

Sympathetic Control of Portal Circulation under Partial Hepatectomy in Rats

Lili LIU¹, Takeo SAKAGUCHI², Tatsuo KANDA¹ and Katsuyoshi HATAKEYAMA¹

¹Division of Digestive and General Surgery, Department of Regenerative and Transplant Medicine, and ²Division of Integrative Physiology, Department of Sensory and Integrative Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Received September 24 2002; accepted April 9 2003

Summary. The effect of a hepatectomy on the sympathetic control of portal circulation was examined in anesthetized rats. Sympathetic activation was obtained by cerebral ischemia. Cerebral ischemia with carotid arterial occlusion (30s) produced a reduction in the portal venous flow (PVF) in the intact liver rats. When the rats received a partial hepatectomy (40, 66 and 90%), there was a proportional relationship between PVF and liver volume, and a significant decrease in PVF due to cerebral ischemia was obtained when 40% of the liver was resected. Cerebral ischemia failed to change PVF under 66% and 90% hepatectomies. Under hepatectomy, nanomolar quantities of adrenaline injected into the portal vein reproduced a decrease in PVF that was seen after cerebral ischemia. A blood flow reduction due to cerebral ischemia was observed in the superior mesenteric vein when 40% and 66% hepatectomies were conducted. Hepatic arterial and splenic venous blood flow were unaffected by cerebral ischemia with or without hepatectomy. Liver functional parameters in the blood were unchanged before and after cerebral ischemia under hepatic resection.

These results suggest that hepatectomy modifies the sympathetic regulation of portal circulation through adrenergic vasoconstriction, and that the neural circulatory system maintains its primary functional efficacy when less than 40% of the liver is resected.

Key words—hepatic resection, sympathetic nerve, liver, circulation, rat.

INTRODUCTION

Liver vascular beds are known to be a major blood reservoir which stabilizes systemic circulation, with blood mobilization controlled by vascular constriction through the sympathetic nerves^{1–4}. Recently, it was observed that activation of the sympathetic nerves with cerebral ischemia or by electrical stimulation of the hepatic splanchnic branch resulted in a decrease in portal blood flow in intact rat livers^{4–7}. Moreover, it was revealed that a partial hepatectomy affected portal venous resistance or portal venous pressure or both^{8,9}. While these findings suggest that a hepatectomy interferes with the sympathetic performance in the mobilization of portal blood, the effect of a hepatectomy on the sympathetic contribution to portal circulation has yet to be examined. In contrast, blood flow in the portal vein is mainly collected from two venous structures¹⁰: the superior mesenteric vein and the splenic vein.

This experiment was designed to investigate whether a hepatectomy influences the sympathetic control of portal blood flow in relation to the two structures.

MATERIALS AND METHODS

Animals

Twenty male Wistar rats weighing 300–330 g were used. They were housed individually (12: 12; light-

Correspondence: Lili Liu, M.D., Division of Digestive and General Surgery, Department of Regenerative and Transplant Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata 951-8510, Japan

Abbreviations—HAF, hepatic arterial flow; PVF, portal venous flow; SMVF, superior mesenteric venous flow; SVF, splenic venous flow.

dark cycle) in a room with the temperature ranging from 22.0 to 24.0°C, and were fed ad lib on a standard diet with free access to tap water before the experiments. The experiments were carried out in the afternoon (13:00-16:00 h) to eliminate diurnal changes in sympathetic activity¹¹.

Anesthesia and general monitoring

The rats were initially anesthetized with pentobarbital sodium (45 mg/kg, i.p.), and an amount of this agent (7.5 mg/kg) was injected intramuscularly every 30 min to maintain the depth of anesthesia⁴. A cannula was inserted into the trachea to allow adequate ventilation. The aortic and carotid sinus nerves were sectioned bilaterally to eliminate any aortic pressure responses which would affect portal blood flow or portal pressure¹¹. The systemic arterial pressure (SAP) was monitored at the right carotid artery. A portion of the portal vein, the hepatic artery, the mesenteric vein, and the splenic vein was cleared, keeping the nerves intact but separated from the surrounding connective and fat tissues. Anal temperature was maintained between 37.0 and 37.5°C with a heating lamp throughout the experiments.

