Capacitance Responses and Fluid Exchange in the Cat Liver during Stimulation of the Hepatic Nerves

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ABSTRACT

Techniques are described to study the hepatic vascular bed in the anesthetized cat without interference with the hepatic artery or portal vein. Liver volume was recorded with a plethysmograph while simultaneous recordings were made of arterial and portal pressures and total hepatic blood flow. Hepatic venous pressure could be raised to any desired level, and capillary filtration coefficients could therefore be determined. The hepatic blood volume was 27 ± 3.3 ml/100 g liver (14% of the total blood volume of the cat). Stimulation of the hepatic nerves decreased liver volume. Maximal responses were reached at frequencies of about 6/sec, and 50% of the blood in the liver was expelled. The hepatic vascular bed is thus an important blood reservoir. The capillary filtration coefficient was 0.30 ± 0.03 ml/min/mm Hg/100 g, and this value was not significantly changed during stimulation of the hepatic nerves.

ADDITIONAL KEY WORDS plethysmograph venous long circuit liver blood volume capillary filtration coefficient

In 1960, Mellander (1) described a technique for the simultaneous measurement of resistance, capacitance, and fluid-exchange responses in the vascular bed of skeletal muscle. This technique was later extended to the intestinal vascular bed (2), and other similar studies have been made on the intestinal vascular bed by Johnson and Hanson (3, 4) and on the spleen by Greenway et al. (5). The results of these investigations have been reviewed recently by Mellander and Johansson (6). Similar information is lacking for the hepatic vascular bed. Although it is frequently stated that the liver is an important blood reservoir controlled by the sympathetic nervous system, that rapid exchanges of fluid can occur across the sinusoidal walls, and that the liver plays a key role in the formation of ascites, these ideas are based on relatively few data. This paper describes a technique by which these problems can be studied and examines the quantitative importance of the liver as a blood reservoir.

In 1930, Griffith and Emery (7) observed a decrease in liver volume when the hepatic sympathetic nerves were stimulated, although no quantitative data were reported.

In a previous paper (8), we described the responses of the resistance vessels of the hepatic artery and portal vein to stimulation of the hepatic nerves. Now we describe a preparation in which the liver volume was recorded continuously with a plethysmograph in the anesthetized cat. Hepatic venous pressure could be varied to allow measurements of the capillary filtration coefficient. The responses to stimulation of the hepatic nerves were studied.

Methods

Cats were anesthetized by injection of sodium pentobarbital (Nembutal), 30 mg/kg ip. When reflex limb and ear movements returned, addi-
tional doses of pentobarbital, 3 mg/kg, were given through a cannula in a forelimb vein. The trachea was cannulated, and mean arterial pressure was recorded from a femoral artery. The abdomen was opened along the midline and right subcostal margin, and the ligaments connecting the central and left lobes of the liver to the diaphragm were ligated and cut. The hepatic nerves were prepared for stimulation (8), and the lymphatics round the hepatic artery were tied. Portal venous pressure was recorded from a cannula inserted through a small vein from the appendix (9).

The liver, with the exception of the right posterior lobe, was then lifted and inserted into a plethysmograph. The attachments of the liver to the posterior abdominal wall and the vessels to and from the liver passed through a 2-cm diameter aperture which was sealed with a plasticized hydrocarbon gel (Plastibase, Squibb). The portal vein and hepatic artery remained intact. The plethysmograph was filled with Ringer-Locke solution at 37 °C and connected to a float recorder operating an isotonic transducer. The pressure within the plethysmograph was adjusted to zero, relative to the right atrium. The arterial and portal pressures and the liver volume were recorded on a polygraph (Grass Instrument Co.).

The cat was artificially ventilated, the chest was opened through the sixth intercostal space, and the thoracic inferior vena cava was exposed. After allowing 30 minutes for hemostasis, heparin, 5 mg/kg, was administered intravenously, and the external jugular and femoral veins were cannulated. The thoracic inferior vena cava was tied, and the blood from the abdominal vena cava was drained through the femoral cannula to a reservoir, previously filled with heparinized blood from a donor cat. From the reservoir, the blood was returned to the cat through the cannulas in the jugular veins by means of a peristaltic pump (Harvard Apparatus Co.). The thoracic inferior vena cava was cannulated, and the abdominal inferior vena cava was occluded between the entrance of the lumboadrenal veins and the point where the cava passes into the substance of the liver. The hepatic blood flow now drained through the cannula in the thoracic inferior vena cava to the reservoir, while the remainder of the inferior vena cava flow drained through the femoral cannulas to the same reservoir. The blood was returned to the heart via the jugular veins. Total hepatic blood flow was measured using an extracorporeal probe of an electromagnetic flow-meter (Nycotton, Oslo). A drain tube was inserted into the pleural cavity, and the chest wall was closed round this tube and the cannula. The drain tube was connected to a suction pump to produce a negative pressure of about 5 cm H2O in the chest. Artificial ventilation was then discontinued, and the animal breathed spontaneously for the remainder of the experiment.

