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Aging and the response of the isolated perfused rat liver to vasoactive drugs

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The clearance of many drugs is dependent on liver blood flow. Therefore, an age-related reduction in liver blood flow has important clinical implications for the elderly patient. Investigations using Indocyanine green clearance have shown that there is a senescence-related decline in liver blood flow of 49–53% in man [1, 2] and 35% in the rat [3]. It has been suggested that this is due mainly to reduced portal venous blood flow [4, 5], for which there are two possible causes: reduced splanchnic outflow or increased hepatic resistance to portal flow. The isolated perfused rat liver has been widely used to study hepatic resistance and its response to vasoactive substances, however little attention has been given to the effects of aging. In this study, we have measured the hepatic resistance in young and aged rats and its modulation by norepinephrine and verapamil. The action of verapamil was studied because it increases hepatic blood flow *in vivo* [6], antagonizes the norepinephrine-induced contractions of the isolated rat portal vein [7], and is used frequently in elderly patients.

Materials and Methods

Female Fischer F344 rats were obtained from Flinders Medical Centre (Adelaide, South Australia).

Liver perfusion. Rats were anaesthetized with pentobarbital (50 mg/kg i.p., Boehringer Ingelheim, Australia) and heparinized (200 U i.v., David Bull Laboratories, Australia). At laparotomy, the portal vein was cannulated with a 16G intravenous catheter and the liver was perfused with albumin-free Krebs–Henseleit buffer (pH 7.4, 1.19 mM $CaCl_2$, equilibrated with 95% O_2/5% CO_2) using a peristaltic pump (Cole-Parmer, IL, U.S.A.) in a non-recirculating system. The bile duct was cannulated with PE10 (Clay Adams, NJ, U.S.A.) and the liver isolated and maintained at 37° within a modified humidicrib. The vena cava was transected flush with the liver so that an outflow cannula was not necessary. Viability was assessed by bile flow, oxygen consumption and macroscopic appearance.

Measurement of portal pressure. A blunted 22G spinal needle was threaded through the portal vein cannula until the tip protruded 3–4 mm into the portal vein. Pressure was taken as the height of the column of perfusate in a vertical tube attached to the needle. Because the zero point was taken as the height of the vena cava outflow orifice, the transhepatic pressure gradient was measured directly. Correction was made for capillary action which was measured separately.

Perfusion protocol. Each liver was perfused with buffer initially and then, in order, with: buffer containing 10 μM norepinephrine (Winthrop, Australia); 10 μM norepinephrine and 20 μM verapamil (Knoll, Australia); 20 μM verapamil; and finally buffer alone to confirm that the pressures had returned to baseline. Pressure readings were taken at flow rates of 5, 10, 20 and 30 mL/min for each perfusate. At the completion of the whole experiment, the inflow line was clamped and the pressure recorded 3 min after cessation of flow. Hepatic resistance was calculated from the transhepatic pressure gradient divided by the flow rate.

Statistical analysis. The Student's *t*-test was used to

compare the pressures of the young and aged rats at each flow rate, and the effects of the vasoactive drugs on resistance. Differences were considered significant when $P < 0.05$.

Results and Discussion

The average age of the young rats was 97 days (range 75–126 days, N = 5) and of the aged rats, 635 days (range 581–676 days, N = 5). When normalized to body weight (young: 179 ± 25 g vs aged: 242 ± 20 g), the liver weights were similar (young: 2.83 ± 0.14% vs aged: 2.65 ± 0.11%). There was no difference in the viability of the young and aged livers as assessed by bile flow (young: 0.75 μL/min/g liver vs aged: 0.72 μL/min/g liver) and oxygen consumption (young: 1.3 μmol/min/g liver vs aged: 1.1 μmol/min/g liver).

Validity of pressure readings. The pressures and resistances recorded here are lower than those often reported for the perfused rat liver (e.g. Refs 8 and 9) because we excluded the artifact introduced by the resistance of inflow and outflow catheters and corrected for the capillary action of the manometer. The main technical disadvantage of our system is that is has a forward-opening probe which tends to underestimate pressures at high flow velocities [10]. Even so, this method has been recommended for the accurate measurement of pressures in the isolated perfused rat kidney [11].

Hepatic resistance to portal flow. There were no significant differences between the transhepatic pressures of the young and aged rats at any flow rate or for any perfusate (Fig. 1). Thus, similar flow–resistance curves were observed in each age group (Fig. 2A, B and C). The lack of an age-related increase in hepatic resistance means that the age-related decrease in liver blood flow must be due to reduced splanchnic outflow *in vivo*. This inference is supported by the findings from a study in which an isotope indicator technique was used to measure liver blood flow in the aged rat [4]. The reduced liver blood flow could be accounted for entirely by a reduction in splanchnic blood flow, which in turn was due to decreased cardiac output and a substantial reduction in the mass of the alimentary tract and spleen [4].

