Effect of a selective rise in sinusoidal norepinephrine on HGP is due to an increase in glycolysis

CHANG AN CHU, DANA K. SINDELAR, DOSS W. NEAL, ERIC J. ALLEN, E. PATRICK DONAHUE, AND ALAN D. CHERRINGTON

Effect of a selective rise in sinusoidal norepinephrine on HGP is due to an increase in glycolysis. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E162–E171, 1998.—To determine the effect of a selective rise in liver sinusoidal norepinephrine (NE) on hepatic glucose production (HGP), norepinephrine (50 ng·kg⁻¹·min⁻¹) was infused intraperitoneally (Po-NE) for 3 h into five 18-h-fasted conscious dogs with a pancreatic clamp. In the control protocol, NE (0.2 ng·kg⁻¹·min⁻¹) and glucose were infused peripherally to match the arterial NE and blood glucose levels in the Po-NE group. Hepatic sinusoidal NE levels rose ~30-fold in the Po-NE group but did not change in the control group. The arterial NE levels did not change significantly in either group. During the portal NE infusion, HGP increased from 1.9 ± 0.2 to 3.5 ± 0.4 mg·kg⁻¹·min⁻¹ (15 min; P < 0.05) and then gradually fell to 2.4 ± 0.4 mg·kg⁻¹·min⁻¹ by 3 h. HGP in the control group did not change over time. Gluconeogenesis did not change significantly in either group. In conclusion, elevation in hepatic sinusoidal NE significantly increases HGP by selectively stimulating glycolysis. Compared with the previously determined effects of epinephrine or glucagon on HGP, the effect of NE is, on a molar basis, less potent but more sustained over time.

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IT IS WELL KNOWN that norepinephrine, acting as a neurotransmitter or circulating hormone, can modify pancreatic hormone secretion and, as a result, indirectly regulate amino acid, fat, and glucose metabolism (40, 41). It is also known that norepinephrine can directly stimulate adipose tissue lipolysis, muscle glycogenolysis, and proteolysis, as well as hepatic glucose production (8, 9, 32, 35). The stimulatory effect of norepinephrine on hepatic glucose production is thought to arise both from its direct action on the liver per se and from its indirect effects arising from an increase in gluconeogenic precursor release from extrahepatic tissues (muscle and adipose tissue).

Previous studies in humans (20, 32, 35) suggested that an increase in circulating norepinephrine can stimulate gluconeogenesis by mobilizing alanine, as well as lactate, from muscle, and glycerol from adipose tissue. Connolly et al. (8, 9) showed in dogs that an increase in arterial norepinephrine (100 ± 24 to 3,244 ± 807 pg/ml) had a pronounced effect on adipose tissue lipolysis (plasma glycerol rose fourfold) and muscle

glycogenolysis (lactate release by nonhepatic tissues increased by 11 µmol·kg⁻¹·min⁻¹). They also showed that hepatic glucose production increased from 2.8 ± 0.2 to 3.4 ± 0.4 mg·kg⁻¹·min⁻¹. At the same time gluconeogenic efficiency rose by 212% and the maximal gluconeogenic rate rose threefold. These data indicate that circulating norepinephrine increased hepatic glucose production by stimulating gluconeogenesis as a result of an increase in gluconeogenic precursor release from muscle and adipose tissue. The greater increase in gluconeogenesis than in hepatic glucose production in that study suggests that gluconeogenesis (indirect effect of norepinephrine) may in fact have suppressed hepatic glycogenolysis. This is further supported by our recent finding (5) that the gluconeogenic effects of epinephrine, which also occur because of an increase in gluconeogenic precursor release from extrahepatic tissues, decrease the catecholamine’s glycogenolytic effect on the liver. Thus the effect of the elevation in hepatic sinusoidal norepinephrine per se (direct effect of norepinephrine) was probably masked by the actions of the rise in arterial norepinephrine on muscle and adipose tissue. Because in all studies that have examined the effects of norepinephrine on glucose production in vivo the catecholamine has been administered peripherally, it has not been possible to evaluate the direct effect of the catecholamine on the liver in vivo in the absence of its overwhelming peripheral effects on glucose utilization, muscle glycogenolysis, and adipose tissue lipolysis.

