Differential action of hepatic sympathetic neuropeptides: metabolic action of galanin, vascular action of NPY

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Mundinger, Thomas O., and Gerald J. Taborsky, Jr. Differential action of hepatic sympathetic neuropeptides: metabolic action of galanin, vascular action of NPY. Am. J. Physiol. Endocrinol. Metab. 278: E390–E397, 2000.—Activation of hepatic nerves increases both hepatic glucose production (HGP) and hepatic arterial vasoconstriction, the latter best described by a decrease of hepatic arterial conductance (HAC). Because activation of canine hepatic nerves releases the neuropeptides galanin and neuropeptide Y (NPY) as well as the classical neurotransmitter norepinephrine (NE), we sought to determine the relative role of these neuropeptides vs. norepinephrine in mediating metabolic and vascular responses of the liver. We studied the effects of local exogenous infusions of galanin and NPY on HGP and HAC to predict the metabolic and vascular function of endogenously released neuropeptide. Galanin (n = 8) or NPY (n = 4) was infused with and without NE directly into the common hepatic artery of halothane-anesthetized dogs, and we measured changes in HGP and HAC. A low dose of exogenous galanin infused directly into the hepatic artery potentiated the HGP response to NE yet had little effect on HGP when infused alone. The same dose of galanin infused into a peripheral vein (n = 8) did not potentiate the HGP response to NE, suggesting that the locally infused galanin acted directly on the liver to modulate NE’s metabolic action. In contrast, a large dose of exogenous NPY failed to influence HGP when infused either alone or in combination with NE. Finally, NPY, but not galanin, tended to decrease HAC when infused alone; neither neuropeptide potentiated the HAC response to NE. Therefore, both hepatic neuropeptides may contribute to the action of sympathetic nerves on liver metabolism and blood flow. It is likely that endogenous hepatic galanin acts directly on the liver to selectively modulate norepinephrine’s metabolic action, whereas endogenous hepatic NPY acts independently of NE to cause vasoconstriction.

hepatic glucose production; hepatic arterial conductance; neurotransmitter; blood flow

GALANIN AND NEUROPEPTIDE Y (NPY) are peptides found in neurons of many mammals, including rats, dogs, and humans. Both neuropeptides are found in the neurons of the brain (35, 39, 42) and in neurons that innervate abdominal tissues such as liver (30), intestine (15, 27), and pancreas (1). In the periphery, NPY and galanin have been predominantly, but not exclusively, associated with sympathetic nerves. For example, the celiac ganglia, which supply postganglionic sympathetic neurons to the liver as well as to the pancreas and other abdominal organs, contain both NPY peptide (1) and NPY mRNA (41). Likewise, galanin peptide (1) and mRNA (41) are found in celiac ganglia of the dog but not of the rat or the monkey. Furthermore, galanin has recently been observed in postganglionic noradrenergic fibers coursing through canine liver parenchyma and adjacent to liver vasculature (30). Similarly, NPY has been previously shown to be colocalized with norepinephrine in hepatic nerves (5). Both neuropeptides are released with norepinephrine from the canine liver upon sympathetic nerve stimulation (20, 38). Therefore, these locally released neuropeptides may influence liver function either by acting independently of norepinephrine or by modulating norepinephrine’s action. In the current study, we investigate their potential role by measuring changes in liver function during local exogenous infusions of these neuropeptides with and without norepinephrine.

Hepatic sympathetic nerves have been implicated in a variety of metabolic and vascular functions of the liver (2, 11, 17, 19, 22), but the relative roles of the classical neurotransmitter norepinephrine vs. the co-released neuropeptides, galanin and NPY, have yet to be determined. For example, activation of hepatic sympathetic nerves leads to increased glucose mobilization due to hepatic glycogenolysis (12, 14, 17, 21) and to decreased arterial blood flow due to hepatic vasoconstriction (44). Although local administration of norepinephrine can both stimulate liver glycogenolysis (7, 13) and cause hepatic arterial vasoconstriction (13), it is currently unknown whether co-released hepatic neuropeptides contribute to these effects. Therefore, in the current study, we infused galanin and NPY directly into the hepatic artery of halothane-anesthetized dogs with and without concomitant norepinephrine infusions. We monitored changes in hepatic glucose production and hepatic arterial conductance during these infusions as our indicators of their metabolic and vascular effects, respectively.

