Sympathetic drive to liver and nonhepatic splanchic tissue during heavy exercise

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Coker, Robert H., Mahesh G. Krishna, D. Brooks Lacy, Eric J. Allen, and David H. Wasserman. Sympathetic drive to liver and nonhepatic splanchic tissue during heavy exercise. J. Appl. Physiol. 82(4): 1244–1249, 1997.—The contribution of sympathetic drive and vascular catecholamine delivery to the splanchic bed during heavy exercise was studied in dogs that underwent a laparotomy during which flow probes were implanted onto the portal vein and hepatic artery and catheters were inserted into the carotid artery, portal vein, and hepatic vein. At least 16 days after surgery, dogs completed a 20-min heavy exercise protocol (mean work rate of 5.7 ± 1 miles/h, 20 ± 2% grade). Arterial epinephrine (Epi) and norepinephrine (NE) increased by ~500 and ~900 pg/ml, respectively, after 20 min of heavy exercise. Because Epi is not released from the splanchic bed and because Epi fractional extraction (FX) = NE FX, NE uptake by splanchic tissue can be calculated despite simultaneous release of NE. Basal nonhepatic splanchic (NHS) FX increased from a basal rate of 0.52 ± 0.09 to a peak of 0.64 ± 0.05 at 10 min of exercise. Hepatic Epi FX increased from a basal rate of 0.68 ± 0.10 to 0.81 ± 0.09 at 20 min of exercise. Even though NHS extraction of Epi reduced portal vein Epi levels by ~60%, the release of NE from NHS tissue maintained portal vein NE at levels similar to those in arterial blood. NHS NE spillover increased from a basal rate of 5.7 ± 1.4 to 11.7 ± 2.8 ng·kg⁻¹·min⁻¹ at 20 min of exercise. Hepatic NE spillover increased from a basal rate of 5.0 ± 1.2 ng·kg⁻¹·min⁻¹ to a peak of 14.2 ± 2.8 ng·kg⁻¹·min⁻¹ at 15 min of exercise. These results show that 1) approximately two- and threefold increases in NHS and hepatic NE spillover occur during heavy exercise, demonstrating that sympathetic drive to these tissues contributes to the increase in circulating NE; 2) the high catecholamine FX by the NHS tissues results in an Epi level at the liver that is considerably lower than that in the arterial blood; and 3) circulating NE delivery to the liver is sustained despite high catecholamine FX due to simultaneous NHS NE release.

catecholamines; fractional extraction; uptake; spillover

BLOOD CATECHOLAMINE LEVELS increase with exercise in direct proportion to work intensity and duration (26). The adrenal medulla is the sole source of epinephrine release into the circulation. On the other hand, the adrenal medulla is only a minor source of circulatory norepinephrine, with the majority derived from sympathetic nerve endings. Because norepinephrine levels rise with exercise beyond what can be accounted for by release from the adrenal medulla, it is clear that sympathetic nerves are activated. The specific sympathetic nerves that are activated by exercise are poorly defined.

Because adrenergic stimulation can cause diverse responses encompassing multiple physiological systems, identifying specific sites of high sympathetic drive is essential in defining the potential impact of the catecholamine response to exercise. At the splanchic bed, sympathetic nerve stimulation has been proposed to be a controller of the increases in glucose production (23), splanchnic lipolysis (27), and proteolysis (28) during exercise. Yet, there is little information regarding how adrenergic drive to nonhepatic splanchic (NHS) and hepatic tissue is affected in the presence of muscular work. The present study was conducted to assess the potential contribution of sympathetic drive and vascular catecholamine delivery to hepatic and NHS tissue during heavy exercise. For this purpose, exercise-induced changes in these variables were assessed by measuring norepinephrine spillover and vascular catecholamine delivery to the splanchic tissue of chronically catheterized dogs.