The question thus arises as to whether, in the absence of its peripheral gluconeogenic action, norepinephrine would increase hepatic glycogenolysis. This possibility is supported by earlier studies in different animal species (11, 15, 18, 22, 33), which showed that electrical stimulation of the distal cut end of the splanchic sympathetic nerves rapidly increases norepinephrine release, glucose output, and the activity of glycogenolytic enzymes in the liver. This question becomes all the more important because norepinephrine released from sympathetic nerve terminals within the liver would selectively alter liver metabolism. The liver removes most (≥95%) of the norepinephrine delivered to it (5), so very little norepinephrine released from hepatic nerve terminals reaches the peripheral tissues. Thus an increase in neural input to the liver would result in selective hepatic activation. Infusion of the catecholamine directly into the hepatic portal vein would allow us to assess the direct effect of norepinephrine on the liver in the absence of its effects on extrahepatic tissues. In view of the difficulties in delivering norepinephrine via the portal vein and in directly
assessing hepatic gluconeogenesis in humans, we decided to address this question in the conscious dog.

A second reason to assess the direct effect of norepinephrine on the liver arises from the finding that the distribution of adrenergic receptors in the canine liver is, like those in humans, predominantly \( \alpha_1 \) and \( \beta_2 \). A previous study (36) showed that epinephrine exerts its effect on hepatocytes predominantly via \( \beta_2 \)-adrenergic receptors. Because the \( \beta_2 \)-adrenergic receptor has a much lower affinity for norepinephrine than epinephrine, the direct effects of norepinephrine on the liver are presumably mediated through \( \alpha_1 \)-receptors. Additionally, our recent unpublished data showed that the effect of norepinephrine on the liver was 85% inhibited by phentolamine (an \( \alpha \)-adrenergic blocker). Furthermore, the intracellular signal pathways of \( \alpha_1 \) and \( \beta_2 \)-adrenergic receptors are quite different; they are mediated through the \( G_S \) protein as well as \( G_0 \) and \( G_8 \) protein as well as adenosine 3',5'-cyclic monophosphate (cAMP), respectively. Thus, although we know that in the absence of its indirect effects on muscle and fat epinephrine's action on the liver is mediated via an effect on glycogenolysis, it is not clear whether such would also be the case for norepinephrine.

The first aim of the present study, therefore, was to determine the direct effects of norepinephrine on hepatic glycogenolysis and gluconeogenesis in vivo in the absence of its action on muscle and adipose tissue (i.e., supply of gluconeogenic precursors reaching the liver), as well as in the absence of its pancreatic effects on insulin and glucagon secretion. The second aim was to test whether there are any differences between the direct effects of norepinephrine and epinephrine on the liver, given their differing affinity for the various subtypes of adrenergic receptors.

**MATERIALS AND METHODS**

Experiments were carried out on ten 18-h-fasted conscious mongrel dogs (20–28 kg) of either sex that had been fed a standard diet of meat and chow described elsewhere (5, 6). The animals 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 Medical Center Animal Care Committee.

A laparotomy was performed 16–18 days before each experiment to implant catheters and Doppler flow probes into or around appropriate blood vessels, as described elsewhere (5, 6). Each dog was used for only one experiment. All dogs studied had (5, 6). Each experiment consisted of a 100-min tracer equilibration and hormone adjustment period (−140 to −40 min), a 40-min basal period (−40 to 0 min), and a 180-min test period (0 to 180 min; Fig. 1). In all studies, a priming dose of purified [\( ^3H \)]glucose (42 \( \mu Ci \)) was given at −140 min, followed by a constant infusion of [\( ^3H \)]glucose (0.35 \( \mu Ci \·min\)), [\( ^{14}C \)]alanine (0.35 \( \mu Ci \·min\)), and indocyanine green dye (ICG; 0.1 mg·m\(^{-2} \)·min\(^{-1} \)). An infusion of somatostatin (0.8 \( \mu g \·kg\(^{-1} \)·min\(^{-1} \)) was started at −130 min to inhibit endogenous insulin and glucagon secretion. Concurrently, intraportal replacement infusions of insulin (300 \( \mu U \·kg\(^{-1} \)·min\(^{-1} \)) and glucagon

<table>
<thead>
<tr>
<th>Time (min) / period</th>
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<th>−40</th>
<th>0</th>
<th>Experimental</th>
</tr>
</thead>
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<tr>
<td>Indocyanine Green + [( ^3H )]Glucose + [( ^{14}C )]Alanine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatostatin + Basal Portal Glucagon and Insulin</td>
<td></td>
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</tr>
<tr>
<td>(0.8 ( \mu g \·kg(^{-1} )·min(^{-1} ))</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(0.65 ( \mu g \·kg(^{-1} )·min(^{-1} ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(245 ( \mu U \·kg(^{-1} )·min(^{-1} ))</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Protocol design. Po-NE group, portal norepinephrine (test) group.