Estimation of blood flow and pressure

The probe for blood flow estimation was placed around the portal vein, and portal venous flow (PVF) was measured with an ultrasonic blood-flow meter (Transonic T201, Advance, NY). Superior mesenteric venous flow (SMVF) or hepatic arterial flow (HAF) or splenic venous flow (SVF) was estimated with the probe^{4,6}. The data were recorded on a pen writing recorder (SR6221, Graphtec, Tokyo). A fine analysis was obtained by evaluating responses in terms of the percent change as necessary.

Sympathetic activation with cerebral ischemia

Cerebral ischemia was obtained by occluding both carotid arteries on each side: the left side artery was closed during the experiment, and the right side artery was closed at the required time. Duration of the occlusion was fixed at 30s based on a report that carotid arterial occlusion for 30s evoked a reproducible reduction in PVF through cerebral sympathetic activation in the rat⁴. A Heifetz clip (Edward Weck, NC) was applied for occlusion. The occlusion was started about 20 min after hepatic surgical treatment.

Hepatectomy

A partial hepatectomy was performed by the methods previously described^{4,6,12}: either the median lobe, constituting about 40%, or the median and left lateral lobes, comprising about 66%, and forming a unit, was ligated and removed. Only the caudal lobe was left in the 90% hepatectomy.

Sectioning nerve

The abdominal sympathetic pathway was sectioned at the required position by the method described previously¹³: a loose thread was looped around the nerve, and then both ends of the thread were passed through a plastic tube so that the nerve could be cut by pulling the loop of the thread.

Adrenaline injection

Adrenaline (Wako Pure Chemical Industries, Ltd., Osaka) dissolved in physiological saline was injected into the portal vein by means of an infusion pump. Physiological saline was used as the control. The test agents were injected through a small 2 mm long catheter (PE-10, Becton, Dickinson and Company, NJ) inserted into the portal vein upstream from the bifurcation of the splenic vein.

Chemical analysis

Blood for chemical analysis (120 micro-l) was drawn off through the portal catheter, and was cooled immediately with iced water and centrifuged at 2,200 rpm for 20 min. Then the separated serum was stored at -20°C until measurement of the following parameters with an auto-analyzer (Hitachi-7070; Hitachi, Tokyo)¹³: glucose (Glu, glucose oxidase method), glutamic pyruvic transaminase (GPT, ultraviolet method), alkaline phosphatase (Alp, Bessy-Lowry method) and total bilirubin (TB, azobilirubin method).

Data analysis

The data were ANOVA analyzed, and specific values were evaluated with Duncan's multiple range test. Regression analysis was also utilized. A value of $p < 0.05$ was defined as significant.