METHODS

Animals and surgical procedures. Experiments were performed on seven mongrel dogs (mean wt 25.0 ± 1.1 kg) of either gender that had been fed a standard diet (Pedigree beef dinner and Wayne Lab Blox: 51% carbohydrate, 31% protein, 11% fat, and 7% fiber based on dry wt). The dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University School of Medicine Animal Care Committee. At least 16 days before each experiment, a laparotomy was performed under general anesthesia (0.04 mg/kg of atropine and 15 mg/kg of thiopental sodium presurgery and 1.0% isofluorene inhalation anesthetic during surgery). Silastic catheters (0.04 in. ID) were inserted in the portal vein and common hepatic vein for sampling. In addition, an incision in the neck region allowed the isolation of the carotid artery into which a Silastic catheter was inserted and advanced to the aortic arch for sampling and hemodynamic measurements during experiments. After insertion, catheters were filled with saline containing heparin (200 U/ml; Abbott Laboratories, North Chicago, IL) and their free ends knotted. Ultrasonic transit time flow probes were used to measure portal vein and hepatic artery blood flow (Transonic Systems, Ithaca, NY). The knotted catheter ends and Transonic probe leads were stored in a subcutaneous pocket in the abdominal region (except for the carotid artery catheter, which was stored in a pocket under the skin of the neck), so that complete closure of the skin incisions was possible.

Beginning 7 days after surgery, dogs were acclimatized to running on a motorized treadmill. Dogs were not exercised 48 h before the experiment. Only animals that consumed all of the daily food ration and had a leukocyte count <18,000 leukocytes/mm³ 3 days before experimentation were used.

All studies were conducted in dogs after an 18-h fast. The free catheter ends were accessed through small skin incisions made under local anesthesia (2% lidocaine; Astra Pharmaceuti-
tichal Products, Worcester, MA) in the abdominal and neck regions immediately before experimentation. Catheters were then aspirated and flushed with saline. The exposed catheters were connected to Silastic tubing and secured to the back of the dog with quick-drying glue.

Experimental procedures. Dogs underwent treadmill tests to determine the work rate at which ~80% of maximum heart rate was achieved. Maximum heart rate was 270 beats/min in dogs of the weight used in these studies (18, 19). Heart rates were monitored by a transducer connected to the carotid arterial catheter. Mean work rate for the high-intensity protocol was 5.7 ± 1.0 miles/h at 20 ± 2% grade. The exercise protocol consisted of basal (–30- to 0-min), exercise (0- to 20-min), and recovery (20- to 50-min) period.

Blood sample collection and analysis. Blood samples were drawn from the carotid artery, hepatic vein, and portal vein to assess catecholamine loads and balances across the NHS tissue and liver. Carotid artery, hepatic vein, and portal vein blood samples were collected every 10 min in the basal state, every 5 min during exercise, and 10 and 30 min after cessation of exercise. Blood samples were collected in tubes containing ethyleneglycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid and glutathione and centrifuged at 4°C. Plasma samples were then stored at –70°C for subsequent analysis of epinephrine and norepinephrine by using high-performance liquid chromatography (17). The coefficients of variation were 5 and 7% for norepinephrine and epinephrine, respectively.

Calculations. The equations described below were used to calculate catecholamine balance, fractional extraction, uptake, and output (spillover) across NHS and hepatic tissue. Net NHS norepinephrine balance was calculated by using the following equation

$$\text{Net NHS norepinephrine balance} = ([N_p] - [N_a]) \cdot P_f$$

where $[N_p]$ is the portal vein plasma norepinephrine concentration, $[N_a]$ is the arterial plasma norepinephrine concentration, and $P_f$ is the portal vein plasma flow normalized for body weight. Net hepatic norepinephrine balance was calculated by using the equation

$$\text{Net hepatic norepinephrine balance} = ([N_n] - [N_h]) \cdot H_f + ([N_n] - [N_p]) \cdot P_f$$

where $[N_n]$ represents hepatic vein plasma norepinephrine concentration, whereas $H_f$ is hepatic artery plasma flow normalized for body weight. NHS and hepatic norepinephrine loads were calculated by using the equations

$$\text{NHS norepinephrine load} = [N_n] \cdot P_f$$

$$\text{Hepatic norepinephrine load} = ([N_a] \cdot A_f) + ([N_n] \cdot P_f)$$

where $A_f$ is arterial plasma flow.

