Preferential Changes in Hepatosplanchnic Hemodynamics in Patients with Borderline Hypertension

Takashi Sugawara, Takao Noshiro, Taku Kusakari, Kazumasa Shimizu, Toshiya Watanabe, Hiroyoshi Akama, Satoru Shibukawa, Wakako Miura, and Yukio Miura*

To investigate changes in systemic and regional hemodynamics during the development of human hypertension, we simultaneously measured cardiac index (CI) by the indocyanine green (ICG) dye dilution method, hepatosplanchnic blood flow (HBF) by the ICG clearance method using a two-compartment model, and renal blood flow (RBF) by the p-aminohippurate clearance method in patients with borderline and essential hypertension. In patients with borderline hypertension (BH, n = 27), HBF (435 ± 15 ml/min/m²) and HBF/CI (16 ± 1%) were significantly (p < 0.05) lower than in age-matched normotensive controls (528 ± 21 and 19 ± 1, respectively, n = 21), while CI, RBF and RBF/CI were similar. In patients with essential hypertension (EH, n = 32), HBF, RBF, and RBF/CI were all significantly (p < 0.01) lower than in the control subjects. Hepatosplanchnic vascular resistance (HVR) in patients with BH was preferentially increased, while total peripheral resistance (TPR) and renal vascular resistance (RVR) remained in the normal range. In patients with EH, TPR, HVR, and RVR were all increased. These results indicate that hemodynamic changes in patients with BH do not occur uniformly among the various regional circulations and suggest that hemodynamic changes in the hepatosplanchnic region precede those in other organ circulations during the development of human hypertension. (Hypertens Res 1997; 20: 201-207)

Key Words: hepatosplanchnic hemodynamics, indocyanine green clearance, borderline hypertension, human hypertension, regional circulation

Essential hypertension has been ascribed to increased cardiac output, increased systemic vascular resistance, or some combination of these two factors (1-3). Although an increase in the vascular resistance of virtually every organ circulation is a well-known characteristic of essential hypertension (4-8), it remains unclear whether this vascular change occurs uniformly in the regional circulation or whether a specific organ circulation is of particular importance during the development of hypertension.

The hepatosplanchnic circulation includes the blood supply of the gastrointestinal tract, spleen, pancreas, and liver. It is the largest regional circulation and receives approximately 20% to 25% of the cardiac output under basal conditions (9, 10). In addition, the organ systems in this region receive a major proportion of total sympathetic outflow (11, 12), which can regulate regional hemodynamics. Despite its importance, information on the splanchnic circulation in humans is sparse, because of difficulties in measuring hepatosplanchnic blood flow (HBF). Conventionally, HBF in humans is measured on the basis of clearance of indocyanine green (ICG) from the plasma, assessed with a single-compartment model. The HBF values measured by this method, however, are consistently underestimated since it does not consider variability in hepatic extraction ratio (ER) of ICG among subjects (9, 13, 14). There have been only a few studies of the hepatosplanchnic circulation in hypertensive patients (6, 15, 16), and their results have been inconsistent. To calculate HBF more precisely, Grainger et al. (17) developed another ICG clearance method based on a two-compartment pharmacokinetic model, which can estimate individual ER values of ICG from the plasma disappearance curve. To our knowledge, no study has examined hepatosplanchnic hemodynamics with this method in hypertensive patients.

The aim of the present study was to determine whether or not regional circulatory changes occur uniformly during the development of hypertension. We simultaneously measured HBF, using Grainger's method, and renal blood flow (RBF) as well as systemic hemodynamics in patients with borderline and...
essential hypertension.

Methods

Subjects
The subjects of this study consisted of 27 patients with borderline hypertension (BH, 17 men and 10 women) aged 18 to 55 yr with a mean age of 35 ± 2 (SEM) yr, and 32 patients with essential hypertension (EH, 19 men and 13 women) aged 24 to 53 yr with a mean age of 39 ± 2 yr. Six EH patients were in World Health Organization (WHO) stage I, and 26 were in WHO stage II. Fifteen had mild left ventricular hypertrophy detected on radiography or electrocardiography, 9 had slightly elevated plasma creatinine concentrations (1.2 to 1.8 mg/dl), and 14 had generalized or focal narrowing of the retinal arteries. These subjects, randomly chosen from among outpatients at the Second Department of Internal Medicine, Tohoku University Hospital, had no serious cardiovascular, renal, or metabolic disorders. We also studied 21 age-matched normotensive healthy volunteers (12 men and 9 women) aged 23 to 60 yr with a mean age of 36 ± 2 yr as control. The results of a routine series of hematologic and blood chemical examinations, including liver function tests (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and alkaline phosphatase), were confirmed to be normal in all subjects. Borderline hypertension was defined as a blood pressure level above 140 mmHg systolic, 90 mmHg diastolic, or both, and essential hypertension was defined as a blood pressure level constantly higher than 160 mmHg systolic, 95 mmHg diastolic, or both, when measured at least three different occasions at the outpatient clinic. Blood pressures were measured at least twice on each visit. thorough clinical evaluations were performed to exclude patients with secondary hypertension and liver diseases. All subjects were on an unrestricted sodium diet and all, if any, medications were discontinued for at least 4 wk before the study. The study protocol was approved by the ethical committee of Tohoku University School of Medicine. Informed consent was obtained from all subjects after they were given a complete explanation of the purpose and protocol of this study.