It has been shown previously (10) that the measurement of hepatic blood flow in this way was not invalidated by the presence of significant venous anastomoses in the cat. Hepatic venous pressure was determined by the position of the hepatic outflow into the reservoir, and the level could be varied at will. Initially this pressure was set below the level of the right atrium. After the preparation was set up, the pressure was increased in steps of 1 cm blood, until a continuous rise in hepatic volume occurred. To obtain an isovolumetric state, the pressure was then reduced 1 cm and kept at that level (usually 0 to 2 cm blood above the right atrium) except when varied for a specific purpose. To determine the capillary filtration coefficient (1, 2), the hepatic venous pressure was increased by 4.7 mm Hg for 1½ minutes.

At the end of each experiment, the inflow and outflow vessels of the liver were simultaneously clamped. The liver was removed from the plethysmograph and weighed, and its vascular bed was perfused with 0.9% saline until the perfusate was clear. The hemoglobin concentration of the washout fluid was compared with that of a sample of arterial blood, and the blood content of the liver was calculated (1, 2).

Results

CONTROL VALUES

In 15 cats (mean weight 2.6 kg), the liver weight was 72 ± 3.8 g (mean ± se), and the portion of the liver placed in the plethysmograph weighed 61 ± 3.1 g (85% of the total). Mean portal venous pressure was 7 ± 0.4 mm Hg before and 8 ± 0.4 mm Hg after insertion of the liver into the plethysmograph. Thus there was minimal obstruction to the portal venous flow by the plethysmograph. After the preparation was set up, mean arterial pressure was 112 ± 2.4 mm Hg, and total hepatic blood flow was 128 ± 12 ml/min/100 g liver. Hepatic blood volume determined at the end of the experiments was 27 ± 3.3 ml/100 g liver.

CAPACITANCE RESPONSES TO HEPATIC NERVE STIMULATION

The hepatic nerves were stimulated with square-wave pulses (15 v, 1-msec duration) at frequencies of 1 to 10/sec for periods of 3 to 20 minutes on 107 occasions in 15 cats. The
Responses in one cat to stimulation of the hepatic nerves (15 v, 1 msec) at frequencies of 2 and 4/sec are shown in Figure 1. The increases in portal pressure were similar to those described previously (8). Liver volume decreased and reached a plateau after about 4 minutes. The decrease was greater and the plateau was reached earlier as the frequency of stimulation was increased until maximal responses were obtained at frequencies of 6 to 8/sec. Complete frequency-response curves were obtained in 12 cats, and the means and standard errors of these results are plotted (Fig. 2). The frequency-response graph described previously (8) for the hepatic arterial resistance vessels is also shown, and the graph for the portal venous resistance vessels was also very similar to this (8).

For each cat, the decrease in volume in response to a maximal frequency of stimulation was expressed as a percentage of the blood content determined at the end of the experiment. Maximal stimulation of the sympathetic nerves expelled 49 ± 4% (mean ± se) of the blood content. This capacitance response was usually well-maintained for up to 20 minutes (the longest period tested), and on cessation of stimulation the volume returned to the control level. However, in two cats, the volume tended to return to control level during the period of stimulation; on cessation...
of stimulation, the volume increased rapidly to above the control level and then continued to increase slowly for several minutes (see discussion).

