepatocyte proliferation following low-dose administration of 5-HT in mice subjected to partial hepatectomy (13). Recent studies with the 5-HT agonist 2,5-dimethoxy-4-iodamphethamine and the 5-HT antagonist ketanserin support a key role for 5-HT2 receptors and its ligand in mediating hepatic regeneration (15, 20). Furthermore, tryptophan hydroxylase 1 ($TPH1$)$^{-/-}$ knockout mice lacking peripheral serotonin, attributable to an absence of the rate limiting synthetic enzyme TPH1, exhibited an impaired liver regeneration after partial hepatectomy. This failure of regeneration was rescued by injection of the serotonin precursor 5-HTP (15). Together these results suggest that 5-HT and its receptors have a key role in mediating liver regeneration.

The plasma membrane serotonin transporter (SERT) is critically important in regulating 5-HT homeostasis by actively removing 5-HT from the extracellular space, thereby storing it into intracellular vesicles where it becomes available for release in response to various stimuli (18). Ninety-five percent of all 5-HT found in blood is stored in platelets via the SERT. Whether continuous SERT activity and 5-HT release from platelets is needed for liver regeneration is unknown.

In the present study, we show that 5-HT levels are almost completely diminished in platelets, blood, and liver of SERT-deficient rats. The SERT-deficient rats exhibit reduced hemostasis, but liver regeneration after partial hepatectomy is not impaired. These findings demonstrate that only very low levels of 5-HT are required for liver regeneration, indicating that an active platelet release of physiological levels of 5-HT is not essential for liver regeneration.

MATERIALS AND METHODS

Animals. All experiments were approved by the Utrecht University Animal Ethics Committee and performed according to institutional and national guidelines. Experiments were designed to minimize the number of required animals and their suffering. The *SERT* knockout rats (Slc6a4^{1Hubr}, Wistar background) were generated by target-selected *N*-ethyl-*N*-nitrosurea-induced mutagenesis and genotyped as described (10). Homozygous *SERT* knockout ($SERT^{-/-}$) rats and wild-type ($SERT^{+/+}$) littermates were obtained by crosses between heterozygous ($SERT^{+/-}$) parents. Animals were housed under standard conditions in groups of two to four per cage per sex under

Address for reprint requests and other correspondence: A. de Bruin, Dept. of Pathobiology, Faculty of Veterinary Medicine, Utrecht Univ., Yalelaan 1, 3508 TD Utrecht, The Netherlands (e-mail: A.debruin@uu.nl).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

controlled experimental conditions (12-h light/dark cycle, 21 ± 1°C, 60% relative humidity, food and water ad libitum). Rats at the age of 12 wk were used for the hepatectomy experiments.

Bleeding time. Assessment of bleeding time was done as previously described with minor modifications (11). Animals were anesthetized with 2% isoflurane in oxygen and air, followed by cleaning of the tail with water and chlorohexidene gluconate (SSL Healthcare, 's-Hertogenbosch, Netherlands). After this step, the tail was dried with a piece of gauze. Bleeding was then assessed by recording time from the moment the tail was incised until bleeding stopped completely.

Platelet counts, platelet volume, density, and aggregation. Blood samples for platelet counts were collected from the tail vein by using a 25-gauge hypodermic needles and then directly mixed with 10% of trisodium citrate dehydrate (TSCD) in a ratio of 1 part TSCD and 9 parts of blood sample. Platelet counting, platelet volume, and density were determined by using an automated Bayer ADVIA 120 analyzer at the Utrecht University, Veterinary Diagnostic Laboratory, as previously described (24). Preparation of platelet-rich plasma, and collagen-induced platelet aggregation assay was performed as described previously with minor modifications (5). Platelet concentration was adjusted to 2.5×10^8 cells/l in presence of 1 mM of $CaCl_2 \cdot 2H_2O$ end concentration. Aliquots of 291 μl were warmed to 37°C for 5 min followed by addition of 3 μl of collagen (10 μg/ml) and 6 μl of fibrinogen (0.5 μg/ml). Aggregation was monitored continuously for 11 min at 900 revolutions per minute in an optical aggregometer (Model 570 VS; Chrono-Log, Havertown, PA).