MATERIALS AND METHODS

Animals and Surgical Procedure

Adult male dogs (28–35 kg) of mixed breed were fasted overnight before surgery and subsequent experimentation. Anesthesia was induced with veterinary sodium thiopental...
(Abbott Laboratories, North Chicago, IL) and maintained with halothane (0.8%) in 100% oxygen administered by positive pressure ventilation from a calibrated vaporizer. Adequate levels of anesthesia during surgery and experimentation were verified by maintenance of normal blood pressure and heart rate and by an absence of a pedal and an eye reflex. A midline laparotomy was performed to allow placement of blood-sampling catheters, a vascular occluder, infusion catheters, and blood flow probes. Blood sampling catheters (Tygon microbore tubing, Norton Performance Plastics, Akron, OH) were placed in the femoral artery, portal vein, and hepatic vein. The portal venous and hepatic venous catheters were inserted at the base of the left lateral lobe of the liver, as described by Chu et al. (7). A vascular occluder (In Vivo Metric, Healdsburg, CA) was placed around the inferior vena cava immediately caudal to the junction with the hepatic vein, as described by Bowden et al. (3). Inferior vena caval blood was occluded only when sampling blood from the hepatic vein to minimize dilution of hepatic venous blood. Infusion catheters were placed in the common hepatic artery for local hepatic administration of neuropeptides and norepinephrine and in the femoral vein for systemic administration of saline or galanin. Ultrasonic blood flow probes (Transonic Systems, Ithaca, NY) were placed around the common hepatic artery and portal vein (30). After the completion of the surgery, a 60-min stabilization period preceded baseline determinations.

All animals included in these studies were certified as healthy by the Veterinary Medical Officer of the Veterans Affairs Puget Sound Health Care System (VAPSHCS) and exhibited normal hematocrit, temperature, food intake, urination, and defecation before acute terminal studies. All research involving animals was conducted in an American Association for Accreditation of Laboratory Animal Care-accredited facility. All protocols were designed to ensure appropriate ethical treatment of the animals and were approved by the Institutional Animal Care and Use Committee of the VAPSHCS.

Experimental Protocol, Blood Sampling, and Assays

Two separate norepinephrine infusions were performed in each animal, one in the absence and one in the presence of a neuropeptide infusion. To ensure that the averaged metabolic and vascular responses to the second norepinephrine infusion were not biased by the antecedent norepinephrine infusion, the order of the neuropeptide infusions was alternated between dogs in each group. Thus, in each group, the number of dogs receiving norepinephrine alone as the first of two infusions was equal to the number of dogs receiving norepinephrine in the presence of neuropeptide as the first of two infusions.

Norepinephrine (2.5 µg/min) was infused at a constant rate of 1 ml/min into the common hepatic artery for 20 min. Blood samples were taken from the femoral artery, portal vein, and hepatic vein, and both hepatic arterial and portal venous blood flows were monitored at each sampling time. Samples were taken at 5- to 10-min intervals immediately before, during, and after the norepinephrine infusion. Neuropeptides (galanin: 25 ng/ml; NPY: 500 pmol/ml) were infused into the hepatic artery or the femoral vein for a total of 55 min, beginning 20 min before the hepatic arterial norepinephrine infusion, continuing throughout, and terminating 15 min after the norepinephrine infusion. Blood samples were taken and blood flows were monitored at 5- to 10-min intervals. Baseline samples for the second norepinephrine infusion were taken 60 min after the last sample of the first infusion, thus allowing the hyperglycemia in response to the first infusion to dissipate.