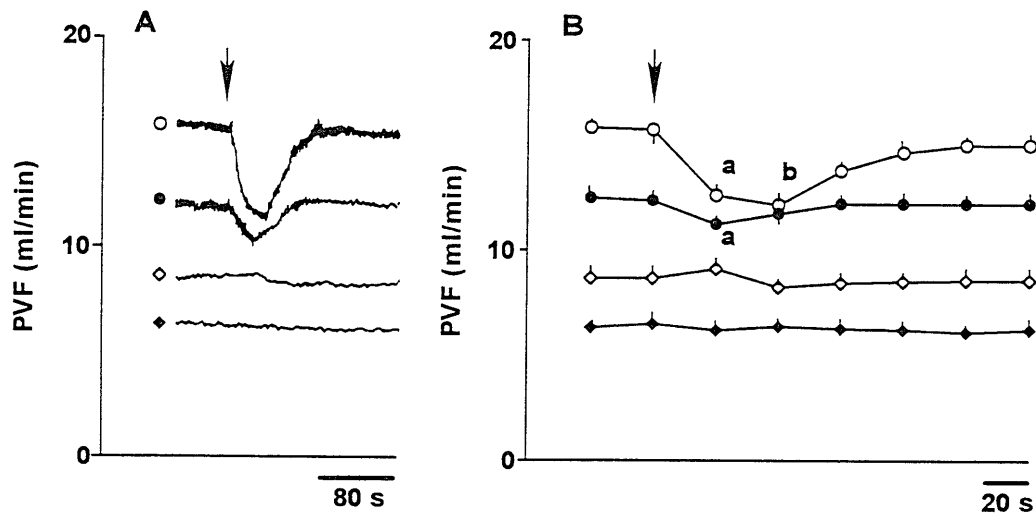


Fig. 1. **A.** Representative response in PVF and hepatectomy to 30s carotid arterial occlusion. Liver intact (○), 40% (●), 66% (◇), and 90% (◆) hepatectomized rats were used. An arrow shows the start of occlusion. **B.** Time course of PVF after the occlusion. Liver intact (○), 40% (●), 66% (◇), and 90% (◆) hepatectomized animals were used. An arrow indicates the start of occlusion. Values are the mean \pm SEM ($n=6$). ^a $p<0.05$ vs value before occlusion. ^b $p<0.01$ vs value before occlusion.

Table 1.

A. Body weight and wet weight of visceral organs				
Body weight (g)	Liver (mg)	Spleen (mg)	Stomach (mg)	
327±4	14519±412	1074±59	1859±43	
B. Basal levels of circulatory parameters				
SAP	PVF	HAF	SMVF	SVF
(mmHg)	(ml/min)			
112±3	15.7±0.4	2.9±0.1	13.3±0.3	2.8±0.2

Values are the mean \pm SEM ($n=6$).

RESULTS

Relationship between PVF and liver volume

Initial levels of PVF were decreased according to the volume of the liver (Fig. 1 and Table 1), and a significant correlation between the PVF and liver volume was established. The regression coefficient ($r=0.940$) was significant, $p<0.01$.

Time course of PVF associated with cerebral ischemia

When carotid arterial occlusion was applied for 30s, PVF was transiently reduced in the liver intact

animals. The response reached its nadir about 40s after the occlusion, and then recovered to the control level within another 2 min (Figs. 1 and 2). The PVF response was completely blocked by sectioning the hepatic splanchnic branch (data not shown).

Time course of PVF due to cerebral ischemia under hepatectomy

Cerebral ischemia transiently reduced PVF, and a hepatectomy altered the PVF response (Fig. 2). When percent changes in PVF were analyzed, a significant reduction was obtained in the intact liver and 40% hepatectomized rats, but no significant change in PVF was detected when 66% and 90% hepatectomies were conducted.

Effects of adrenaline and hepatectomy

Adrenaline injection into the portal vein induced a fall in PVF. When percent changes in PVF were analyzed 40s after injection, the decrease was dose-dependent in the intact liver and 40% hepatectomized rats but not in the 66% hepatectomized rats (Fig. 3).