ELISA. For ELISA, blood was drawn from portal vein and vena cava. All blood collection was performed under 2% isoflurane (Abbott, England) anesthesia, 0.4 and 0.3 l/min of oxygen and air, respectively. Preparation of serum and plasma samples as well as determination of 5-HT in blood by ELISA were performed according to the manufacturer's protocol (KAPL 10-0900; Biosource, Nivelles, Belgium). To determine 5-HT in the liver, 100 mg of liver was homogenized in 1 ml ice-cold 0.05 N HCl. Amount of protein in the samples were normalized after quantifying the total protein by Pierce BCA protein assay reagent kit. ELISA assays for 5-HT in the liver were performed as described in the manufacturer's protocol (Biosource).

Partial hepatectomy. Anesthesia in rats was induced by isoflurane 3%, oxygen 0.4 l/min, and air 0.3 l/min. Preparation of incision area was followed by injection of buprenophine hydrochloride (Temgesic) analgesic at the dosage of 0.01 mg/100 g body wt before starting surgery. Seventy percent partial hepatectomy was performed at 2% isoflurane. Surgical procedures involved midline incision of skin ~3–4 cm from xiphoid region, followed by muscles and peritoneum. The ligament connecting diaphragm and median lobes was removed as previously described (6). Gentle pressure was applied to exteriorize the liver. To remove median lobes, two ligatures were applied on the vessel ~5 mm apart on its visceral surface close to the hilus. The blood vessel was freed from other tissue carefully to permit the passage suture (Mersilene) size 4–0 (Johnson & Johnson, Dilbeek, Belgium) with the help of tissue forceps. The vessel was then separated by cutting between the two ligatures. A third ligature was laid by hand by passing the suture around the common pedicle of the median lobes. Finally a knot was tied between the first two ligatures and the lobes resected proximal to the third ligature. After cutting the ligament on the visceral surface of the left lower lobe, a ligature was laid with hands around its pedicle, and the lobe was ligated and resected.

Determination of BrdU incorporation. Two hours before the rats were euthanized, BrdU (Sigma-Aldrich, St. Louis, MO) dissolved in PBS was injected at a dose of 50 mg/kg body wt ip, and a carbon dioxide chamber was used for euthanasia at 20, 24, 48, and 96 h, respectively, after 70% partial hepatectomy. After the animals were euthanized, liver tissues were fixated in 10% formalin and imbedded in paraffin. Sections (4 μm) were mounted on Super Frost Plus Slides (Menzel-Gläser, Braunschweig, Germany) and deparaffinized. Endogenous peroxidase activity was blocked by 0.3% H_2O_2 in methanol for 30 min at room temperature. Antigen retrieval was performed by heating the slides in preheated 10 mM citrate, pH 6.0, in a microwave oven for 15 min, cooled down for 30 min at room temperature. DNA denaturing was performed by incubating the slides in 2 N hydrochloric acid for 30 min at 37°C. After being washed with PBS buffer containing 0.1% Tween-20, background staining was blocked by incubating the slides with normal goat serum (1:10 diluted). Slides were incubated overnight at 4°C with the mouse anti-BrdU antibody, clone Bu20a (Dako, Glostrup, Denmark), diluted 1:50. After being washed in PBS-Tween-20 buffer, the slides were incubated in EnVision+ System horseradish peroxidase-labeled polymer anti-mouse (Dako) for 30 min. After the washing in PBS buffer, color was developed in 3–3′diaminobenzidine (Sigma-Aldrich). Sections were counterstained with 10% Mayer's haematoxylin (Merck KgaA, Darmstadt, Germany), dehydrated, and mounted in Eukitt mounting medium (O. Kindler, Freiburg, Germany). Percentages of BrdU-positive cells per slide were calculated after counting 1,000 nuclei per animal.

Statistics. Data are presented as means ± SD. Further statistical analysis to compare SERT-deficient and SERT-proficient rats was done by using Student's *t-test* and Mann-Whitney *U*-test method with R software version 2.7.0 for Windows. Results were considered significant when $P < 0.05$, at a 95% confidence interval.