Blood samples drawn for measurement of glucose and insulin were immediately placed in tubes containing EDTA (Becton Dickinson, Franklin Lakes, NJ). Blood samples for glucagon were drawn on heparin (Elkins-Sinn, Cherry Hill, NJ) and the proteolytic inhibitor benzamidine HCl (Aldrich, Milwaukee, WI). All blood samples were kept on ice until centrifugation (3,000 rpm, 20 min, 2°C). Centrifuged plasma was then decanted and frozen at −20°C until assayed. Plasma glucose was assayed by a glucose oxidase method (ICN Biomedicals, Costa Mesa, CA), and plasma insulin was measured by a modification of the double-antibody RIA method of Morgan and Lazarow (28), as described previously (37). Plasma glucagon was measured by an RIA using COOH-terminally directed antiserum, as described previously (37).

Data Analysis

Changes in hepatic glucose production (HGP) and hepatic arterial conductance (HAC) were used to define the metabolic and vascular responses of the liver to exogenous norepinephrine, galanin, and NPY. HGP was calculated by the method proposed and verified by Myers et al. (31)

\[
\text{HGP} = \left( \frac{\left[ (G_{hv} \times (HABF + PVBF)) \times 0.72 \right]}{H_{ha} \times HABF} \times 0.73 \right) \times \text{weight}
\]

where \(G_{hv}, G_{ha}\), and \(G_p\) are plasma glucose concentrations in the hepatic vein, hepatic artery, and portal vein, respectively; HABF is hepatic arterial blood flow; and PVBF is portal venous blood flow. The factors 0.72 and 0.73 are used to convert plasma glucose to blood glucose. HAC was calculated as HABF/MAP, where MAP is mean arterial blood pressure. HAC is preferred as an index of local hepatic vascular tension because it factors out the changes in blood flow that are due solely to changes in hydrostatic driving pressure (21, 36, 44).

Data are expressed as means ± SE. The “average change from baseline” was calculated as the average of the four time points during norepinephrine infusion (t = 5, 10, 15, and 20) minus the average of the three time points immediately preceding the norepinephrine infusion (t = −10, −5, and 0). A single-tailed paired t-test was used to compare the mean of the baseline period with that during exogenous infusions. Comparison of the average change from baseline in the absence vs. presence of neuropeptides was also made by a single-tailed paired t-test.

RESULTS

Effects of Hepatic Arterial Galanin

HGP and arterial glucose. To define the metabolic response of the liver to locally infused, exogenous norepinephrine, we calculated HGP before and during a hepatic arterial infusion of norepinephrine. During the 20 min of norepinephrine infusion, HGP increased from a baseline of 1.20 ± 0.07 mg·kg⁻¹·min⁻¹ to an average of 4.87 ± 1.01 mg·kg⁻¹·min⁻¹ (Δ = +3.67 ± 1.00 mg·kg⁻¹·min⁻¹, P < 0.005, n = 8; Fig. 1A).

To assess the effect of galanin on the norepinephrine-stimulated increase in HGP, we infused galanin into the hepatic artery before and during the hepatic arterial infusion of norepinephrine. When norepinephrine was infused in the presence of galanin, HGP increased from
1.29 ± 0.15 mg·kg⁻¹·min⁻¹ to an average of 6.81 ± 1.24 mg·kg⁻¹·min⁻¹ (Δ = +5.51 ± 1.27 mg·kg⁻¹·min⁻¹, P < 0.005, n = 8, Fig. 1A). This latter response was significantly larger than the HGP response to norepinephrine alone (P < 0.025 vs. norepinephrine alone).