Study Protocol
The subjects fasted overnight and were asked to refrain from smoking on the morning of the study. The study was started at 9:00 AM with the subjects resting in the supine position in a quiet air-conditioned room to minimize diurnal variations of blood pressure. Blood pressure and heart rate were measured every 5 min with an automatic sphygmomanometer (BP-203NP, Nippon Kolin Co., Komaki) on the upper arm. An antecubital vein of each arm was catheterized for blood collection and injection of test agents. After the subjects rested quietly for at least 60 min, blood samples were obtained for measurement of plasma catecholamines, plasma renin activity, and plasma aldosterone. Initial doses of p-aminohippurate (PAH, 7 mg/kg) and ICG (0.5 mg/kg) were then infused as a bolus over 15 s. Ten percent PAH dissolved in saline was subsequently infused by a syringe pump (Terfusion; TERUMO Co., Tokyo), to produce a plasma PAH concentration of 2 to 3 mg/dl. Blood samples for the measurement of HBF were collected via an indwelling venous catheter placed in the opposite arm to that for ICG infusion. Samples were taken 5, 8, 12, 20, 25, 30, and 35 min after the injection of ICG. After an equilibration period of at least 60 min, blood samples and urine were collected for the measurement of PAH and creatinine concentrations. Cardiac output was measured at the end of each procedure.

Systemic Hemodynamics
Cardiac output was measured by the ICG dye dilution method using a cardiac computing system (Cardiac Output Computer MLC-4100, Nihon Koden Co., Tokyo). The values measured by this system showed good reproducibility: the standard deviation of measured cardiac output in 40 subjects (10 with BH, 21 with EH, and 9 controls) was 8.8 ± 6.6%. The results were expressed as the mean of three determinations in ml/min/m² (cardiac index, CI) after correction for body surface area. The total peripheral resistance (TPR) was calculated by dividing the mean blood pressure by CI.

Hepatosplanchic Hemodynamics
HBF was determined from the ICG clearance by Grainger's method (17). Immediately after each examination, blood samples for the measurement of HBF were centrifuged at 3,000 rpm for 20 min, and 1 ml of each plasma sample was transferred to a test tube. The plasma was diluted with 2 ml of saline and re-centrifuged for 20 min. The absorbance of each sample was read at 805 nm against each patient’s own plasma as control, using a spectrophotometer (Hitachi Model 100-60, Tokyo). The concentration of ICG was calculated from the absorbance using a standard curve constructed from the patient’s own plasma. ICG clearance was determined from the ICG disappearance curve using a two-compartment model as follows:

The plasma disappearance curves after bolus intravenous injection were determined using equation (1):

$$[ICG](t) = A e^{-\alpha t} + B e^{-\beta t}$$

where [ICG](t) is the plasma concentration of ICG at time t, A and B are the zero-time intercepts, and $\alpha$ and $\beta$ are the slopes of the two exponentials.

The hepatic extraction ratio of ICG, ER, can be determined from the formulated variables for the disappearance curve. This method employs the exponents describing the ICG plasma disappearance curve to obtain values for the rate constants of the transfer of ICG between the plasma and the liver ($k_{12}$ and $k_{21}$) and its elimination from the liver ($k_{20}$). These values are used to derive the ER using equation (2):
The plasma clearance of ICG (CIcG) was calculated using equation (3):

$$C_{\text{ICG}} = \frac{\text{Dose (mg)}}{\text{AUC}_0^\infty (\text{mg-min}^{-1}l^{-1})}$$

