Hepatic and Gut Clearance of Catecholamines in the Conscious Dog

Chang An Chu, Dana K. Sindelar, Doss W. Neal, and Alan D. Cherrington

Our aim was to assess hepatic and gut catecholamine clearance under normal and simulated stress conditions. Following a 90-minute saline infusion period, epinephrine ([EPI] 180 ng/kg · min) and norepinephrine ([NE] 500 ng/kg · min) were infused peripherally for 90 minutes into five 18-hour fasted, conscious dogs undergoing a pancreatic clamp (somatostatin plus basal insulin and glucagon). Arterial plasma levels of EPI and NE increased from 44 ± 9 to 2,961 ± 445 and 96 ± 6 to 6,467 ± 571 pg/mL, respectively (both $P < .05$). Portal vein plasma levels of EPI and NE increased from 23 ± 8 to 1,311 ± 173 and 79 ± 10 to 3,477 ± 380 pg/mL, respectively (both $P < .05$). Hepatic vein plasma levels of EPI and NE increased from 5 ± 2 to 117 ± 33 and 48 ± 10 to 448 ± 59 pg/mL, respectively (both $P < .05$). Net hepatic and gut EPI uptake increased from 0.5 ± 0.1 to 30.0 ± 3.0 and 0.4 ± 0.1 to 26.3 ± 4.0 ng/kg · min, respectively (both $P < .05$). Net hepatic and gut NE uptake increased from 1.5 ± 0.4 to 74.7 ± 8.4 and 0.8 ± 0.2 to 57.9 ± 7.6 ng/kg · min, respectively (both $P < .05$). Neither the net hepatic (0.86 ± 0.05 to 0.93 ± 0.02) nor gut (0.45 ± 0.10 to 0.55 ± 0.04) fractional extraction of EPI changed significantly during the simulated stress condition. Net hepatic and gut spillover of NE increased from 0.8 ± 0.2 to 3.5 ± 1.3 and 0.6 ± 0.2 to 8.8 ± 2.0 ng/kg · min, respectively, during catecholamine infusion (both $P < .05$). These results indicate that (1) approximately 30% of circulating catecholamines are cleared by the splanchnic bed (16% and 14% by the liver and gut, respectively); (2) the liver and gut remove a large proportion (approximately 86% to 93% and 45% to 55%, respectively) of the catecholamines delivered to them on first pass; and (3) high levels of plasma catecholamines increase NE spillover from both the liver and gut, suggesting that the percentage of NE released from the presynaptic neuron that escapes the synaptic cleft is increased in the presence of high circulating catecholamine levels.

CATECHOLAMINES play an important role in the regulation of hemodynamics and lipid, protein, and carbohydrate metabolism. During physiological and stressful conditions, an increase in circulating catecholamines stimulates hepatic glucose production, lipolysis, and proteolysis.[1-8] The basal arterial plasma level of epinephrine (EPI) in humans and dogs is approximately 60 pg/mL.[7-9] The basal arterial plasma level of norepinephrine (NE) is approximately 100 to 200 pg/mL.[6-9] In extremely stressful situations (ie, severe hypoglycemia, exhaustive exercise, or hemorrhagic shock), plasma levels of catecholamines can increase 50- to 100-fold.[1,2,8] EPI is released from the adrenal medulla into the circulation, while the majority of circulating NE is derived from sympathetic nerve terminals, with only a small amount originating from the adrenal medulla. Plasma catecholamines are quickly cleared or removed from the circulation.[10,11] Although the whole-body clearance of catecholamines in the human has been determined,[10-12] the clearance of catecholamines by the liver and gut have been poorly defined in both physiological and stressful conditions. Henriksen et al[13] used the arteriovenous (arterial–hepatic vein)-difference technique to assess splanchnic extraction of catecholamines in the human. Since no portal vein (which accounts for 80% of hepatic flow) samples were drawn, they could not differentiate liver and gut uptake.