Time course of SMVF associated with cerebral ischemia

When blood flow was analyzed by percent change, a

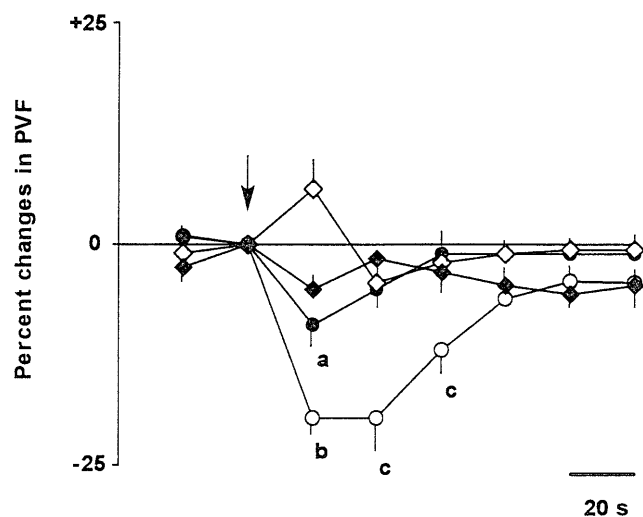


Fig. 2. Percent changes in PVF after 30 s carotid arterial occlusion. Liver intact (\circ), 40% (\bullet), 66% (\diamond), and 90% (\blacklozenge) hepatectomized rats were used. An arrow indicates the start of occlusion. Values are the mean \pm SEM ($n=6$). ^a $p<0.01$ vs \diamond and \blacklozenge . ^b $p<0.01$ vs \bullet . ^c $p<0.01$ vs \bullet , \diamond and \blacklozenge .

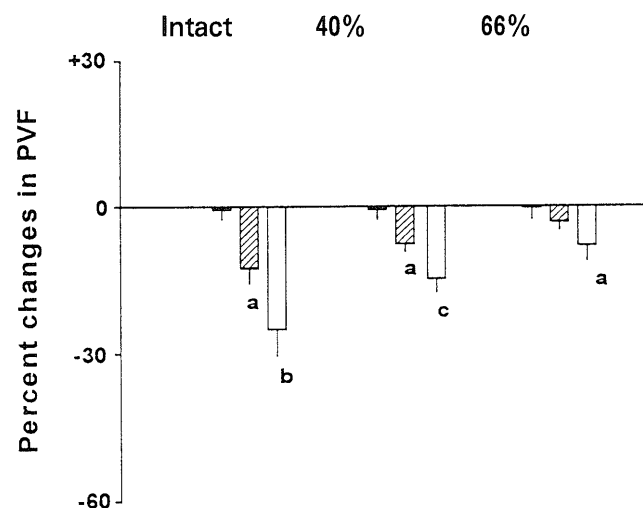


Fig. 3. PVF in response to adrenaline under hepatectomy. Adrenalin (hatched , 1×10^{-9} M; diagonal lines , 8×10^{-9} M) or saline (white) was portally injected. PVF values 40s after adrenalin injection are for the liver intact, 40%, and 66% hepatectomized rats. Values are the mean \pm SEM ($n=6$). ^a $p<0.01$ vs white . ^b $p<0.01$ vs adrenalin hatched . ^c $p<0.05$ vs adrenalin hatched .

transient fall in SMVF was seen after cerebral ischemia in the intact liver animals. A significant fall in SMVF was also detected in 40% and 66% hepatectomized rats. No significant change in SMVF was seen after 90% hepatectomy (Fig. 4).

Cerebral ischemic effect on PVF, SMVF and SVF under hepatectomy

When the levels of blood flow 40s after cerebral ischemia were analyzed by percent change and the values were plotted under 40%, 66% and 90% he-

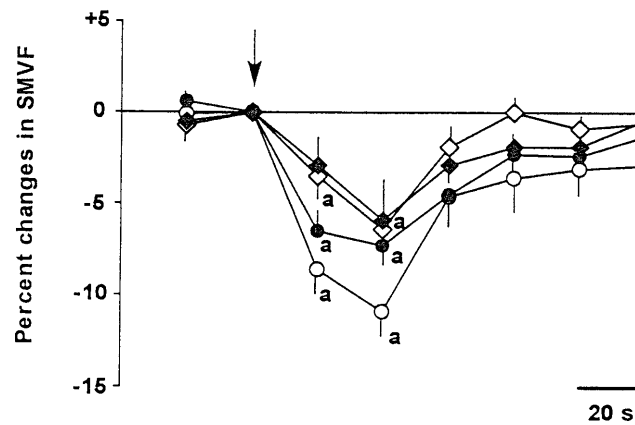


Fig. 4. Percent changes in SMVF after 30 s carotid arterial occlusion. Liver intact (\circ), 40% (\bullet), 66% (\diamond), and 90% (\blacklozenge) hepatectomized rats were used. An arrow shows the start of occlusion. Values are the mean \pm SEM ($n=6$). ^a $p < 0.01$ vs immediately before occlusion.