The area under the curve (AUC$_0^\infty$) was computed by integration of the equation describing the curve. Dose represents the amount of ICG injected. The hepatosplanchnic plasma flow was then calculated from equation (4):

$$\text{hepatosplanchnic plasma flow} = \frac{C_{\text{ICG}}}{\text{ER}}$$

HBF was estimated from the hepatosplanchnic plasma flow and hematocrit. Hepatosplanchnic vascular resistance (HVR) was calculated by dividing the mean blood pressure by HBF and was expressed in dynes·s·cm$^{-5}$·m$^{-2}$. The calculated ER showed relatively large individual differences (74.5% to 97.6%), but the mean ER values were similar among the three groups (normal controls, 89.5 ± 1.4%; BH, 87.5 ± 1.1%; EH, 88.6 ± 0.8%). The values of CIcG were all within normal range (greater than 0.168) in the subjects studied.

### Renal Hemodynamics

Renal plasma flow was measured by the PAH clearance method using a continuous infusion technique. Endogenous creatinine clearance was determined from the urinary creatinine excretion rate and plasma creatinine concentration. PAH and creatinine were analyzed according to the methods of Brun (18) and Bonsnes et al. (19), respectively. PAH and creatinine clearance were corrected for body surface area. RBF was estimated from the renal plasma flow and hematocrit. The renal vascular resistance (RVR) was calculated by dividing the mean blood pressure by RBF and was expressed in dynes·s·cm$^{-5}$·m$^{-2}$.

### Analytical Methods

Catecholamine concentrations were measured by our sensitive fluorometric method as described previously (20). Plasma renin activity and aldosterone concentration were determined by radioimmunoassay. Urinary sodium and potassium levels were determined by flame photometry.

### Statistical Analysis

Results are expressed as the means ± SEM. Analysis of variance followed by Duncan's multiple range test was used to determine the significance of differences among the three groups studied. The minimum level of statistical significance was $p < 0.05$.

### Results

#### Patient Characteristics

The clinical characteristics of the subjects are summarized in Table 1. There were no significant differences among the three groups in height, weight, body surface area, hematocrit, plasma norepinephrine and epinephrine levels, plasma renin activity, plasma aldosterone concentration, endogenous creatinine clearance, and urinary potassium excretion. Urinary sodium excretion was lower in BH patients than in the normal controls.

#### Systemic Hemodynamics

As shown in Table 2, systolic, diastolic, and mean blood pressure levels were higher in both BH and EH patients than in the control subjects. Heart rate and CI did not differ among the three groups. TPR was significantly greater in EH patients (3,620 ± 124 dynes·s·cm$^{-5}$·m$^{-2}$, $p < 0.001$), but not in BH patients (2,790 ± 140), than in the control subjects (2,510 ± 100).

#### Regional Hemodynamics

The HBF and RBF levels in the subjects are shown in Fig. 1. In BH patients, HBF (435 ± 15 ml/min/m$^2$) was significantly ($p < 0.01$) lower than in the

### Table 1. Clinical Characteristics in Normal and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal controls</th>
<th>Borderline hypertensives</th>
<th>Essential hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects (male/female)</td>
<td>21 (12/9)</td>
<td>27 (17/10)</td>
<td>32 (19/13)</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>36±2.2</td>
<td>35±2.2</td>
<td>39±1.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164±1.4</td>
<td>163±1.8</td>
<td>163±1.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59±1.2</td>
<td>62±1.9</td>
<td>64±1.7</td>
</tr>
<tr>
<td>Body surface area (m$^2$)</td>
<td>1.67±0.02</td>
<td>1.70±0.03</td>
<td>1.73±0.03</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40±1.1</td>
<td>40±1.0</td>
<td>39±1.0</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml/h)</td>
<td>1.22±0.23</td>
<td>1.79±0.36</td>
<td>1.31±0.21</td>
</tr>
<tr>
<td>Plasma aldosterone (ng/dl)</td>
<td>5.3±0.7</td>
<td>7.0±0.7</td>
<td>7.1±0.9</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/ml)</td>
<td>133±10</td>
<td>138±11</td>
<td>143±12</td>
</tr>
<tr>
<td>Plasma epinephrine (pg/ml)</td>
<td>38±7</td>
<td>34±4</td>
<td>35±4</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/m$^2$)</td>
<td>61.9±2.1 (n=12)</td>
<td>58.0±1.6 (n=16)</td>
<td>55.4±2.3 (n=20)</td>
</tr>
<tr>
<td>Urinary sodium excretion (mEq/min)</td>
<td>0.239±0.024 (n=12)</td>
<td>0.172±0.018* (n=16)</td>
<td>0.204±0.020 (n=20)</td>
</tr>
<tr>
<td>Urinary potassium excretion (mEq/min)</td>
<td>0.080±0.009 (n=12)</td>
<td>0.065±0.006 (n=16)</td>
<td>0.056±0.005 (n=20)</td>
</tr>
</tbody>
</table>

Values are means±SEM. *p<0.05 vs. normal controls.
of regional vascular resistances, HVR was significantly (p < 0.001) greater in both BH and EH patients than in the normal controls. Of the regional vascular resistances, HVR was significantly (p < 0.001) greater in both BH and EH patients than in the normal controls.