In view of the difficulty in obtaining plasma samples from the portal vein and thus in directly assessing hepatic and gut catecholamine extraction in the human, we decided to address this issue in the conscious dog. Stevenson et al[4] showed that high levels of catecholamines can alter endogenous pancreatic hormone secretion both directly and indirectly through hyperglycemia in the dog. Since it has been reported[14-18] that an increase in plasma insulin can activate the sympathetic nervous system, we decided to use a pancreatic clamp to simplify data interpretation.

The aims of the present study therefore were (1) to assess catecholamine clearance by the liver and gut in the presence of low and high catecholamine levels, and (2) to determine the effect of high circulating catecholamine levels on NE spillover from the gut and liver.

MATERIALS AND METHODS

Experiments were performed on five eighteen-hour fasted, conscious mongrel dogs (22 to 29 kg) of either sex that were fed a standard diet of meat and chow as described elsewhere.[7,8] The animals were housed in a facility that meets the American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee.

A laparotomy was performed 16 to 18 days before each experiment to implant catheters and Doppler flow probes in or around appropriate blood vessels as described elsewhere.[7,8] Each dog was used for only one experiment. All dogs studied had (1) a leukocyte count less than 18,000/μl, (2) a hematocrit greater than 35%, (3) a good appetite, and (4) normal stools.

Each experiment consisted of a 100-minute equilibration and hormone adjustment period (−140 to −40 minutes), a 40-minute basal period (−40 to 0 minutes), a 90-minute saline + ascorbic acid (70 mg/dL) control, and a 90-minute test period (0 to 90 and 90 to 180 minutes, respectively). An infusion of somatostatin with intraportal replacement infusion of insulin and glucagon were performed as described elsewhere.[7,8] EPI (180 ng/kg · min) and NE (600 ng/kg · min)

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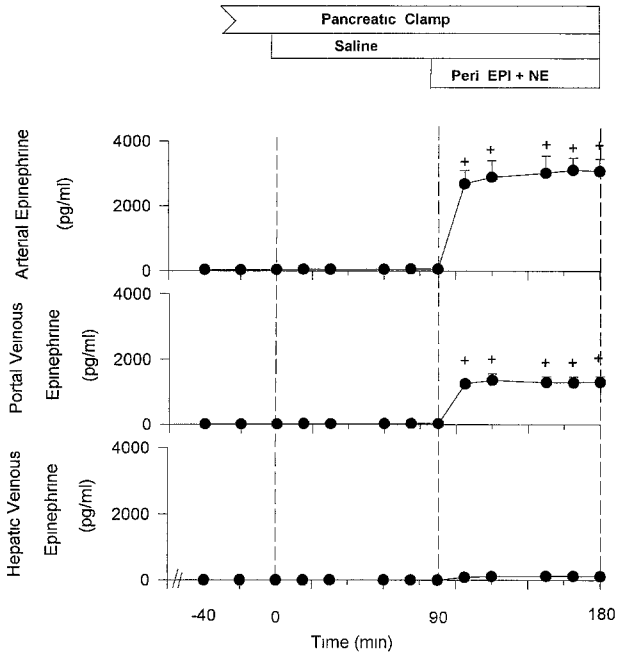

Fig 1. Plasma EPI levels in the artery, portal vein, and hepatic vein during basal, saline control, and test periods in conscious 18-hour fasted dogs. $^{+}P < .05$ *v* corresponding saline period.

were infused in the solution of ascorbic acid via cephalic catheters during the test period.