The increase of HGP in response to the local norepinephrine infusion resulted in an increase of arterial plasma glucose concentration. When norepinephrine was infused alone, arterial glucose increased from a baseline of 102 ± 3 mg/dl to an average of 118 ± 4 mg/dl (Δ = +16 ± 3 mg/dl, P < 0.0005, Fig. 2A). When norepinephrine was infused in the presence of galanin, arterial glucose increased from an average of 106 ± 3 mg/dl to an average of 129 ± 5 mg/dl (Δ = +23 ± 4 mg·kg⁻¹·min⁻¹, P < 0.0005; Fig. 2A). This latter response was significantly larger than the arterial glucose response to norepinephrine alone (P < 0.025 vs. norepinephrine alone).

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The local hepatic arterial infusion of galanin alone increased basal HGP and arterial glucose slightly. When galanin was infused alone for the 20 min immediately preceding the norepinephrine infusion, HGP increased from a baseline of 1.15 ± 0.14 mg·kg⁻¹·min⁻¹ to an average of 1.29 ± 0.15 mg·kg⁻¹·min⁻¹ (Δ = +0.14 ± 0.05 mg·kg⁻¹·min⁻¹, P < 0.025; Fig. 1A), and arterial glucose increased from a baseline of 103 ± 3 mg/dl to an average of 106 ± 3 mg/dl (Δ = +3 ± 1 mg/dl, P < 0.01 vs. baseline; Fig. 2A).

Insulin and glucagon. To determine whether the potentiating effect of galanin on the HGP response to norepinephrine was due to decreased insulin or increased glucagon secretion in the presence vs. absence of galanin, we measured the concentration of these pancreatic hormones in portal venous plasma. The increment of portal venous insulin concentration during the combined galanin plus norepinephrine infusion (Δ = +30 ± 10 µU/ml) was not lower than the increment during norepinephrine alone (Δ = +25 ± 9 µU/ml). Likewise, the increment of portal venous glucagon concentration during the combined galanin plus norepinephrine infusion (Δ = +22 ± 5 ng/l) was not significantly greater than the increment during norepinephrine alone (Δ = +20 ± 14 ng/l).

Blood flow and HAC. To define the hepatic vascular response to local exogenous norepinephrine and the effect of galanin thereon, we calculated HAC by measuring HABF and dividing it by mean arterial blood pressure (MAP) before and during the two norepinephrine infusions. When norepinephrine was infused alone, HABF decreased (Δ = −25 ± 9 ml/min, P < 0.025), MAP did not change, and therefore HAC decreased from a baseline of 0.65 ± 0.10 ml·min⁻¹·mmHg to an average of 0.37 ± 0.05 ml·min⁻¹·mmHg (Δ = −0.28 ± 0.10 ml·min⁻¹·mmHg, P < 0.025, Fig. 3A). The HAC response to norepinephrine during the hepatic arterial infusion of galanin (Δ HAC = −0.32 ± 0.07 ml·min⁻¹·mmHg, P < 0.005; Fig. 3A) was not different from the HAC response to norepinephrine alone (P = NS vs. norepinephrine alone).

The local hepatic arterial infusion of galanin alone did not have significant effects on HAC.

**Fig. 1.** Change in hepatic glucose production (HGP) during hepatic arterial (HA) infusion of norepinephrine (NE, 25 µg/min) alone and in combination with HA infusion of galanin (GAL, 25 ng/min; A), iv infusion of galanin (GAL, 25 ng/min; B), and HA infusion of neuropeptide Y (NPY, 500 pmol/min; C). Values are means ± SE.
Effects of Intravenous Galanin