Norepinephrine is concurrently taken up and released from tissue beds. As a consequence, tissue norepinephrine release and removal cannot be distinguished from measurements of norepinephrine arteriovenous differences alone. Because epinephrine is not released from splanchic tissues and epinephrine fractional extraction (FX) equals norepinephrine FX independent of plasma catecholamine concentration (2, 3, 4, 10), simultaneous tissue uptake and output (spillover) of norepinephrine can be assessed. NHS epinephrine FX was calculated by using the equation

$$\text{NHS epinephrine FX} = ([E_n] - [E_h])/[E_n]$$

where $[E_a]$ and $[E_p]$ represent the arterial and portal vein plasma concentrations of epinephrine, respectively. Hepatic epinephrine FX is calculated by the following equation

$$\text{Hepatic epinephrine FX} = \frac{[E_n] \times A_f/(A_f + P_f) + [E_p] \times P_f/(A_f + P_f) - [E_h] \times H_f/(H_f + P_f)}{[E_n] \times A_f/(A_f + P_f) + [E_h] \times H_f/(H_f + P_f)}$$

where $[E_n]$ represents hepatic vein plasma epinephrine concentration. NHS and hepatic norepinephrine uptakes were calculated by using the equations

$$\text{NHS norepinephrine uptake} = \text{Eq. 3} \cdot \text{Eq. 5}$$

$$\text{Hepatic norepinephrine uptake} = \text{Eq. 4} \cdot \text{Eq. 6}$$

NHS and hepatic norepinephrine spillovers were calculated by the equations

$$\text{NHS norepinephrine spillover} = \text{Eq. 1} + \text{Eq. 7}$$

$$\text{Hepatic norepinephrine spillover} = \text{Eq. 2} + \text{Eq. 8}$$

Statistical analysis. Data are presented as means ± SE for seven dogs. Statistical analyses were done by using one-way analysis of variance with repeated measures. Statistical significance was defined as $P < 0.05$.

RESULTS

Arterial, portal vein, and hepatic vein plasma epinephrine and norepinephrine concentrations. Arterial plasma epinephrine concentrations rose ($P < 0.05$) sixfold from a basal level of 100 ± 23 to 610 ± 146 pg/ml at 20 min of exercise. Portal vein plasma epinephrine concentrations increased ($P < 0.05$) fourfold from a basal level of 52 ± 18 to 200 ± 37 pg/ml at 20 min of exercise. Basal hepatic vein plasma epinephrine concentrations were 22 ± 9 pg/ml at rest and increased ($P < 0.05$) to 50 ± 17 pg/ml at 20 min of exercise (Fig. 1). Arterial plasma norepinephrine concentrations rose ($P < 0.05$) fourfold from a basal level of 321 ± 75 to 1,221 ± 214 pg/ml at 20 min of exercise. Portal vein plasma norepinephrine concentrations increased ($P < 0.05$) from a basal level of 443 ± 100 pg/ml to a peak of 1,163 ± 195 pg/ml at 15 min of exercise. Finally, hepatic vein plasma norepinephrine concentrations rose ($P < 0.05$) twofold, increasing from 209 ± 52 to 457 ± 73 pg/ml at 20 min of exercise (Fig. 1).

NHS and hepatic epinephrine FX. NHS epinephrine FX increased ($P < 0.05$) from a basal rate of 0.52 ± 0.09 to a peak of 0.64 ± 0.05 at 10 min of exercise. Hepatic epinephrine FX increased ($P < 0.05$) from a basal rate of 0.68 ± 0.10 to 0.81 ± 0.09 at 20 min of exercise (Fig. 2).