Plasma levels of EPI and NE were assessed using high-performance liquid chromatography as previously described.[19] Catecholamine concentrations were measured based on linear regression using dihydroxybenzylamine as an internal standard. The recovery of NE and EPI was virtually complete. The sensitivity of the assay is approximately 20 and 30 pg/mL for NE and EPI, respectively. The coefficient of variation using this method was 5% and 7% for NE and EPI, respectively. The blood pressure and heart rate were measured using methods described elsewhere.[7,8]

Doppler flow probes and indocyanine green dye (ICG) were used to estimate total hepatic blood flow.[7,8] Total hepatic blood flow in the basal, saline control, and test periods was 31 ± 4, 33 ± 3, and 31 ± 5 mL/kg · min, respectively, when measured with Doppler flow probes and 26 ± 3, 28 ± 5, and 25 ± 6 mL/kg · min, respectively, when measured with ICG. Since in our studies hepatic blood flow measured using the Doppler method was more stable than the flow determined using the ICG method, and because the former did not require an assumption about the distribution of liver blood flow (A *v* P), the data in the Figures were calculated with Doppler-measured flows.

Net hepatic EPI uptake and net EPI fractional extraction were calculated as follows: net hepatic EPI uptake = [H · (Fa + Fp) − (A · Fa + P · Fp)], net hepatic EPI fractional extraction (HEFE) = [H · (Fa + Fp) − (A · Fa + P · Fp)]/(A · Fa + P · Fp), net gut EPI uptake = (P − A) · Fp, and gut EPI fractional extraction (GEFE) = (P − A)/A. A, P, and H are the arterial, portal vein, and hepatic vein plasma EPI concentrations, and Fa and Fp are the hepatic arterial and portal vein plasma flow measured by Doppler flow probes.

Since EPI is not released from the liver and the net hepatic fractional extraction of EPI equals that of NE and is independent of the plasma EPI concentration,[10,20] hepatic NE spillover can be calculated as follows: hepatic NE spillover (HNS) = $H \cdot (Fa + Fp)$ − [(A · Fa + P · Fp) · (1-HEFE)], gut NE spillover (GNS) = $P \cdot Fp$ − [A · Fp · (1 − GEFE)], net hepatic NE uptake = [$H \cdot (Fa + Fp)$ − (A · Fa + P · Fp)] + HNS, and net gut NE uptake = $(P - A) \cdot Fp$ + GNS. *A*, *P*, and *H* are the arterial, portal vein, and hepatic vein plasma NE concentrations.

Statistical Analysis

All statistical comparisons were made using repeated-measures ANOVA with post hoc analysis by univariate F tests. Statistical significance was accepted at a *P* level less than .05. Data are expressed as the mean ± SE.

RESULTS

Arterial plasma insulin remained at basal levels (6 to 10 μU/mL) throughout the study. Likewise, arterial plasma glucagon remained at basal levels (51 to 56 pg/mL) throughout the study. The arterial blood glucose remained unchanged during the basal and control periods, but increased from 75 ± 4 to 140 ± 16 mg/dL during catecholamine infusion. The arterial, portal vein, and hepatic vein plasma levels of EPI and NE did not change significantly during the basal and control periods. Peripheral infusion of catecholamines increased the arterial plasma level of EPI and NE from 44 ± 9 to 2,961 ± 445 and 96 ± 6 to 6,467 ± 571 pg/mL, respectively, during the test period (both $P < .05$). Portal vein plasma levels of EPI and NE increased from 23 ± 8 to 1,311 ± 173 and 79 ± 10 to 3,477 ± 380 pg/mL, respectively (both $P < .05$), while hepatic vein plasma levels of EPI and NE increased from 5 ± 2 to 117 ± 33 and 48 ± 10 to 448 ± 59 pg/mL, respectively (both $P < .05$) (Figs 1 and 2).

Net Hepatic and Gut Uptake and Fractional Extraction of EPI

The net hepatic and gut uptake of EPI did not change significantly during the basal or saline control periods, but increased from 0.5 ± 0.1 to 30.0 ± 3.3 and 0.4 ± 0.1 to 26.3 ± 4.0 ng/kg · min, respectively, during the catecholamine infusion