Of the regional vascular resistances, HVR was significantly (p < 0.001) greater in both BH and EH patients than in the normal controls (normal controls, 13,300 ± 640 dynes•s•cm⁻⁵•m²; BH, 18,000 ± 590; EH, 21,100 ± 720; Fig. 2). RVR (25,000 ± 1,140 dynes•s•cm⁻⁵•m²) was significantly greater in EH patients than in BH patients (15,600 ± 880, p < 0.01) and the normal controls (14,000 ± 670, p < 0.001). There were no significant differences in the ER values of ICG among the three groups.

As for the regional distribution of cardiac output, the HBF/CI was significantly lower in BH patients than in the normal controls (15.8 ± 0.9% vs. 19.4 ± 1.0%, p < 0.01), whereas the HBF/CI in EH patients (17.3 ± 0.7%) did not differ from that in the normal controls. The RBF/CI in EH patients was significantly lower than that in the normal controls (13.9 ± 1.1% vs. 17.3 ± 1.2%, p < 0.001), whereas the RBF/CI in BH patients (17.0 ± 1.5%) did not differ from that in the normal controls. There was no significant correlation between plasma noradrenaline concentration and HBF, HVR, RBF, or RVR in any of the three groups.

In all subjects, regression analysis showed a significant positive correlation between the levels of the mean arterial pressure and HVR (r = 0.687, p < 0.001, n = 80) or RVR (r = 0.736, p < 0.001, n = 48). Scatterplots in Fig. 3 illustrate the relationship between the mean arterial pressure and regional hemodynamics in the three groups.

Table 2. Systemic Hemodynamic Variables in Normal and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal controls (n=21)</th>
<th>Borderline hypertensives (n=27)</th>
<th>Essential hypertensives (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
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</tr>
<tr>
<td>systolic</td>
<td>112±2.0</td>
<td>138±2.0**</td>
<td>148±2.2***</td>
</tr>
<tr>
<td>diastolic</td>
<td>70±2.0</td>
<td>86±1.3***</td>
<td>99±1.9***</td>
</tr>
<tr>
<td>mean</td>
<td>85±1.7</td>
<td>102±1.4***</td>
<td>116±1.8***</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>61±1.5</td>
<td>61±1.5</td>
<td>61±1.5</td>
</tr>
<tr>
<td>CI (l/min/m²)</td>
<td>2.77±0.09</td>
<td>2.91±0.14</td>
<td>2.69±0.09</td>
</tr>
<tr>
<td>TPR (dynes•s•cm⁻⁵•m²)</td>
<td>2,510±100</td>
<td>2,790±140</td>
<td>3,620±124***</td>
</tr>
</tbody>
</table>

Values are means±SEM. All values were determined while subjects were resting supine for 60 min. CI, cardiac index; TPR, total peripheral resistance. **p<0.001 vs. normal controls. †p<0.01 vs. borderline hypertensives.
Discussion

The present study clearly demonstrated that HBF/CI in BH patients was significantly reduced and that only HVR was preferentially increased in BH patients, while TPR, RVR, and HVR were all increased in EH patients as compared with normotensive subjects. These findings suggest that hemodynamic changes in BH patients may occur preferentially in the hepatosplanchnic region.

Our measurement of HBF represents the blood flow that passes through the gastrointestinal tract, spleen, pancreas, and liver. These organ blood flows are supplied via numerous arteries and then ultimately pass through the liver and return to the inferior vena cava through the hepatic veins. The organs of the hepatosplanchnic region receive 20% to 25% of the resting cardiac output and hold close to 30% of the total blood volume (9, 10, 21).