NHS norepinephrine uptake and spillover. NHS norepinephrine uptake increased ($P < 0.05$) from a basal rate of 3.8 ± 1.0 to 11.9 ± 1.8 ng·kg⁻¹·min⁻¹ at 20 min of exercise (Fig. 3). NHS norepinephrine spillover increased ($P < 0.05$) from a basal rate of 5.7 ± 1.4 ng·kg⁻¹·min⁻¹ to a peak rate of 11.7 ± 2.8 ng·kg⁻¹·min⁻¹ at 20 min of exercise (Fig. 4).
Hepatic norepinephrine uptake and spillover. Hepatic norepinephrine uptake increased significantly ($P < 0.05$) from a basal rate of $9.8 \pm 2.1$ ng·kg$^{-1}$·min$^{-1}$ to a peak rate of $23.2 \pm 4.3$ ng·kg$^{-1}$·min$^{-1}$ at 15 min of exercise (Fig. 3). Hepatic norepinephrine spillover increased ($P < 0.05$) from a basal rate of $5.0 \pm 1.2$ ng·kg$^{-1}$·min$^{-1}$ to a peak rate of $14.2 \pm 2.8$ ng·kg$^{-1}$·min$^{-1}$ at 15 min of exercise (Fig. 4).

Hemodynamic measurements. Heart rates increased ($P < 0.05$) from a basal rate of $107 \pm 12$ beats/min to a peak of $220 \pm 5$ beats/min at 15 min of exercise. Portal vein blood flow fell ($P < 0.05$) from a basal rate of $25 \pm 3$ to $18 \pm 2$ ml·kg$^{-1}$·min$^{-1}$ at 20 min of exercise, whereas hepatic artery blood flow remained stable during the basal and exercise periods (Table 1).

**DISCUSSION**

Heavy exercise in the dog increased arterial norepinephrine approximately fourfold, reaching levels $>1,200$ pg/ml in just 20 min. The present experiment shows that both NHS and hepatic tissues are sources of the increased circulating norepinephrine during intense exercise, as spillovers from these tissues were increased approximately twofold and approximately threefold, respectively. This adds to the observation in humans that whole body norepinephrine spillover is increased by high-intensity exercise (15). These data support earlier work in the dog that suggests that there is an increase in total splanchnic norepinephrine spillover during light exercise (20). However, splanchnic plasma flow was not measured in the earlier work, as it was in the present study, and NHS and liver were not distinguished as portal vein sampling was not performed.

NHS balance, measured by assessing arterial catecholamine inflow and portal venous catecholamine outflow, reflects contributions from several tissues. Among these are the pancreas, spleen, adipose tissue, and gastrointestinal tract. The most substantial of these tissues, based on mass and metabolic activity, is the latter. The finding of increased NHS norepinephrine spillover is consistent with anatomic considerations and a number of physiological observations. From an anatomic standpoint, the potential for a considerable increase in sympathetic drive exists because the splanchnic bed is extensively innervated by sympathetic nerves. Celiac and superior mesenteric ganglia provide sympathetic input to the small intestine while superior and inferior mesenteric ganglia supply sympathetic input to the colon. The pancreas...
and spleen are innervated by nerves from the celiac ganglion. The notion that this extensive neural network is activated by exercise is consistent with several characteristics of the exercise response. Glucagon is increased and insulin is decreased with exercise, as it is with sympathetic nerve stimulation (21). These responses have been shown to be prevented by adrenergic blockade in some studies (12, 16) but are intact in humans adrenalectomized for treatment of pheochromocytoma (11, 12). This suggests that sympathetic innervation is controlling the pancreatic hormone response to exercise. Circulating red cell volume is increased during exercise and is consistent with the response that is seen with sympathetic nerve stimulation of the spleen (1).