HGP and arterial glucose. To determine whether the effect of galanin to potentiate the norepinephrine-stimulated increase in HGP was due to a nonhepatic effect of galanin, we repeated the hepatic arterial infusions of norepinephrine in the presence and absence of the same dose of galanin infused into a peripheral vein, not the hepatic artery. During the norepinephrine infusion alone, HGP increased from a baseline of 1.12 ± 0.16 to 5.22 ± 0.96 mg·kg⁻¹·min⁻¹ (Δ = 4.10 ± 1.04 mg·kg⁻¹·min⁻¹, P < 0.005; Fig. 1B). When norepinephrine was infused in the presence of intravenous galanin, HGP increased from 1.31 ± 0.13 mg·kg⁻¹·min⁻¹ to an average of 6.31 ± 1.15 mg·kg⁻¹·min⁻¹ (Δ = 5.00 ± 1.22 mg·kg⁻¹·min⁻¹, P < 0.005; Fig. 1B). In contrast to the HGP response to norepinephrine during the hepatic arterial infusion of galanin (see above), the HGP response to norepinephrine during the intravenous infusion of galanin was not significantly greater than the HGP response to norepinephrine alone (P = NS vs. norepinephrine alone).

As before, the increase of HGP in response to the local norepinephrine infusion alone resulted in an increase of arterial glucose (Δ = +18 ± 2 mg/dl, P < 0.0005; Fig. 2B). In the presence of intravenous galanin, local norepinephrine increased arterial glucose by an increment (Δ = +20 ± 3 mg/dl, P < 0.0005; Fig. 2B) that was not significantly larger than that seen during norepinephrine alone. Thus the intravenous infusion of galanin did not significantly increase either the HGP or the arterial glucose response to norepinephrine.

When galanin was infused alone intravenously, neither HGP nor arterial glucose changed from their baseline values.

Blood flow and HAC. When norepinephrine was infused alone, HAC decreased by 0.34 ± 0.14 ml·min⁻¹·mmHg (P < 0.025, Fig. 3B). When norepinephrine was infused during intravenous galanin, HAC decreased by 0.29 ± 0.10 ml·min⁻¹·mmHg (P < 0.01; Fig. 3B). Thus the HAC response to norepinephrine during the intravenous infusion of galanin was not significantly different from the HAC response to norepinephrine alone (P = NS vs. norepinephrine alone).

Effects of Hepatic Arterial NPY

HGP and arterial glucose. To assess the effect of another hepatic neuropeptide, NPY, on the metabolic and vascular responses of the liver to locally infused norepinephrine, we infused NPY into the hepatic artery before and during a hepatic arterial infusion of norepinephrine. As in the other groups, norepinephrine alone increased HGP from a baseline of 1.62 ± 0.21 to 5.12 ± 1.07 mg·kg⁻¹·min⁻¹ (Δ = 3.50 ± 1.33 mg·kg⁻¹·min⁻¹, P < 0.025, n = 4; Fig. 1C). When norepinephrine was infused during the NPY infusion, HGP tended to increase from 1.34 ± 0.24 to 4.89 ± 1.59 mg·kg⁻¹·min⁻¹ (Δ = 3.55 ± 1.73 mg·kg⁻¹·min⁻¹, P = NS, n = 4; Fig. 1C), a response that was not significantly different from that of HGP to norepinephrine alone (P = NS vs. norepinephrine alone). However, the time course of the
HGP response in the presence of NPY appeared to be delayed.

As in the other groups, when norepinephrine was infused alone, arterial glucose increased from a baseline of 100 ± 4 mg/dl to an average of 117 ± 6 mg/dl (Δ = +17 ± 2 mg/dl, P < 0.005, Fig. 2C). The magnitude of the arterial glucose response to norepinephrine during the hepatic arterial infusion of NPY (Δ = +13 ± 3 mg/dl, P < 0.01; Fig. 2C) was not significantly larger than the arterial glucose response to norepinephrine alone (P = NS vs. norepinephrine alone), but the time course of the arterial glucose response appeared to be delayed.

The hepatic arterial infusion of NPY alone decreased HGP and arterial glucose slightly. When NPY was infused alone, HGP decreased from a baseline of 1.73 ± 0.29 mg·kg⁻¹·min⁻¹ to an average of 1.34 ± 0.24 mg·kg⁻¹·min⁻¹ (Fig. 1C, P < 0.01), and arterial glucose decreased from a baseline of 104 ± 7 mg/dl to an average of 102 ± 6 mg/dl (Fig. 2C, P < 0.025).