RESULTS

Bleeding time prolongation and platelet aggregation in $SERT^{-/-}$ rats. To evaluate the role for SERT in blood hemostasis, we determined the bleeding time from wild-type rats and

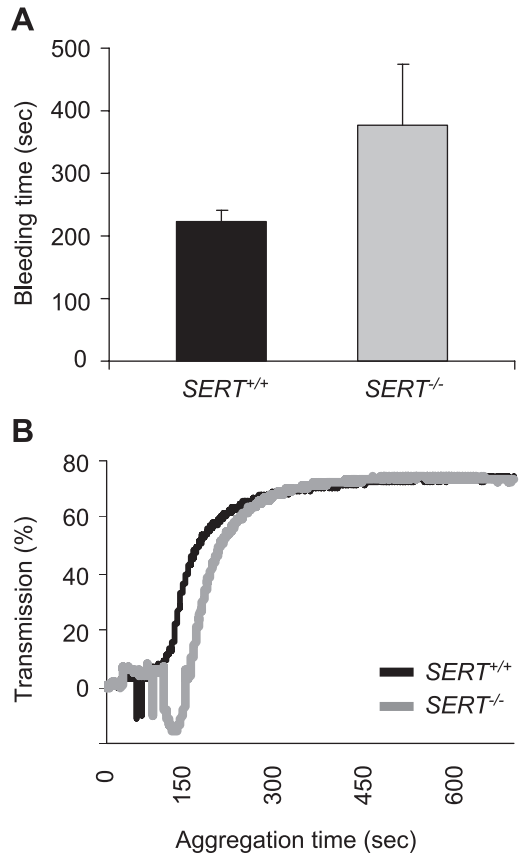

Fig. 1. Bleeding time prolongation and platelet aggregation in serotonin transporter ($SERT$)$^{-/-}$ rats. *A*: prolonged bleeding time of $SERT^{-/-}$ rats (gray bar) compared with $SERT^{+/+}$ rats (solid bar). $P < 0.05$; $n = 4$; Student's *t-test*. *B*: representative platelet aggregation plots with collagen as proaggregatory substance. $SERT^{-/-}$ and $SERT^{+/+}$ platelets aggregated to the same extent under the investigated conditions.

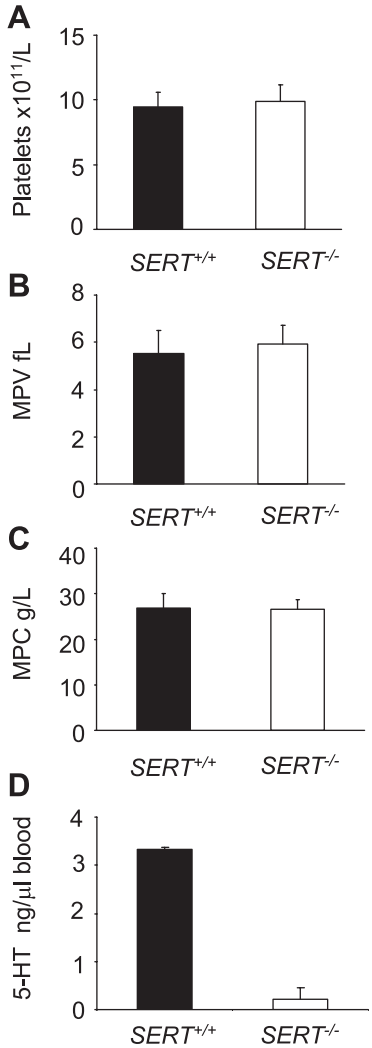

Fig. 2. Platelet numbers and reduced 5-HT blood levels in $SERT^{-/-}$ rats. $SERT^{-/-}$ and $SERT^{+/+}$ rats show same levels of platelet counts (*A*), mean platelet volume (MPV) (*B*), and mean platelet content (MPC) (*C*). 5-HT levels (*D*) are reduced in platelet-free plasma of $SERT^{-/-}$ rats (mean 381 pg/μl) vs. $SERT^{+/+}$ rats (mean 3,364 pg/μl) measured by ELISA assay.

rats lacking SERT. Tail tips served on $SERT^{+/+}$ rats resulted in a mean bleeding time of 223 s (Fig. 1*A*). In contrast, the mean bleeding time of $SERT^{-/-}$ rats extended to 376 s, indicating that SERT function is critical for regulating blood hemostasis.