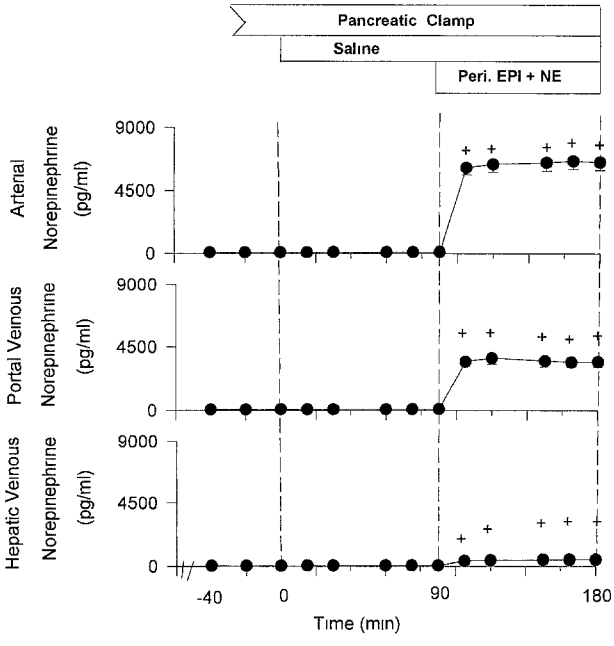

Fig 2. Plasma NE levels in the artery, portal vein, and hepatic vein during basal, saline control, and test periods in conscious 18-hour fasted dogs. $^{+}P < .05$ *v* corresponding saline period.

period (both $P < .05$) Net hepatic and gut fractional extraction of EPI did not change significantly in any period (0.86 ± 0.06, 0.87 ± 0.05 and 0.93 ± 0.02 and 0.45 ± 0.09, 0.42 ± 0.10, and 0.55 ± 0.04, respectively) (Fig 3).

Net Hepatic and Gut Uptake, Spillover, and Fractional Extraction of NE

Net hepatic and gut uptake of NE did not change significantly during the basal and saline control periods, but increased from 1.5 ± 0.4 to 74.7 ± 8.4 and 0.8 ± 0.2 to 57.9 ± 7.6 ng/kg · min, respectively, during the catecholamine infusion period (both $P < .05$). Net hepatic and gut spillover of NE did not change significantly during the basal and control periods, but increased from 0.8 ± 0.2 to 3.5 ± 1.3 and 0.6 ± 0.2 to 8.8 ± 2.0 ng/kg · min, respectively, during infusion of catecholamines ($P < .05$) (Fig 4).

Hepatic Blood Flow, Arterial Blood Pressure, and Heart Rate

Neither the hepatic blood flow (30 ± 4, 31 ± 4, and 32 ± 5 mL/kg · min) nor the mean arterial blood pressure (110 ± 7, 105 ± 8, and 116 ± 8 mm Hg) changed significantly during the experiment. The heart rate did not change significantly during the basal and saline control periods, but increased from 76 ± 9 to 104 ± 10 beats per minute in response to catecholamine infusion ($P < .05$) (Fig 5).

DISCUSSION

The first aim of the present study was to assess hepatic and gut catecholamine clearance under normal and simulated stress conditions. During peripheral infusion of EPI (180 ng/kg · min) and NE (500 ng/kg · min), net hepatic uptake of the two catecholamines was 30.0 ± 3.3 and 74.7 ± 8.4 ng/kg · min, respectively; the gut uptake of EPI and NE was 26.3 ± 4.0 and 57.9 ± 7.6 ng/kg · min, respectively. Thus, the liver and gut cleared 17% and 15%, respectively, of the infused EPI and 15% and 12% of the infused NE. Overall, the splanchnic bed removed approximately one third of the infused catecholamines.