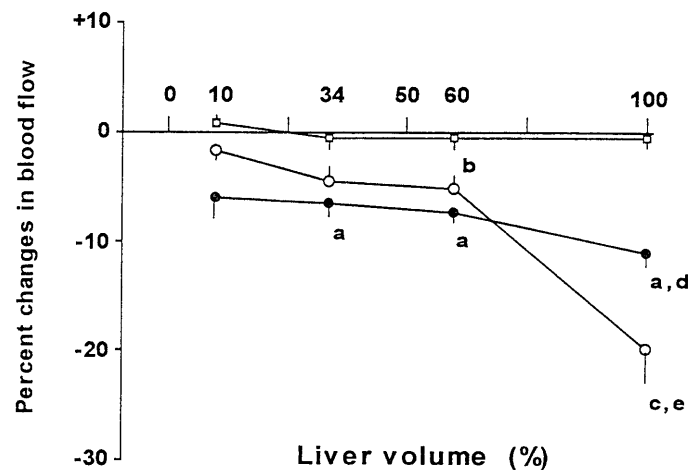


Fig. 5. Percent changes in blood flow 40 s after cerebral ischemia. PVF (\circ), SMVF (\bullet) and SVF (\square) values are for different volumes of the liver. Values are the mean \pm SEM ($n=6$). ^a $p < 0.01$ vs \square . ^b $p < 0.05$ vs \square . ^c $p < 0.01$ vs \bullet . ^d $p < 0.05$ vs \bullet (10, 34 and 60%). ^e $p < 0.01$ vs \circ (10, 34 and 60%).

patectomies, the magnitude of flow response was different among PVF, SMVF and SVF. The neural efficacy of a hepatectomy was in the order of PVF, SMVF to SVF, and the efficacy was reduced when the liver volume was lessened (Fig. 5).

Hepatic arterial and splenic venous blood flow with cerebral ischemia

Blood flow in the hepatic artery and the splenic vein was unchanged after cerebral ischemia (data not shown).

Hepatic functional parameters after cerebral ischemia

Serum concentrations of GPT, Alp and TB were unchanged before and 60 min after cerebral ischemia (Table 2).

Table 2. Chemical parameters during experiments under 66% hepatectomy

Parameter	Before	60 min later
Glu (mg/dl)	141±9	133±8
GPT (U/l)	25±2	27±4
Alp (U/l)	326±42	315±43
TB (mg/dl)	0.1±0.0	0.1±0.0

Values are the mean±SEM (n=6).

DISCUSSION

We found that a hepatectomy modifies the sympathetic performance of portal blood mobilization: sympathetic activation with cerebral ischemia resulted in a decrease in the portal blood flow dependent on the volume of the liver. This is partially consistent with the view that sympathetic activation is effective in reducing portal circulation through vasoconstriction in liver intact animals^{4,6,14}.

Rheologically, portal circulation has been considered to be determined by two components - portal venous pressure and portal venous resistance^{8,9}, and a hepatectomy has been regarded as an obstacle^{9,14}. In this study, portal blood flow was decreased after a partial hepatectomy, and a positive relationship between levels of portal blood flow and the volume of the liver was obtained so that increased resistance due to a hepatectomy may have introduced decreased basal levels of portal blood flow. Moreover, it was noted that the peak effect of the sympathetic drive on blood flow was attained more rapidly in the hepatectomized rats than in the liver intact rats. One reason for this might be the volume of the liver serving as a factor which determines the speed of liver blood mobilization by the nerves.