(0.65 ng·kg\(^{-1} \)·min\(^{-1} \)) were started. The plasma glucose level was monitored every 5 min, and euglycemia was maintained by adjusting the rate of insulin infusion. The final alteration in the insulin infusion rate was made ≥30 min before the start of the basal period, and the rate of insulin infusion (mean of 245 \( \mu U \·kg\(^{-1} \)·min\(^{-1} \)) remained unchanged thereafter. The study included two groups. In the first group (Po-NE), norepinephrine (50 ng·kg\(^{-1} \)·min\(^{-1} \)) was delivered via the right celiac vein catheter to match the arterial norepinephrine level in the Po-NE group. The peripheral NE infusion was based on our previous studies (5, 6), which showed that the liver extracts almost all (>98%) of the catecholamine delivered intraortally. Arterial glucose levels in the control group were clamped to the level in the Po-NE group by an infusion of exogenous glucose (20% dextrose) via the right celiac vein. Blood pressure and heart rate were measured using methods described elsewhere (5, 6).

Plasma and blood glucose, plasma [\( ^{3}H \)]- and [\( ^{14}C \)]glucose, blood lactate, glyceral, \( \beta \)-hydroxybutyrate (BOHB), alanine, glutamine, glutamate, glycine, serine, threonine, and plasma free fatty acid (FFA) were determined using previously described methods (5, 6). The levels of insulin, glucagon, cortisol, epinephrine, and norepinephrine were also determined as described elsewhere (5, 6).

Doppler flow probes and ICG were used to estimate total hepatic blood flow (5, 6). The total hepatic blood flows in the test periods of the two groups were 27 ± 2 or 29 ± 3 ml·kg\(^{-1} \)·min\(^{-1} \) when measured with Doppler flow probes and 24 ± 3 or 26 ± 2 ml·kg\(^{-1} \)·min\(^{-1} \) when measured with ICG (Po-NE and Con groups, respectively). Because in our studies hepatic blood flows measured using the Doppler method were more stable than those determined with the ICG method, data in Figs. 1–5 and Tables 1–4 are those calculated with Doppler-measured flows.
E164 EFFECTS OF NOREPINEPHRINE ON GLUCOSE PRODUCTION

dal plasma norepinephrine levels were calculated using Dopp-

Hepatic sinusoidal level = A · Fa/(F_a + F_p) + P · Fp/(F_a + F_p)

where A and P are the arterial and portal vein plasma

norepinephrine concentrations, and F_a and F_p are the hepatic

arterial and portal vein plasma flows measured by the

Doppler flow probes.

It should be noted, to the extent that there is hepatic

glucose uptake (HGU), net hepatic glucose output (NHGO)

slightly underestimates total hepatic glucose release

(NHGO + HGU). On the basis of an earlier study (27), HGU in

the control period of the present protocols was ~0.2

mg·kg⁻¹·min⁻¹. However, because the increment in hepatic

sinusoidal norepinephrine level increased hepatic glucose

production, it is likely that HGU fell slightly during norepi-

nephrine infusion, so that total hepatic glucose release during

the test period was probably within ~0.1 mg·kg⁻¹·min⁻¹ of

net hepatic glucose output. In the control group, hyperglyce-

mia (~16 mg/dl in blood glucose) would be expected to

increase HGU slightly (~0.1 mg·kg⁻¹·min⁻¹), as indicated by

the data of Pagliassotti et al. (30). This would cause NHGO to

underestimate total hepatic glucose release by ~0.3

mg·kg⁻¹·min⁻¹.