The arterial concentration of EPI and NE increased approximately 65-fold during catecholamine infusion. Such levels are only found during extreme stress (ie, exhaustive exercise[21] and hemorrhagic shock[1,4]). In response to high circulating catechol-

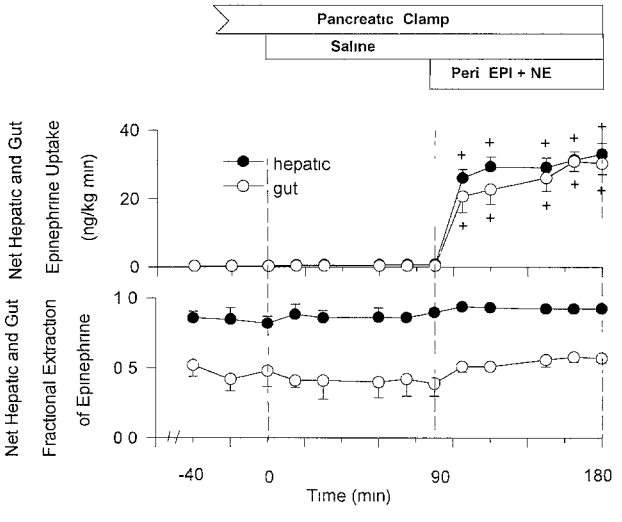

Fig 3. Net hepatic and gut uptake and fractional extraction of EPI during basal, saline control, and test periods in conscious 18-hour fasted dogs. $^{+}P < .05$ *v* corresponding saline period.

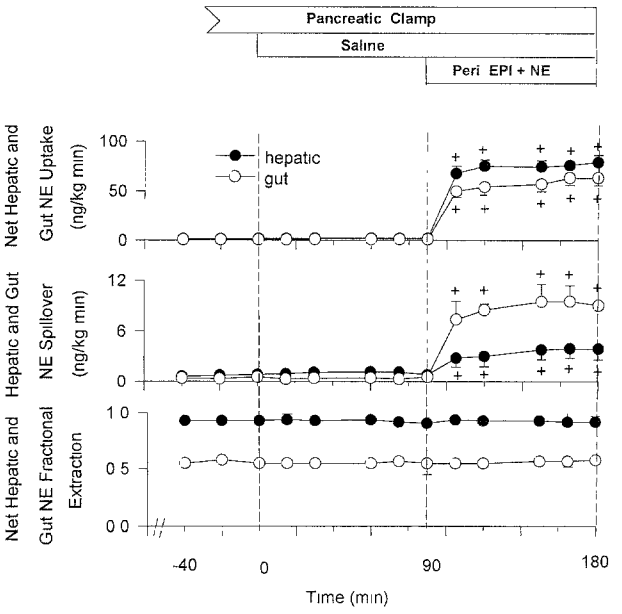

Fig 4. Net hepatic and gut uptake, spillover, and fractional extraction of NE during basal, saline control, and test periods in conscious 18-hour fasted dogs. $^{+}P < .05$ *v* corresponding saline period.

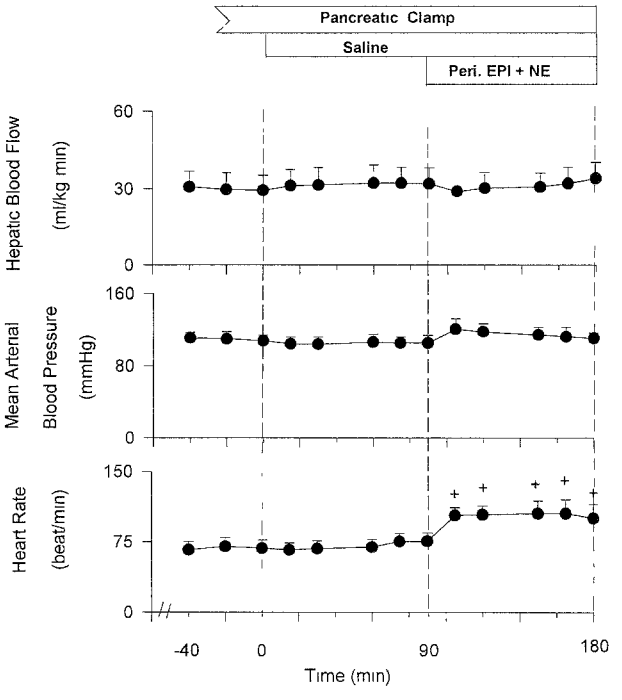

Fig 5. Hepatic blood flow, mean arterial blood pressure, and heart rate during basal, saline control, and test periods in conscious 18-hour fasted dogs. $^{+}P < .05$ *v* corresponding saline period.