**CAPILLARY FILTRATION COEFFICIENT**

The capillary filtration coefficient was determined on 6 to 22 occasions in each of 7 cats. The determination in one cat is shown in Figure 3. On raising the hepatic venous pressure by 4.7 mm Hg, the portal pressure increased by 3.0 ± 0.4 mm Hg (mean ± SE), and the initial rapid increase in volume was 3.5 ± 0.3 ml. The capillary filtration coefficient was calculated from the slow component of the volume record, shown by the broken line in Figure 3, assuming that 100% of the rise in hepatic venous pressure was transmitted to the sinusoids (see discussion). The mean value for each cat varied from 0.2 to 0.51 ml/min/mm Hg/100 g liver, and the standard error in each animal did not exceed ±0.01. The mean value for the 7 cats was 0.30 ml/min/mm Hg/100 g liver and the standard error was ±0.03. On nine occasions, the coefficient was determined with increases in hepatic venous pressure of 2.3 and 7.0 mm Hg. The coefficients were not statistically different from those determined with increases of 4.7 mm Hg.

When the hepatic venous pressure was restored, portal pressure returned to its previous value but the liver volume did not return completely (Fig. 3). The gain in volume 2 minutes after restoration of the venous pressure represented 66 ± 1% of the slow increase in volume during the period of raised venous pressure.

**CAPILLARY FILTRATION COEFFICIENT DURING HEPATIC NERVE STIMULATION**

It was not possible to determine the capillary filtration coefficient during the first 2 minutes after the onset of nerve stimulation since the liver volume was changing rapidly. On 28 occasions in 4 cats, the coefficient was determined 2 to 5 minutes after the onset of nerve stimulation at frequencies of 1 to 10/sec. One such determination is shown in...
Figure 3. The coefficient did not differ significantly from the previous control value (paired t-test, \( P > 0.8 \)). Similarly, on 14 occasions, the coefficients determined 5 to 10 minutes after the onset of nerve stimulation did not differ significantly \((P > 0.8)\) from either the previous control or the coefficient determined 2 to 5 minutes after the onset of stimulation.

Since the volume recovered only slowly after the cessation of nerve stimulation (Fig. 1), the coefficient could not be determined until 2 to 5 minutes after cessation. These values did not differ significantly \((P > 0.8)\) from those during the control period or during nerve stimulation.

**Discussion**

The techniques described allow quantitative measurement of the liver volume without surgical interference with the hepatic artery or portal vein. The general state of the animals was good. Corneal, ear flick, and swallowing reflexes returned repeatedly as the effects of each supplementary dose of pentobarbital wore off. The measured variables remained steady, and consistent responses to nerve stimulation were obtained for at least 4 hours after completion of the preparation. The cats were breathing spontaneously, and although we did not measure the blood gas tensions in these experiments, we hoped to avoid the hypocapnia frequently produced by artificial ventilation. Hemostasis was good provided the 30-minute period was allowed between completion of the surgery and administration of heparin.

Sealing the plethysmograph proved easier than expected. The right posterior lobe of the liver is separate in the cat and could be excluded from the plethysmograph without damage to liver tissue. The remainder of the liver was fully enclosed, and tissue did not herniate through the opening. The plastibase produced an excellent seal without obstruction. Portal venous pressure provided a good indication of obstruction to the portal or hepatic veins and minimal obstruction occurred. The volume baseline was very stable during the control periods, after the initial decrease in volume on beginning stimulation of the nerves and after return to the control level on cessation of stimulation. These facts suggest that the plethysmograph did not leak either during the control periods or during nerve stimulation, and on two occasions, dye (Evans Blue) introduced into the plethysmograph did not leak into the abdominal cavity. It was not possible to distinguish between changes in blood volume in the sinusoids and in the larger portal venous and hepatic venous channels within the liver. Changes in volume of the very short lengths of extrahepatic vessels within the plethysmograph are unlikely to form a significant part of the recorded responses. The gallbladder usually contained 2 to 3 ml of bile, and it was visible through the transparent wall of the plethysmograph. No obvious changes in its volume occurred during the experiments. The major lymphatic drainage from the liver was occluded. This was done to prevent the possible loss of fluid filtered during the periods of raised venous pressure. It is unlikely that occlusion of the lymphatics modified the results although no data are available on hepatic lymph flow in the cat. The lymphatics were also occluded in the similar experiments on the vascular beds of skeletal muscle (1) and intestine (2, 11).

The hepatic blood volume was 27 ml/100 g liver or 7.5 ml/kg body weight. This may be an underestimate since it was assumed in the calculation that the intrahepatic hematocrit is the same as that of arterial blood and this may not be true (12, 13). However, it seems unlikely that the error is large when the determination includes all the intrahepatic vessels and not just the sinusoids. Since the blood volume of the cat is 53 ± 7 ml/kg body weight (14), the hepatic blood volume represents 14% of the total in the cat. Values reported for the hepatic blood volume in the dog and rat are very variable but average 15 ml/100 g liver (12, 15-17). However, these values may not include the blood volume of the larger intrahepatic vessels, and hepatic venous pressures are not usually stated.