One reason for the impaired primary hemostasis could be that $SERT^{-/-}$ platelets lost the ability to aggregate. Platelets carry 5-HT in the blood and release it at sites of tissue injury as part of their action on hemostasis. Our previous work has shown that $SERT^{-/-}$ rats contained about 1% of wild-type 5-HT levels in platelets (9), demonstrating that SERT is critical for transferring 5-HT from the blood into platelets. To examine the role of SERT in platelet aggregation, we isolated rat platelets and performed collagen induced aggregation assays. $SERT^{+/+}$ and $SERT^{-/-}$ platelets demonstrated equal responses of ~70% aggregation within 5 min (Fig. 1*B*), indicating that exacerbate bleeding in rats lacking SERT is not a consequence of reduced platelet aggregability.

Next, we quantified the numbers, volume (mean platelet volume), and density (mean platelet contents) of $SERT^{-/-}$ platelets and compared them with wild-type littermates. The platelet measurements were within physiological boundaries for $SERT^{+/+}$ as well as $SERT^{-/-}$ rats (Fig. 2, *A–C*), suggesting that *SERT* deletion does not alter number, size, and density of platelets.

Since vasoconstriction occurs during hemostasis to reduce the flow of blood toward the damaged vessel and is mediated by a number of factors including 5-HT, we measured the levels of 5-HT in the peripheral circulation. The 5-HT concentration in platelet-free plasma of $SERT^{-/-}$ rats was reduced to about 6% of wild-type 5-HT levels (Fig. 2*D*). These findings suggest

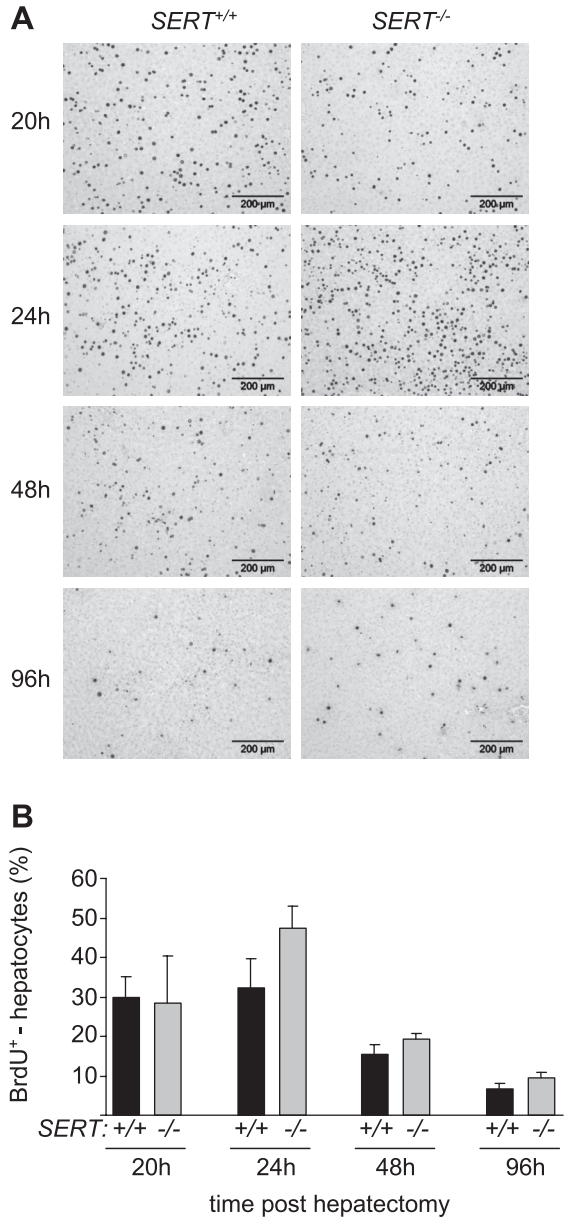

Fig. 3. Liver regeneration in $SERT^{-/-}$ rats. *A*: histological examples of BrdU-stained sections of remnant livers at different time points after 70% partial hepatectomy. *B*: average number of BrdU-positive hepatocytes was counted in livers of $SERT^{-/-}$ and $SERT^{+/+}$ rats, expressed at percentage of total hepatocyte nuclei at different time points following partial hepatectomy. $P < 0.01$ is for $SERT^{-/-}$ vs. $SERT^{+/+}$ rats at 24 h posthepatectomy; $n = 5$ animals per group; Mann-Whitney *U*-test.

that low 5-HT blood levels impair vasoconstriction in *SERT*-deficient rats, resulting in prolongation of the bleeding time.