Although the ICG clearance method is commonly used and is reliable for measurement of HBF in humans (9), this method requires hepatic venous catheterization or adoption of several assumptions because the ER of ICG is not uniform. However, Grainger (17) developed an ICG clearance method using a two-compartment pharmacokinetic model. This method can be used to calculate the ER of ICG from its disappearance curve alone and provides a more accurate estimation of plasma clearance than that calculated from a traditional single-compartment model. Grainger demonstrated that the ER was comparable to that measured by hepatic venous catheterization. In the present study, we determined HBF using Grainger’s method. Although individual variations in ER were relatively large (74.1% to 97.6%) in this study, we could measure HBF more accurately by correcting the values with individual ER.

In the present study, HBF and HBF/CI decreased in BH patients, but RBF remained in the normal range. Previous studies have reported inconsistent results for RBF in BH patients, with some reporting an increase (15, 22) or no change (6, 23). However, only a few studies have examined the hepatosplanchnic circulation in patients with BH (6, 15). Temmar et al. (6) found that men with BH showed a significant rise in cardiac output associated with normal HBF and RBF as compared with those of male control subjects. Messerli et al. (15) reported that HBF was significantly higher in BH patients with high cardiac output than in those with low cardiac output. In contrast, EH patients in the present study showed decreased HBF, RBF, and RBF/CI, with no change in HBF/CI. The HBF values in EH patients have been reported as unchanged (6, 7) or decreased (16, 24). These discrepancies with our results can be explained by differences in subject demographics and in the method for measurement of HBF. First, our subjects comprised 17 men and 10 women with BH and 20 men and 17 women with EH, whereas in Temmar’s study the 16 patients with BH and 16 with EH were all men. Our results, however, did not change even if the values of HBF were analyzed only for male subjects. There were no significant differences in CI among the three groups in our study and only 7 (26%) of 27 BH patients showed increased cardiac index (3.28 l/min/m² or higher), whereas the CI values in Messerli’s paper (16) were lower in EH patients than in the control subjects. Second, in previous studies, HBF was measured by ICG (15, 16) or D-propranolol (6, 7) clearance using a single compartment model, whereas we measured HBF by ICG clearance using a two-compartment model (17).

Although there have been many investigations of splanchnic blood flow in experimental hypertension, the results have been inconsistent, with splanchnic blood flow remaining unchanged (25-27), increasing (28-30), or decreasing (31). These disparate results may be related to differences in the model and stage of hypertension (21, 30), differences in the methods for determining blood flow, differences in the animal species studied, and differences among the various splanchnic organs.

As for the mechanisms whereby the hepatosplanchnic circulation was preferentially changed in the
BH patients studied, we cannot provide a well-founded explanation from our present study. Several mechanisms, however, might be involved. First, the sympathetic nervous system plays a key role in the control of splanchnic vascular beds richly innervated by sympathetic nerves (11, 12). Increased sympathetic nerve activity has been frequently reported in BH patients (2, 4, 32-37). Sympathetic activation may occur systemically or regionally (37), suggesting the possibility that sympathetically-mediated vasoconstriction prevails in the hepatosplanchnic vasculature in BH patients. In the BH patients in our study, however, increased plasma catecholamine concentrations were not confirmed, at least in the peripheral venous blood, although regional changes in plasma catecholamines in the hepatosplanchnic circulation may not accurately reflect those in the peripheral venous blood (37). Second, plasma renin activity tended to be higher in the BH patients than in the normal controls in our study (Table 1). Messerli et al. (16) reported that HBF/CI was significantly decreased in patients with renal artery stenosis who had high plasma renin activity. As compared with other vascular beds, splanchnic vessels have been reported to be more sensitive to angiotensin II in animals (38) and in normotensive men (39). To our knowledge, however, there have been no reports documenting differences in sensitivity to angiotensin II between the renal and hepatosplanchnic vessels. Further studies are needed to clarify the precise mechanisms underlying the regional changes in the hepatosplanchnic circulation in patients with BH, which may represent an early stage of essential hypertension.

Urinary sodium excretion was lower in the BH patients than in the normal controls. This difference is difficult to explain and might have occurred accidentally, because the number of BH patients was relatively small and the daily sodium intake was not controlled in this study.

In conclusion, we have demonstrated that BH patients showed a selective increase in HVR associated with a normal RVR and TPR, while EH patients exhibited generalized increases in HVR, RVR, and TPR. These results suggest that regional hemodynamic changes may occur preferentially in the hepatosplanchnic circulation in the early stage of essential hypertension, assuming that borderline hypertension represents an antecedent form of sustained hypertension.

References
23. Tuck ML, Sullivan HM, Hollenberg NK, Dluhy RG, Williams GH: Hemodynamic and endocrine response