Total glucose production (R_a) and utilization (R_d) were
determined using both one- and two-compartment models, as
previously described (5, 6). The results were similar regard-
less of which approach was employed because the deviations
from steady state were minimal. The R_a and R_d data shown in
Fig. 3 and Table 2, respectively, are those calculated with the

two-compartment method. It should also be noted, because
the kidneys produce a small amount of glucose, that the rate
of endogenous glucose production determined by the tracer
method slightly (0.3 mg·kg⁻¹·min⁻¹) overestimates total

hepatic glucose release (27). This overestimate, however,
should have been equal in the two groups and would not have
been expected to change during the test period in either

group. Gluconeogenic efficiency was assessed using a double-

isotope technique described elsewhere (5, 6). Because the

conversion of [¹⁴C]alanine to [¹³C]glucose by the kidney is

minimal (28), [¹³C]glucose production in our study was almost

exclusively attributable to the liver. Maximal and minimal

rates of gluconeogenesis from circulating gluconeogenic pre-
cursors were calculated using the methods described previ-
ously (5, 6). Once the maximal and minimal gluconeogenic
rates were obtained, hepatic glycolysis was estimated by

subtracting either the maximal or minimal gluconeogenic
rate from either NHGO or total endogenous glucose produc-

tion. Because a recent study in the dog (14) showed that the

maximal gluconeogenic rate is closer to the gluconeogenic
rate determined using the biopsy and high-performance

liquid chromatography method of Giacchi and Rossetti (13),
hepatic glycolysis presented (see Fig. 4) was calculated by

subtracting the maximal gluconeogenic rate from NHGO

or tracer-determined glucose production.

Statistical analysis. All statistical comparisons were made
using repeated-measures analysis of variance with post hoc
analysis by univariate F tests or the paired Student’s t-test
where appropriate. Statistical significance was accepted at
P < 0.05. Data are expressed as means ± SE.

RESULTS

Hormone levels. The arterial and portal plasma lev-

els of insulin and glucagon remained at basal values in

both groups throughout the study (Table 1). The arte-

rial plasma levels of epinephrine and cortisol also

remained unchanged in both groups (Table 1). During

the test period the arterial plasma levels of norepineph-

rine increased slightly in both the Po-NE group [188 ±

24 to 231 ± 28 pg/ml; not significant (NS)] and the

control group [150 ± 10 to 206 ± 13 pg/ml; NS] (Fig. 2).

The portal vein and hepatic sinusoidal plasma levels of

norepinephrine increased from 126 ± 16 to 4,951 ± 666

pg/ml (P < 0.05) and from 139 ± 16 to 4,154 ± 559

pg/ml (P < 0.05), respectively, in the Po-NE group, but

did not change (108 ± 8 to 102 ± 7 pg/ml and 121 ± 11

to 123 ± 8 pg/ml, respectively) in the control group

(Fig. 2).

Hepatic blood flow, arterial blood pressure, and heart
rate. Hepatic blood flow remained stable in both groups.
The systolic, diastolic, and mean arterial blood pres-

sures, as well as heart rate, did not change significantly

in either group.

Glucose levels and kinetics. During portal norepineph-

rine infusion, the arterial blood glucose level gradually

rose from 79 ± 5 to 93 ± 7 mg/dl (P < 0.05) by 60 min

and remained constant thereafter (Fig. 3). The arterial

glucose level in the control group (76 ± 3 to 94 ± 4

mg/dl by 60 min, P < 0.05) was clamped to that seen in

the Po-NE group. In response to the portal infusion of

norepinephrine, NHGO increased from 1.9 ± 0.2 to

3.5 ± 0.4 mg·kg⁻¹·min⁻¹ by 15 min (P < 0.05) and then

gradually fell back to 2.4 ± 0.4 mg·kg⁻¹·min⁻¹ by the

end of the study (Fig. 3). In the presence of the hyper-
glycemic clamp alone (control group), NHGO did not

change initially, but gradually fell to 1.1 ± 0.2

mg·kg⁻¹·min⁻¹ by the end of the study (P < 0.05; Fig.

3). Because the fall in NHGO from 15 min on was

parallel in the two groups, the effect of norepinephrine

on net hepatic glucose production (the difference be-

 tween the Po-NE and control groups) was sustained

over time [Δ1.4 and Δ1.3 mg·kg⁻¹·min⁻¹ initially (15–

30 min) and during the last 30 min of the test period,

respectively; Fig. 3]. The changes in tracer-determined

endogenous glucose production paralleled those in

NHGO in both groups and also indicated that the effect

of norepinephrine did not wane with time (Δ1.1 and

Δ1.1 mg·kg⁻¹·min⁻¹ initially and during the last 30

min of the test period, respectively; Fig. 3). Tracer-
determined glucose utilization remained essentially

unchanged in each group (Table 2). Glucose clearance

fell from 2.2 ± 0.2 to 1.8 ± 0.2 ml·kg⁻¹·min⁻¹ (P < 0.05)

and from 2.4 ± 0.2 to 1.9 ± 0.1 ml·kg⁻¹·min⁻¹ (P <

0.05) in the Po-NE and control groups, respectively

(Table 2).