The increase in hepatic norepinephrine spillover observed in the present study is consistent with the demonstration that the rat liver is depleted of norepinephrine during prolonged exercise due, presumably, to norepinephrine release from sympathetic nerve terminals in the liver (29). Sympathetic innervation of the liver has been proposed to have an important function in the stimulation of hepatic glucose production during exercise on the basis of two premises. First, increases in phosphorylase activity (9, 22) and hepatic glycogenolysis (8, 9, 14) occur with direct hepatic nerve stimulation. Second, the exercise-induced increase in glucose production is more rapid than changes in arterial glucagon, insulin, and epinephrine levels (7). The results of the present study show that the increase in sympathetic nerve activity is sufficiently prompt to contribute to the increase in hepatic glucose production. Nevertheless, despite the circumstantial evidence outlined above and documented by the present study, there is still no direct experimental evidence implicating the sympathetic nerves in the control of hepatic glucose production during exercise (25).

Arterial plasma epinephrine concentrations were increased sixfold to over 600 pg/ml at 20 min of heavy exercise. The plasma epinephrine concentrations increased to peak levels of only 200 and 50 pg/ml in the portal vein and hepatic vein, respectively. The lower plasma epinephrine concentrations in these vessels illustrate the high FX of epinephrine by the NHS and

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### Table 1: Hemodynamic measurements during heavy exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Basal Level</th>
<th>Heavy Exercise Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>107 ± 12</td>
<td>211 ± 11*</td>
</tr>
<tr>
<td>Blood flow, ml·kg⁻¹·min⁻¹</td>
<td>209 ± 10*</td>
<td>220 ± 5*</td>
</tr>
<tr>
<td>Portal vein</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Hepatic artery</td>
<td>25 ± 3</td>
<td>20 ± 2*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 7 dogs. *Significantly different from basal levels, P < 0.05.
hepatic tissues. A result of this is that the arterial levels of epinephrine do not reflect and are, in fact, much higher than the concentrations of this hormone in the blood perfusing the liver. A corollary to this is that hepatic catecholamine action assessed by using a peripheral infusion underestimates the sensitivity of the liver to catecholamines if splanchic catecholamine extraction is not considered. For example, one study showed that an increase in arterial epinephrine levels to ~1,800 pg/ml by using a peripheral venous infusion increased hepatic glucose production transiently by ~2 mg·kg⁻¹·min⁻¹ in the dog (24). Because NHS extracts ~60% of the epinephrine presented to it, the concentration in the portal vein was considerably less than that in the artery, only ~600 pg/ml. Because of the higher arterial norepinephrine levels and increased NHS norepinephrine spillover, exercise resulted in a much greater increment in vascular norepinephrine delivery compared with that for epinephrine. Nevertheless, the sensitivity of the liver to norepinephrine, at least with respect to glucose production, is considerably lower than that of epinephrine. An intraportal infusion of norepinephrine that increased portal vein norepinephrine by 3,000 pg/ml increased glucose production by only 1.0 mg·kg⁻¹·min⁻¹ in resting dogs (5).

In the resting state, splanchnic tissues are a main site of catecholamine removal from the circulation (6, 20). The important role of splanchnic tissue in control of norepinephrine uptake is probably related to the high density of sympathetic nerves, which have terminals that take up catecholamines, and the high activity of catechol-O-methyltransferase in the liver (13). This experiment shows that ~60% of the splanchic catecholamine uptake is due to uptake by NHS during rest and exercise. The rate of tissue uptake is a result of tissue delivery and FX. Although catecholamine delivery to the liver is somewhat lower than NHS tissues, this is counterbalanced by an ~25% higher FX. Norepinephrine and epinephrine uptakes by NHS and hepatic tissues both rise in response to exercise, mainly because of an increase in their circulating concentrations. In addition, uptake was facilitated by exercise-induced increases in FX of ~15% at both sites.

In summary, adrenergic stimulation of the liver during heavy exercise is determined by hepatic sympathetic nerve activity and vascular catecholamine delivery. NHS and hepatic norepinephrine spillovers increase approximately two- and approximately threefold during heavy exercise and contribute to the increase in circulating norepinephrine. Although vascular epinephrine delivery is increased by exercise, arterial levels greatly overestimate the magnitude of the increment due to an ~60% FX by the NHS tissues. Finally, despite the high catecholamine FX by splanchnic tissue, the delivery of norepinephrine to the liver is maintained because of simultaneous NHS norepinephrine release.

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