Efficient liver regeneration in SERT$^{-/-}$ rats. Previous studies suggested that 5-HT is involved in liver regeneration. To test whether reduction of 5-HT levels impairs hepatocyte proliferation in vivo, we performed 70% partial hepatectomy on *SERT*$^{-/-}$ rats. *SERT*$^{-/-}$ livers were histologically normal before and during partial hepatectomy and exhibited no differences in liver weight/body weight ratio compared with *SERT*$^{+/+}$ littermates (data not shown). To monitor hepatocyte DNA replication, we measured BrdU incorporation in livers of homozygous and control rats at 0, 20, 24, 48, and 96 h after partial hepatectomy. Regenerating livers from *SERT*$^{-/-}$ and *SERT*$^{+/+}$ rats exhibited an S-phase peak in hepatocyte DNA replication between 20–24 h after partial hepatectomy (Fig. 3, *A* and *B*). Interestingly, regenerating *SERT*$^{-/-}$ livers displayed an increase in DNA replication at 24 h, whereas at other time points no differences were detectable.

On the basis of the fact that *SERT*$^{-/-}$ rats are incapable of transporting 5-HT into platelets, we investigated whether a potential local increase of 5-HT in the liver could explain the temporary accelerated DNA replication during liver regeneration. Since 5-HT has been reported to increase and attain maximum level in rat livers 24 h after partial hepatectomy (20), we measured 5-HT in the liver 24 h posthepatectomy. ELISA assays revealed decreased levels of 5-HT levels in *SERT*$^{-/-}$ livers compared with wild-type livers (Fig. 4*A*). Moreover, we quantified the 5-HT levels in the portal vein, which provides the direct connection between 5-HT-producing enterochromaffin cells in the intestine and the liver. The portal vein of *SERT*$^{-/-}$ rats contained 6% of wild-type 5-HT levels, whereas the vena cava carried 1% of wild-type levels (Fig. 4*B*). These results demonstrated that there was a decrease of 5-HT levels in the liver as well as in the portal vein of *SERT*$^{-/-}$ rats, indicating that the temporary increase in DNA replication is not due to local increase of 5-HT levels.

Progression into mitosis was not altered in regenerating hepatocytes of *SERT*$^{-/-}$ and *SERT*$^{+/+}$ rats, as evident by similar numbers of mitotic figures at 48 h after partial hepatectomy (data not shown). Taken together, these results suggest that low levels of 5-HT in platelets, blood, and liver are sufficient for liver regeneration and that the activity of SERT is not required for liver regeneration.

DISCUSSION

It was not surprising that *SERT*$^{-/-}$ rats with diminished levels of 5-HT in platelets and blood exhibited a significant prolongation of experimental bleeding times (Fig. 1*A*). Before gaining attention as a neurotransmitter, 5-HT was first detected in the blood and initially studied for its ability to trigger vasoconstriction (7). 5-HT is known to constrict endothelium-denuded arteries, an important mechanism for the closure of vessels at sites of tissue injury (3).

It was surprising that *SERT*$^{-/-}$ platelets did not fail to aggregate in vitro upon stimulation with collagen (Fig. 1*B*). Collagen stimulation induced a pronounced shape change and culminated in aggregation in both *SERT*$^{+/+}$ and *SERT*$^{-/-}$ platelets. Previous studies have shown that an initial dense body secretion, liberating 5-HT, ADP, and Ca^{2+}, drives this reaction, which in turn induces α-granule release (22, 23). The synergistic action of ADP, Ca^{2+}, and 5-HT may explain the preserved aggregation of *SERT*$^{-/-}$ platelets in vitro, given that they contain about 1% of wild-type 5-HT amounts (9), which appeared to be still enough to support the reaction together with the other secretagogs. Concordant with our findings in *SERT*$^{-/-}$ rats, *TPH*$^{-/-}$ mice, which contained about 5% of wild-type 5-HT levels in platelets, showed normal collagen-induced aggregation and exhibited a prolonged bleeding time (8, 28). Interestingly, recent studies performed on platelets derived from *SERT*$^{-/-}$ mice demonstrated a slower rate of ADP-induced aggregation (2). In contrast to ADP, thrombin did not induce a decrease in aggregation rates in *SERT*$^{-/-}$ platelets of mice (2), indicating an agonist-dependent role of the SERT transporter in maintaining platelet aggregation. A more detailed study of how various platelet agonists trigger platelet aggregation will provide a deeper understanding of how SERT activity collaborates with the multiple pathways that drive platelet aggregation.