Half of the hepatic blood volume (7% of the total blood volume) was expelled from the
capacitance vessels of the liver by maximal stimulation. In the cat, such maximal stimulation expels 25% to 30% of the 3 ml/100 g in skeletal muscle and 35% of the 8 ml/100 g in intestine (6) while 10% to 15% of the total blood volume can be expelled from the spleen (5, 18). The hepatic capacitance response to sympathetic stimulation is therefore large.

This response was not a passive consequence of a decrease in hepatic blood flow since stimulation of the hepatic nerves produced no change in portal venous flow and only a brief decrease in hepatic arterial flow (8). In contrast to these flow responses, the capacitance responses were large and well maintained. This also suggests that net fluid movements across the sinusoidal walls were not occurring and therefore that sinusoidal hydrostatic pressure did not change. In this respect, the liver resembles intestine (11) and spleen (5) rather than skeletal muscle (1).

The capillary filtration coefficient was determined by a method comparable to that used in skeletal muscle (1) and intestine (2). In these organs, the early rapid increase in volume following a rise in venous pressure is due to an increase in blood content while the later, slow increase is due to filtration of interstitial fluid. Since the comparable response in the liver has not yet been proved to consist of these components, the data presented must be regarded as preliminary. However, the general similarity of the responses suggests that a similar interpretation is reasonable. The calculation of the capillary filtration coefficient requires knowledge of the proportion of the rise in venous pressure which is transmitted back to the sinusoids. This value is not known for the liver. However, since approximately 64% (3 mm Hg) of the rise in hepatic venous pressure is transmitted back to the portal vein and since the greatest pressure drop between the portal and hepatic veins appears to occur in the portal venous radicals within the liver (19), the proportion transmitted to the sinusoids is probably very high. We have assumed 100% transmission. If the value is subsequently found to be less than this, the coefficients reported are too low.

The hepatic capillary filtration coefficient (0.3 ml/min/mm Hg/100 g liver) is large compared to that in intestine (0.1 ml/min/mm Hg/100 g) and in skeletal muscle (0.01 ml/min/mm Hg/100 g) (6). This suggests that the vascular bed of the liver has a higher permeability to small molecules or a larger surface area. The resting hepatic blood flow per 100 g tissue is also 2 to 3 times greater than that in intestine and 20 to 30 times greater than that in skeletal muscle (6).

In two cats, after the initial decrease in volume on beginning nerve stimulation, the volume steadily increased, and after the initial rapid increase in volume on cessation of stimulation, the volume continued to rise for several minutes. Similar responses have been observed in the intestinal vascular bed of sick cats (20, and Greenway and McNeill, unpublished observations), and the mechanism of these changes may be of pathophysiological significance. The capillary filtration coefficient was not abnormal, and this suggests that a rise in sinusoidal hydrostatic pressure had occurred.

The capillary filtration coefficient during and after stimulation of the hepatic nerves was not significantly different from that during the control periods. This suggests that a maintained contraction of the presinusoidal sphincters, as occurs in the intestinal vascular bed (21), is not produced in the hepatic vascular bed by sympathetic nerve stimulation. However, it does not exclude the possibility that short-lasting changes occur at the onset of stimulation, as in the vascular bed of skeletal muscle (22). If this interpretation of our results is correct, maintained changes in sinusoidal surface area do not occur during sympathetic nerve stimulation, and this would suggest that a gross redistribution of portal venous flow within the liver, as suggested by Daniel and Prichard (23, 24), does not occur.

The data presented are consistent with the following conclusions on the effects on the hepatic vascular bed of stimulation of the
sympathetic nerves. Hepatic arterial resistance increases and flow decreases but this is not maintained and autoregulatory escape occurs. Portal venous resistance increases and portal pressure rises. Portal flow does not change unless there is a simultaneous change in intestinal or splenic blood flows. Sinusoidal pressure rises. Portal flow does not change to the hepatic parenchyma. The blood expelled from the capacitance vessels will increase venous return without impairment of the flow to the hepatic parenchyma.

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