An isolated portal vein preparation has been shown to contract in response to biologically active catecholamines such as adrenaline and noradrenaline¹⁵. In this study, because adrenaline directly injected into the portal vein decreased portal blood flow in a dose-dependent fashion, the decrease in portal blood flow due to the agent may be ascribed to adrenergic vasoconstriction of the portal venules within the liver¹⁶. In connection with this, the injected concentration of adrenaline was at a level which was released into the circulation in response to electrical stimulation of the nerves innervating the adrenal gland.¹⁷ Considering these findings together with the present results showing that decreased portal blood flow due to adrenaline was dose-dependent when less

than 40% of the liver was resected, it is possible to propose that the active concentration of catecholamines inducing portal vasoconstriction is dependent on the net volume of adrenergic fibers in the liver.

The portal blood stream followed the mesenteric blood stream, and a synchronized interaction between portal and superior mesenteric vasoconstriction existed when cerebral ischemia was introduced in the liver intact animals, though the sympathetic nerve innervating the superior mesenteric vein directly regulates the vascular tone independent of the nerve controlling the portal venous tone^{4,6}. In this study, different peak responses in blood flow between the two veins were observed after cerebral ischemia under a hepatectomy. It is likely that the volume of the liver modifies the sympathetic function which synchronizes the two venous constrictions.

Of particular interest is the finding that the efficacy of the sympathetic drive constricting the venous walls differs in the portal from that in the superior mesenteric veins. As shown in Fig. 5, the former introduced a precipitous decline and the latter a mild decline according to the liver volume. It is not easy to explain this. One interpretation is that, if a hepatectomy evenly increases venous resistance in the portal and mesenteric areas, vascular constriction due to the nerves is primarily differential according to the terminating area. In fact, it has been shown that the visceral organs are innervated with several sympathetic structures^{6,10}: the splanchnic nerves, the celiac ganglions and the lumbar sympathetic chain. It can be deduced that these various neural networks determine the final performance of the nerves, and this may also hold true for the portal and mesenteric vascular beds.

The spleen has been shown to be an organ reserving blood volume: a constrictor response of the splenic veins was provoked when the sympathetic nerves were activated in dogs^{18,19}, and it has been presumed that this nerve takes part in blood mobilization from the spleen to the portal vein. In this study, however, blood flow in the splenic vein was entirely unaffected by sympathetic activation with or without a hepatectomy. This is in keeping with the previous finding that sympathetic activation had no effect on splenic venous circulation in intact liver rats⁴. There may be a specific difference among individual animals.

Although the hepatic branch of the sympathetic nerves supplying the hepatic artery has been reported to have some neural fibers which run through pathways other than the major splanchnic nerves in rats²⁰, in this study, the sympathetic activation failed

to change hepatic arterial blood flow. There may be no neural circulatory system in the hepatic artery of rats.

Finding that a hepatectomy had no influence on hepatic functional scores in the blood after cerebral ischemia suggests that liver parenchymal substances released from the liver were not involved in the sympathetic circulatory response.

This study simulated the acute phase of enterohepatic circulation caused by the sympathetic nerves after liver trauma, liver resection and liver transplantation. Consequently, the liver blood reservoir function by the nerve¹⁻⁴⁾ characteristically deteriorated after the loss of liver volume. In relation to this, there are liver diseases which cause portal hypertension in humans^{14,21)} and, experimentally, portal pressure can determine portal circulation¹⁻⁴⁾. However, the pathophysiological connection between portal hypertension and liver volume with sympathetic activation remains to be clarified. Further study on this aspect is required.

From these observations, we conclude that a hepatectomy changes the sympathetic regulation of portal circulation, and the neural effect of the regulatory system including mesenteric circulation is determined by the volume of the liver.

Acknowledgments. The authors are greatly indebted to Mr. H. Higuchi (Div. of Surg.) for his assistance.