amine levels, hepatic and gut EPI uptake increased nearly 65-fold. Therefore, no significant change was found for hepatic (0.86 ± 0.05 to 0.93 ± 0.02) or gut (0.45 ± 0.10 to 0.55 ± 0.04) fractional extraction of EPI in the presence of high circulating catecholamines. This explains why the liver and gut are equal in the overall clearance of EPI. Although the liver extracts a higher proportion on the first pass, 80% of the blood reaching it (from the portal vein) has first passed through the vasculature of the gut. Thus, the plasma EPI level within hepatic sinusoids is approximately 60% of the arterial EPI level. Unlike the case with the pancreatic hormones, the arterial and portal vein EPI ratio is positive. This also means that after an overnight fast the liver is normally (in a nonstressed state) exposed to little EPI.

Hepatic and gut NE uptake increased approximately 50- and 70-fold, respectively, in the presence of high plasma catecholamine levels. Since NE is concurrently taken up by and released from tissue beds, tissue NE release and removal cannot be distinguished from measurements of NE arteriovenous differences alone. Our data indicate clearly that the liver and gut remove a large proportion (~90% and 50%, respectively) of the catecholamines delivered to them on first pass. In a recent dog study, Coker et al[22] showed that arterial plasma EPI and NE levels increased twofold and threefold, respectively, during moderate exercise. The liver and gut removed a similar proportion (87% and 50%, respectively) of the catecholamines delivered to them, as we report here. Since the increment in arterial plasma catecholamine levels in the present study was approximately 20- to 30-fold over the increase in the study by Coker et al,[22] it appears that the net hepatic and gut fractional extraction of catecholamines is independent of plasma catecholamine levels.

It should be noted that the liver and gut uptake of catecholamines may also be influenced by the blood flow to these organs. In the current study, hepatic blood flow did not change significantly during catecholamine infusion. The reason for this is that the adrenergic receptor subtypes on the hepatic portal vein (which provides 80% of the blood flow to the liver) are $\alpha 1$ and $\beta 2$.[23] Stimulation of the former and the latter on the portal vein would cause constriction and dilation, respectively. EPI stimulates both receptors, while NE exerts its effect predominantly through $\alpha 1$ receptors. In all likelihood, the effects of NE and EPI on the portal vein were offset during the infusion of a catecholamine mixture.

Previous studies by Haefely et al[24] and Yamaguchi et al[25] showed that the tissue spillover of NE increases as the activity of sympathetic nerves to the tissue increase. In the present study, the liver and gut spillover of NE increased fourfold and 15-fold, respectively, during catecholamine infusion. The mechanism for the increase in NE spillover is not clear. Several possible explanations can be proposed. First, in theory, infusion of catecholamines may have stimulated the sympathetic nervous system, thereby increasing NE release from nerve terminals within the liver and gut. This seems unlikely since plasma cortisol levels did not change, suggesting the animals were not stressed. Secondly, high levels of circulating catecholamines may have decreased the reuptake of endogenously produced NE and thus caused the exit of a greater percentage of released NE from the synapses. Finally, metabolism of the exogenous catecholamines within the nerve terminals could have decreased the deamination of endogenous NE, which would thus result in an increase of NE release from nerve terminals. Regardless of which of the latter two suggestions is correct, our data suggest that NE spillover from the liver and gut that occurs in response to increased nerve stimulation will increase to a greater extent than the true increase in nerve firing.

In conclusion, our study shows that (1) approximately 30% of circulating catecholamines are cleared by the splanchnic bed (16% and 14% by the liver and gut, respectively); (2) the liver and gut remove a large proportion (approximately 90% and 50%, respectively) of catecholamines delivered to them on first pass; and (3) NE spillover from the liver and gut increases in the presence of high levels of plasma catecholamines.

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