Blood flow and HAC. Norepinephrine alone decreased HABF and slightly increased MAP (Δ = +3 ± 1 mmHg, P < 0.025). Consequently, HAC decreased during norepinephrine alone from a baseline of 0.87 ± 0.15 ml·min⁻¹·mmHg to an average of 0.57 ± 0.16 ml·min⁻¹·mmHg (Δ = −0.30 ± 0.02 ml·min⁻¹·mmHg, P < 0.0005, Fig. 3C). When norepinephrine was infused in the presence of NPY, HAC decreased by 0.27 ± 0.08 ml·min⁻¹·mmHg (P < 0.025, Fig. 3C), a response that was not different from the HAC response to norepinephrine alone (P = NS vs. norepinephrine alone).

Although NPY had no effect on the norepinephrine-stimulated decrease of HAC, NPY alone tended to decrease HAC (Fig. 3C). When NPY was infused alone, HAC decreased from a baseline of 0.81 ± 0.03 ml·min⁻¹·mmHg to an average of 0.70 ± 0.04 ml·min⁻¹·mmHg (Fig. 3C; Δ = −0.12 ± 0.07, P < 0.10 vs. baseline). NPY alone did not increase MAP (Δ = −4 ± 3 mmHg, P = NS); therefore, the decrease of HAC was due to a decrease of HABF (Δ = −17 ± 8 ml/min, P < 0.05 vs. baseline).

**DISCUSSION**

Hepatic sympathetic nerves are activated during stress (29), and the classical sympathetic neurotransmitter norepinephrine has been implicated in mediating both the resultant increase of HGP (4) and the decrease of hepatic blood flow (21, 43). However, the neuropeptides galanin and NPY are co-released with norepinephrine from sympathetic nerves of the dog liver (20, 38) and thus may either modulate norepinephrine's action or act independently of it to contribute to neurally-mediated changes in liver function. To test for such potential roles of endogenously released hepatic galanin and NPY, we infused these neuropeptides locally to the liver before and during a concomitant infusion of norepinephrine, and we compared the metabolic and vascular responses with those seen during norepinephrine alone. The doses of galanin and norepinephrine were chosen to approximate the amount spilling over from the liver during hepatic nerve stimu-
lation (20), with the galanin dose restricted somewhat to avoid inhibition of insulin and stimulation of glucagon secretion upon recirculation (10); the NPY dose was chosen to demonstrate its known vasoconstrictive effect and was substantially greater than that spilling over during nerve stimulation (38). We found that our dose of locally infused galanin potentiated the HGP and arterial glucose responses to norepinephrine but did not potentiate the HAC response. This potentiation of norepinephrine's metabolic action was not seen during a systemic administration of galanin, suggesting that galanin acted directly on the liver. In contrast, NPY did not potentiate either the HGP or HAC response to norepinephrine, yet it tended to decrease HAC by itself. We conclude that it is likely that hepatically released galanin contributes to norepinephrine's metabolic action, whereas hepatically released NPY makes an independent contribution to vasoconstriction.

**Metabolic Responses**

The hepatic arterial norepinephrine infusion produced a rapid and sustained increase of HGP. The arterial glucose response lagged behind the HGP response, presumably due to the time needed for heptically released glucose to mix and be distributed in arterial plasma. Furthermore, the arterial glucose level tended to plateau at the end of the 20 min of norepinephrine infusion despite a sustained increase of HGP, suggesting that the rate of glucose disappearance (not measured) had increased in response to the hyperglycemia and/or resultant hyperinsulinemia. Because hyperglycemia per se can suppress HGP in an autoregulatory fashion (18), the HGP response during the latter part of the norepinephrine infusion may underestimate the degree of direct adrenergic stimulation of the liver. Furthermore, because the increment of arterial glucose in the presence of local galanin was greater than during norepinephrine alone, the potential autoregulatory suppression may also have been greater, leading us to underestimate the potentiating effect of galanin on HGP. Nonetheless, there was a significant potentiation of the HGP response to norepinephrine during local galanin infusion.