REFERENCES

- 1) Greenway CV, Stark RD: Hepatic vascular bed. *Physiol Rev* **51**: 23-65, 1971.
- 2) Ljung B: Local transmitter concentrations in vascular smooth muscle during vasoconstrictor nerve activity. *Acta Physiol Scand* **77**: 212-223, 1969.
- 3) Ljung B, Stage D: Postnatal ontogenetic development of neurogenic and myogenic control in the rat portal vein. *Acta Physiol Scand* **94**: 112-127, 1975.
- 4) Aono T, Ohtake M, Sakaguchi T, Sandoh N, Tsukada K, Hatakeyama K: Responses in portal venous flow and pressure produced by cerebral sympathetic activation in rats. *Acta Med Biol* **41**: 97-103, 1993.
- 5) Takeuchi T, Horiuchi J, Terada N: Central vasomotor control of the rabbit portal vein. *Pflügers Arch* **413**: 348-353, 1989.
- 6) Ohtake M: The role of the abdominal sympathetic nervous system in regulating portal venous flow and its functional distribution. *Surg Today* **22**: 128-136, 1992.
- 7) Greenway CV, Lawson AE, Mellander S: The effects of stimulation of the hepatic nerves, infusions of noradrenaline and occlusion of the carotid arteries on liver blood flow in the anaesthetized cat. *J Physiol (Lond)* **192**: 21-41, 1967.
- 8) Lauth WW, Greenway CV, Legare DJ, Weisman H: Localization of intra-hepatic portal vascular resistance. *Am J Physiol* **251**: G375-381, 1986.
- 9) Richardson PDI, Withington PG: Liver blood flow. I. Intrinsic and nervous control of liver blood flow. *Gastroenterology* **81**: 159-173, 1981.
- 10) Lambert R: Surgery of the digestive system in the rat. Charles C Thomas Publisher, Springfield, 1965.
- 11) Sakaguchi T, Takahashi M, Bray GA: Diurnal changes in sympathetic activity: relation to food intake and to insulin injected to the ventromedial or suprachiasmatic nucleus. *J Clin Invest* **82**: 282-286, 1988.
- 12) Gaub J, Iversen J: Rat liver regeneration after 90% partial hepatectomy. *Hepatology* **4**: 902-904, 1984.
- 13) Sakaguchi T, Liu L: Hepatic branch vagotomy can block liver regeneration enhanced by ursodesoxycholic acid in 66% hepatectomized rats. *Auton Neurosci: Basic and Clinic* **99**: 54-57, 2002.
- 14) Mathie RT, Nagorney DM, Blumgart LH: Liver blood flow: physiology, measurement and clinical relevance. In: Blumgart LH (ed), *Surgery of the liver and biliary tract*. Churchill Livingstone, London 1988, p73-87.
- 15) Ljung B, Bevan JA, Pegram BL, Purdy RE, Su M: Vasomotor nerve control of isolated arteries and veins. *Acta Physiol Scand* **94**: 506-516, 1975.
- 16) Schwartz SI: Influence of vasoactive drugs on portal circulation. *Ann NY Acad Sci* **170**: 296-314, 1970.
- 17) Warashina A, Fujiwara N, Shimoji K: Characteristics of nicotinic and muscarinic secretory responses in the rat adrenal medulla studied by real-time monitoring of catecholamine release. *Biomed Res* **10**: 157-164, 1989.
- 18) Carneiro JJ, Donald DE: Blood reservoir function of dog spleen, liver, and intestine. *Am J Physiol* **232**: H67-72, 1977.
- 19) Iizuka T, Mark AL, Wendling MG, Schmid PG, Eckstein JW: Differences in responses of saphenous and mesenteric veins to reflex stimuli. *Am J Physiol* **219**: 1066-1070, 1970.
- 20) Reilly FD, McCuskey PA, McCuskey RS: Intrahepatic distribution of nerves in the rat. *Anat Rec* **191**: 55-68, 1978.
- 21) Yachha SK, Chetri K, Lal R: Management of portal hypertension. *Indian J Pediatr* **69**: 809-813, 2002.