Several recent papers highlighted the importance of 5-HT in rodent hepatic wound healing (1, 15, 20). The SERT is the major protein responsible for uptake and release of 5-HT (19). 5-HT is present in high concentration in platelets, where it accumulates from the plasma via the active transport system SERT. To determine whether SERT activity is essential for liver regeneration, we performed partial hepatectomies on *SERT*$^{-/-}$ rats. Remarkably, liver regeneration was not impaired in *SERT*$^{-/-}$ rats, though 5-HT levels in blood, platelets, and liver were markedly diminished. These findings indicate that SERT function, as well as 5-HT released from platelets, is not critical for liver regeneration. The extreme low levels of 5-HT in blood, platelets, and liver of *SERT*$^{-/-}$ rats might even indicate that 5-HT is not required at all for liver regeneration. However, on the basis of the previous studies performed by Lesurtel et al. (15) showing that 5-HT agonist or 5-HT precur-

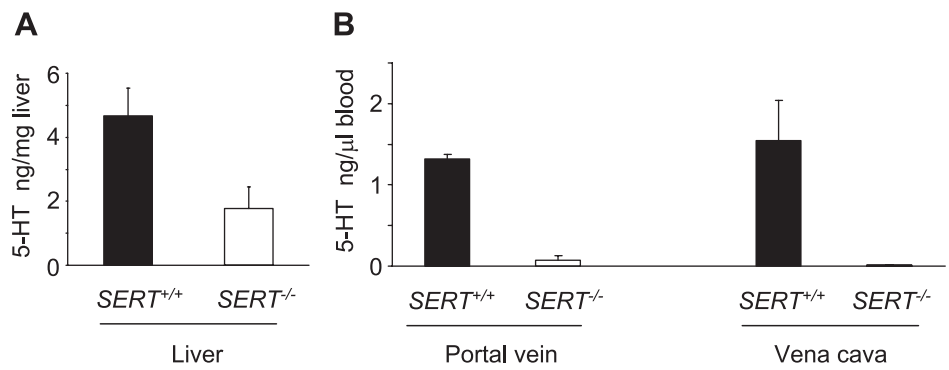

Fig. 4. Reduced 5-HT levels in livers and portal veins of *SERT*$^{-/-}$ rats. *A*: 5-HT levels are reduced in *SERT*$^{-/-}$ livers 24 h after partial hepatectomy. *B*: 5-HT levels of *SERT*$^{-/-}$ rats are reduced in serum derived from portal veins (mean = 75 pg/μl) and vena cava (mean = 15 pg/μl) compared with serum levels of *SERT*$^{+/+}$ animals from portal vein (mean = 1321 pg/μl) and vena cava (mean = 1547 pg/μl).

sor molecules can reconstitute liver proliferation in *TPH1*$^{-/-}$ mice, this suggests that 5-HT is essential for mediating liver regeneration. Therefore, our studies now indicate that very low blood levels of 5-HT are sufficient to support liver regeneration. This is supported by the fact that our *SERT*$^{-/-}$ rats contained about 1–6% of wild-type 5-HT levels in the blood (Figs. 2*D* and 4*B*), whereas in *TPH1*$^{-/-}$ mice 5-HT was not detectable in blood (27) and liver regeneration was impaired. Interestingly, low levels of 5-HT have been detected in platelets of *TPH1*$^{-/-}$ mice and *SERT*$^{-/-}$ rats. These findings indicate that 5-HT levels in platelets are not relevant, but rather threshold levels within the blood are critical for efficient liver regeneration.