Although galanin was administered locally via the hepatic artery in an attempt to elicit its direct effect on the liver, because little galanin is extracted by the liver (20), recirculating galanin could potentially decrease insulin and increase glucagon secretion (10, 26), thereby potentiating norepinephrine-stimulated HGP indirectly. To avoid these potential indirect effects, the dose of galanin employed in these studies was chosen so that the systemic levels of galanin achieved would not affect pancreatic hormone secretion. Indeed, the potentiation of norepinephrine-stimulated HGP and arterial glucose responses during the hepatic arterial infusion of galanin was not accompanied by either lower insulin or higher glucagon increments than those seen during norepinephrine alone. Nonetheless, to more convincingly rule out an indirect effect of galanin, we infused this same dose of galanin systemically in a separate group of dogs during the local infusion of norepinephrine. In this control experiment, the intravenous infusion of galanin did not potentiate the norepinephrine-stimulated increase of either HGP or arterial glucose. We conclude that low levels of galanin act directly on the liver to potentiate norepinephrine-stimulated HGP, and therefore the amount of galanin released from the liver during sympathetic activation is likely sufficient to contribute to norepinephrine's direct stimulation of HGP.

Further studies are required to elucidate the mechanism responsible for galanin's direct effect on the liver to potentiate norepinephrine-stimulated HGP. Norepinephrine is known to stimulate HGP by activating α-1 receptors (8), which leads to the activation of the glycogenolytic enzyme glycogen phosphorylase. Galanin receptors in the liver, their associated intracellular pathways, and their possible interaction with α-adrenergic receptor pathways have yet to be described.

Although this low dose of galanin acts at the liver to potentiate the HGP and arterial glucose responses to infused norepinephrine, it produced little mobilization of glucose when infused alone: galanin, given alone in the hepatic artery, produced <4% of the HGP response to norepinephrine alone. Perhaps the exogenous galanin did not stimulate HGP on its own but simply potentiated the metabolic effect of the endogenous norepinephrine, which is tonically released by hepatic sympathetic nerves. Such a selectively potentiating action of galanin suggests that this neuropeptide may be a sympathetic neuromodulator rather than a true neurotransmitter capable of independent action. However, infusing higher doses of galanin would be required to definitively rule out the possibility of an independent stimulatory effect. Such an experiment would require a different experimental protocol, because higher doses of galanin would recirculate and stimulate HGP indirectly via galanin's known effects on pancreatic hormone secretion (10).

In contrast to galanin, a high dose of NPY infused via the hepatic artery did not potentiate either the HGP or arterial glucose response to local norepinephrine despite a larger glucagon response in the presence of NPY (data not shown). The lack of effect of NPY on the magnitude of the HGP and arterial glucose responses was not due to administration of a biologically ineffective dose of NPY, because this dose produced a noticeable vascular response when infused alone. NPY did, however, seem to produce a slight delay in both the HGP and arterial glucose responses to norepinephrine (see Figs. 1C and 2C). This delayed HGP response in the presence of NPY may be secondary to the tendency toward a small inhibition of basal HGP that occurred during the preceding infusion of NPY alone. This small inhibition of baseline HGP was not due to either an increase of insulin or a decrease of glucagon secretion but may be due to an effect of NPY to activate Y2 receptors on presynaptic noradrenergic nerve terminals. Presynaptic Y2 activation can inhibit endogenous norepinephrine release (24), possibly withdrawing tonic noradrenergic stimulation of basal HGP. Alternatively, the small inhibition of HGP by NPY alone and the
subsequently delayed HGP response to norepinephrine may be unrelated. Nonetheless, during NPY infusion, the HGP and arterial glucose responses to norepinephrine were different only in timing, not magnitude. We conclude that, because the substantial dose of NPY had neither an independent nor a norepinephrine-dependent modulatory role to increase HGP, it is very unlikely that endogenous hepatic NPY has a physiological role in mediating HGP responses during activation of hepatic sympathetic nerves.