Response to norepinephrine. Norepinephrine (10 μM) produced an average increase in resistance of 198 ± 74% in the young rat and 218 ± 66% in the aged rat. These values lie between those reported previously for the adult rat (82% [8], 345% [12]). In our study, the difference in response to norepinephrine between young and aged was not significant. Similar age-independent responses to norepinephrine have been reported in the rat renal and femoral arteries and veins [13] and in the human dorsal hand vein [14].

Response to verapamil. Verapamil (20 μM) alone had no effect on hepatic resistance. However, when perfused in conjunction with norepinephrine, verapamil reduced the norepinephrine-induced increase in resistance significantly, by 19 ± 11% in the young rats and 21 ± 10% in the aged rats. Therefore, it is possible that the verapamil-induced increase in liver blood flow *in vivo* [6] is due to its partial reversal of norepinephrine-induced hepatic venous tone.

Closing pressures. The transhepatic pressures at zero flow were −0.3 cm H_2O and −0.2 cm H_2O in the young and aged rats, respectively. This is an unexpected finding because the zero-flow pressure has been found previously to be positive in the liver [9, 15] and other capillary beds [16]. This positive pressure, the "critical closing pressure" has been used as evidence that capillary collapse occurs at low flow rates [10, 16]. However, the previously reported closing pressures in the liver have been extrapolated values [15] or were uncorrected for capillary action [9]. Measuring this pressure directly allowed the demonstration that it is not positive. This is consistent with the anatomy of the hepatic microcirculation because the sinusoidal cells are

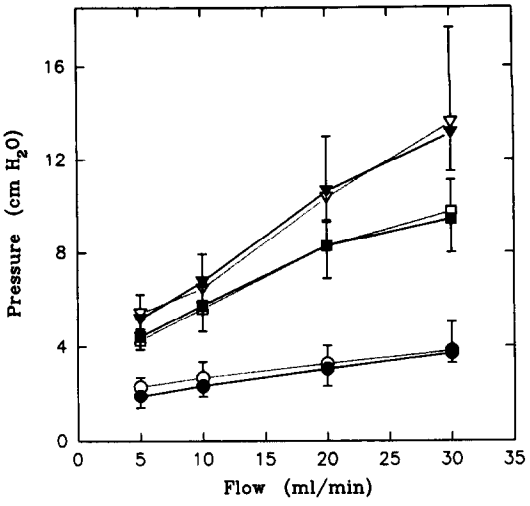

Fig. 1. Transhepatic pressure–flow relationships in the isolated perfused liver of young (empty symbols) and aged (filled symbols) rats, in the presence of buffer only (circles), 10 μM noradrenaline (triangles), and 10 μM noradrenaline with 20 μM verapamil (squares). There were no significant differences between young and aged livers for any of the perfusion conditions (mean ± SD).

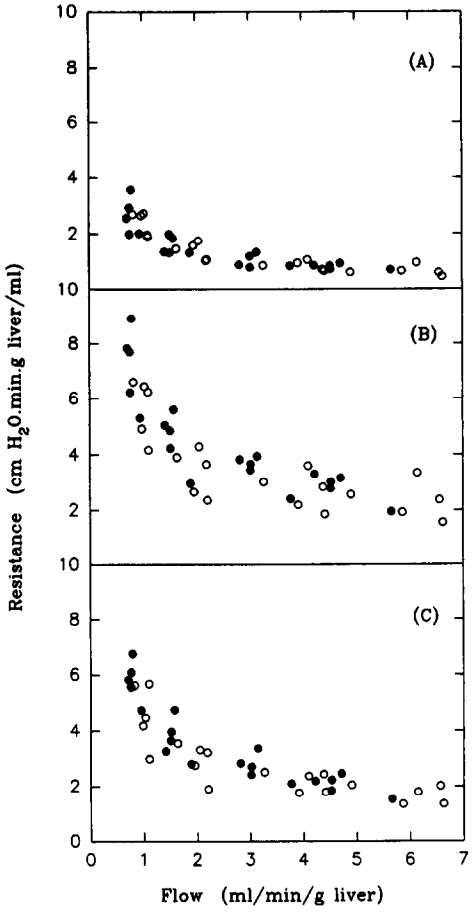

Fig. 2. Calculated hepatic vascular resistances as a function of hepatic flow normalized for liver weight (young = empty symbols, aged = filled symbols). (A) Buffer only, (B) noradrenaline, (C) noradrenaline and verapamil.

highly fenestrated [17], and a positive closing pressure would infer that there is an inward collapse of the surrounding sheets of parenchymal cells, which is unlikely. The slightly negative values recorded in the current study indicate that the albumin-free perfusate is able to drain via extravascular routes, presumably through the lymphatics.

In conclusion, there were no age-related changes in hepatic resistance to portal blood flow in the isolated perfused rat liver, nor in the liver's response to norepinephrine and verapamil. Therefore, it would appear that the age-related decline in hepatic blood flow [1–3] is due to reduced splanchnic outflow *in vivo*.

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