Another interesting finding is that DNA replication was enhanced after 24-h hepatectomy in *SERT*$^{-/-}$ rats compared with wild-type rats, whereas at other time points no differences were observed (Fig. 3, *A* and *B*). In a previous study on regeneration of liver in rats, it was noted that splenoectomy increased platelet counts and accelerated liver regeneration via an unclear mechanism (26). In another study, thrombocytotic mice exhibited increased liver regeneration while a thrombocytopenic group showed impaired regeneration (17). In our study, there was no significant difference detected in the number of platelets between *SERT*$^{+/+}$ and *SERT*$^{-/-}$ rats (Fig. 2*A*), which could explain the accelerated liver regeneration. Since 5-HT is not stored in *SERT*-deficient platelets, we investigated whether the accelerated liver regeneration may be caused by an increased flow of 5-HT from the enterochromaffin cells of the intestine toward the liver via the portal vein. Compared with other peripheral vessels, 5-HT levels were higher in the portal vein, but 5-HT levels in *SERT*-deficient rats were still lower compared with wild-type littermates (Fig. 3*B*). It has been reported that liver 5-HT levels peaked at 24 h after partial hepatectomy, remained at high levels up to 40 h, and declined to normal levels thereafter (20). We considered the possibility of a temporal accelerated increase of 5-HT in the liver 24 h after partial hepatectomy, but our studies revealed that 5-HT levels in liver were lower in *SERT*$^{-/-}$ rats compared with the control rats (Fig. 4*A*). Hepatocytes could be sensitized attributable to adaptation to continuous lower 5-HT levels; however, the mechanism remains unknown for the accelerated DNA replication observed in the *SERT*$^{-/-}$ rats after partial hepatectomy.

Our studies provide important insights in the understanding of the physiological role of serotonin in mediating liver regeneration. Previous work has documented an important role of 5-HT in liver regeneration and concluded that platelet-derived 5-HT is involved in the initiation of liver regeneration (15). We demonstrate now that *SERT*$^{-/-}$ rats with 5-HT-depleted platelets exhibit efficient liver regeneration, indicating that the active release of serotonin from platelets upon liver injury is not required for liver regeneration. Our studies rather suggest that liver regeneration is dependent on sufficient levels of serotonin in the serum and independent of platelet-derived serotonin.