Vascular Responses

Because activation of sympathetic nerves causes vasoconstriction in addition to an increase of HGP, we also investigated the effects of galanin and NPY on the hepatic vascular responses. We report changes of HAC as our index of hepatic vasoconstriction, because HABF can be influenced by blood pressure in addition to local vasoconstriction (36). As expected (13), norepinephrine infused into the hepatic artery decreased HAC, yet local galanin infusion did not augment this response. Additionally, galanin infused alone had no effect on HAC, demonstrating that the dose of galanin that potentiates norepinephrine-stimulated increase of HGP lacks vascular effects. These findings are surprising, because a recent immunohistochemical study shows dense galaninergic innervation of hepatic blood vessels, as well as hepatocytes, in the dog liver (30). It may simply be that the vascular smooth muscle of the dog liver does not express galanin receptors, whereas hepatocytes do. If true, this raises the possibility that receptor expression on a target tissue, not the degree of innervation, determines the local role of endogenous neuropeptides. In any case, because the hepatic vasculature is more sensitive than hepatocytes to low levels of nerve stimulation (30), and the low dose of galanin modulated HGP in the absence of vasoconstrictive effects, we think it is unlikely that heparatically released galanin has vascular action. However, we cannot definitively rule out vascular effects of hepatic galanin without testing higher doses.

The absence of a vasoconstrictor action of galanin alone or in combination with norepinephrine is consistent with previous reports showing little vascular action of galanin in other organs (10, 34). NPY, on the other hand, has been shown to produce vasoconstriction in many tissues (9, 23, 33) by activating Y1 receptors on vascular smooth muscle (6). Indeed, in the present study, HAC tended to decrease when NPY was infused alone into the hepatic artery, suggesting that NPY could possibly be a true neurotransmitter in the dog liver. It is unclear, however, whether these data reflect a physiological role of hepatic NPY, because the dose infused was substantially greater than the amount of NPY spilling over during hepatic nerve stimulation. Unexpectedly, this dose of NPY did not potentiate norepinephrine's vascular action, as has been reported in certain other tissues (see below). Figure 3C is formatted to highlight this lack of potentiation, yet the effect of NPY alone to decrease baseline HAC is clearly visible.

Our finding that NPY does not potentiate norepinephrine-stimulated vasoconstriction in the dog liver in vivo is in contrast to previously published reports using cell culture or in vitro preparations (16, 40). This discrepancy may be due to 1) the different experimental preparations, with in vivo preparations more similar to the natural milieu of hepatic vasculature, 2) the level of stimulation by norepinephrine and NPY (32), or 3) the common use of a specific Y1 agonist, usually (Leu 31, Pro 34) NPY, in in vitro experiments, thereby eliminating the possible influence of Y2-receptor activation, which would inhibit endogenous norepinephrine release. This potential Y2-mediated withdrawal of noradrenergic tone by use of NPY peptide in vivo may mask the clear potentiation seen with Y1-specific agonists in vitro. Therefore, we conclude that, if NPY has a physiological role in the liver, it is to act independently of norepinephrine to cause vasoconstriction.

In all three groups of dogs, there is a tendency for HAC to partially recover after reaching a nadir at the 5-min time point despite the continued norepinephrine infusion. Furthermore, after the discontinuation of the local hepatic norepinephrine infusion, HAC rose sharply above baseline (see Fig. 3, A, B, and C). Both effects may be due to the release of vasodilatory factors during the norepinephrine infusion. One such vasodilatory factor, nitric oxide, is released from hepatic arteries during norepinephrine infusions and during hepatic nerve stimulation (25). If nitric oxide is responsible for the postnorepinephrine vasodilation, then apparently its vasodilatory action persists for some time after norepinephrine is cleared.

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