REFERENCES

1. **Balasubramanian S, Paulose CS.** Induction of DNA synthesis in primary cultures of rat hepatocytes by serotonin: possible involvement of serotonin S2 receptor. *Hepatology* 27: 62–66, 1998.
2. **Carneiro A, Cook E, Murphy DL, Blakely R.** Interaction between integrinαIIbβ3 and the serotonin transporter regulate serotonin transport and platelet aggregation in mice and humans. *J Clin Invest* 118: 1544–1552, 2008.
3. **Cohen R, Shepherd J, Vanhoutte P.** Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets. *Science* 221: 273–274, 1983.
4. **Fausto N, Campbell JS, Riehle KJ.** Liver regeneration. *Hepatology* 43: S45–S53, 2006.
5. **Ferreira IA, Mocking AIM, Feijge MAH, Gorter G, van Haeften TW, Heemskerk JWM, Akkerman JWN.** Platelet inhibition by insulin is absent in type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol* 26: 417–422, 2006.
6. **Greene A, Puder M.** Partial hepaectomy in the mouse: technique and perioperative management. *J Invest Surg* 16: 99–102, 2003.
7. **Hilton BP, Cumings JN.** An assessment of platelet aggregation induced by 5-hydroxytryptamine: part I after pre-incubation with 5-hydroxytryptamine and reserpine. *J Clin Pathol* 24: 250–258, 1971.
8. **Holtje M, Winter S, Walther D, Pahner I, Hortnagl H, Ottersen OP, Bader M, Ahnert-Hilger G.** The vesicular monoamine content regulates VMAT2 activity through G alpha q in mouse platelets. *J Biol Chem* 278: 15850–15858, 2003.
9. **Homberg J, Mudde J, Braam B, Ellenbroek B, Cuppen E, Joles JA.** Blood pressure in mutant rats lacking the 5-hydroxytryptamine transporter. *Hypertension* 48: e115–e116, 2006.
10. **Homberg JR, Olivier JDA, Smits BMG, Mul JD, Mudde J, Verheul M, Nieuwenhuizen OFM, Cools AR, Ronken E, Cremers T, Schoffelmeer ANM, Ellenbroek BA, Cuppen E.** Characterization of the serotonin transporter knockout rat: a selective change in the functioning of the serotonergic system. *Neuroscience* 146: 1662–1676, 2007.
11. **Kihara H, Koganei H, Hirose K, Yamamoto H, Yoshimoto R.** Antithrombotic activity of AT-1015, a potent 5-HT2A receptor antagonist, in rat arterial thrombosis model and its effect on bleeding time. *Eur J Pharmacol* 433: 157–162, 2001.
12. **Kim DK, Tolliver TJ, Huang SJ, Martin BJ, Andrews AM, Wichems C, Holmes A, Lesch KP, Murphy DL.** Altered serotonin synthesis, turnover and dynamic regulation in multiple brain regions of mice lacking the serotonin transporter. *Neuropharmacology* 49: 798–810, 2005.
13. **Kulinskii V, Saratikov A, Vstavskaia IA, Udovitsina T.** Receptors mediating serotonin-stimulating liver regeneration in mice. *Biull Eksp Biol Med* 95: 89–91, 1983.
14. **Kulinskii V, Udovitsina T, Vstavskaia IA, Rykov S.** Comparison of the changes in mitotic activity and in serotonin concentration in regenerating liver. *Vopr Med Khim* 29: 104–107, 1983.
15. **Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, Gachet C, Bader M, Clavien PA.** Platelet-derived serotonin mediates liver regeneration. *Science* 312: 104–107, 2006.
16. **Michalopoulos GK.** Liver regeneration. *J Cell Physiol* 213: 286–300, 2007.
17. **Murata S, Ohkohchi N, Matsuo R, Ikeda O, Myronovych A, Hoshi R.** Platelets promote liver regeneration in early period after hepatectomy in mice. *World J Surg* 31: 808–816, 2007.
18. **Murphy DL, Lerner A, Rudnick G, Lesch KP.** Serotonin transporter: gene, genetic disorders, and pharmacogenetics. *Mol Interv* 4: 109–123, 2004.
19. **Ni W, Watts SW.** 5-Hydroxytryptamine in the cardiovascular system: focus on the serotonin transporter (SERT). *Clin Exp Pharmacol Physiol* 33: 575–583, 2006.
20. **Papadimas GK, Tzirogiannis KN, Panoutsopoulos GI, Demonakou MD, Skaltsas SD, Hereti RI, Papadopoulou-Daifoti Z, Mykoniatis MG.** Effect of serotonin receptor 2 blockage on liver regeneration after partial hepatectomy in the rat liver. *Liver Int* 26: 352–361, 2006.
21. **Ruddell RG, Mann DA, Ramm GA.** The function of serotonin within the liver. *J Hepatol* 48: 666–675, 2008.
22. **Shirakawa R, Yoshioka A, Horiuchi H, Nishioka H, Tabuchi A, Kita T.** Small GTPase Rab4 regulates Ca2+-induced alpha-granule secretion in platelets. *J Biol Chem* 275: 33844–33849, 2000.
23. **Siess W.** Molecular mechanisms of platelet activation. *Physiol Rev* 69: 58–178, 1989.
24. **Stanworth SJ, Denton K, Monteath J, Patton WN.** Automated counting of platelets on the Bayer ADVIATTMM 120 analyser. *Clin Lab Haematol* 21: 113–118, 1999.

25. **Taub R.** Liver regeneration: from myth to mechanism. *Science* 5: 836–847, 2004.
26. **Tomikawa M, MH, Highashi H, Ohta M, Sugimachi K.** The role of the spleen, platelets and plasma hepatocyte growth factor activity on hepatic regeneration in rats. *J Am Coll Surg* 182: 12–16, 1996.
27. **Walther DJ, Peter JU, Bashammakh S, Hortnagl H, Voits M, Fink H, Bader M.** Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* 299: 76, 2003.
28. **Walther DJ, Peter JU, Winter S, Höltje M, Paulmann N, Grohmann M, Vowinckel J, Alamo-Bethencourt V, Wilhelm CS, Ahnert-Hilger G, Bader M.** Serotonylation of small GTPases is a signal transduction pathway that triggers platelet [alpha]-granule release. *Cell* 115: 851–862, 2003.
29. **Yu PL, Fujimura M, Hayashi N, Nakamura T, Fujimiya M.** Mechanisms in regulating the release of serotonin from the perfused rat stomach. *Am J Physiol Gastrointest Liver Physiol* 280: G1099–G1105, 2001.