Arterial blood level, net hepatic uptake, and frac-
tional extraction of alanine. The arterial level, net

hepatic uptake, and fractional extraction of alanine did

not change significantly in both groups throughout (Table 3).

Arterial blood level and net hepatic balance of lactate.
The arterial blood levels and net hepatic uptake of

lactate did not change significantly in either group

(Table 3).

Arterial blood level, net hepatic uptake, and frac-
tional extraction of glycerol, FFA, and BOHB. Neither

the blood level nor the net uptake or fractional extrac-

tion of glycerol by the liver changed in either group.
Table 1. Arterial and portal plasma insulin and glucagon and arterial epinephrine and cortisol during basal and test periods of groups in which norepinephrine or saline + glucose were given in the presence of a pancreatic clamp in conscious 18-h-fasted dogs

<table>
<thead>
<tr>
<th></th>
<th>Basal Period, min</th>
<th>Test Period, min</th>
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<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td><strong>Insulin, U/ml</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Arterial</td>
<td>9±1</td>
<td>10±2</td>
</tr>
<tr>
<td>Control Portal</td>
<td>22±4</td>
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</tr>
<tr>
<td>Po-NE Arterial</td>
<td>8±2</td>
<td>9±2</td>
</tr>
<tr>
<td>Po-NE Portal</td>
<td>28±6</td>
<td>27±7</td>
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<tr>
<td><strong>Glucagon, pg/ml</strong></td>
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</tr>
<tr>
<td>Control Arterial</td>
<td>47±7</td>
<td>43±9</td>
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<tr>
<td>Control Portal</td>
<td>62±5</td>
<td>61±5</td>
</tr>
<tr>
<td>Po-NE Arterial</td>
<td>42±4</td>
<td>41±5</td>
</tr>
<tr>
<td>Po-NE Portal</td>
<td>61±4</td>
<td>65±5</td>
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<tr>
<td><strong>Epinephrine, pg/ml</strong></td>
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<tr>
<td>Control Arterial</td>
<td>52±18</td>
<td>55±15</td>
</tr>
<tr>
<td>Control Portal</td>
<td>56±14</td>
<td>54±20</td>
</tr>
<tr>
<td>Po-NE Arterial</td>
<td>0.7±0.2</td>
<td>0.8±0.3*</td>
</tr>
<tr>
<td>Po-NE Portal</td>
<td>1.0±0.1</td>
<td>1.6±0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Groups were given portal norepinephrine (Po-NE) or saline and glucose (Control). *P < 0.05 difference between 2 groups.

(Table 3). Similarly, there were no changes in FFA or BOHB metabolism (Table 3).

Arterial blood levels and net hepatic balances of gluconeogenic amino acids. The arterial blood glucose levels remained essentially unchanged in the Po-NE and control groups (Table 4). Net hepatic glucose balance, on the other hand, switched from output to uptake in both groups (0.43 ± 0.67 to −0.75 ± 0.41 μmol·kg⁻¹·min⁻¹ in Po-NE and from 0.71 ± 0.74 to −0.51 ± 1.15 μmol·kg⁻¹·min⁻¹ in control), but there was no effect of portal norepinephrine infusion (Table 4). The arterial blood levels and net hepatic balances of the other gluconeogenic amino acids were not significantly altered by saline or norepinephrine infusion (Table 4). In the control group the net fractional extraction of the gluconeogenic amino acids by the liver rose slightly, but not significantly, by the end of the study (Table 4). In the Po-NE group the changes were slightly greater than those in the control group so that they achieved significance relative to the control period but not to the saline group (Table 4). Taken together, the amino acid data indicate that the intraportal infusion of norepinephrine had no detectable effect on gluconeogenic amino acid metabolism.

Gluconeogenic parameters. Hepatic gluconeogenic efficiency (24 ± 8 to 28 ± 7% and 24 ± 9 to 28 ± 10%), as well as the maximal (0.6 ± 0.2 to 0.6 ± 0.2 mg·kg⁻¹·min⁻¹ and 0.5 ± 0.2 to 0.6 ± 0.2 mg·kg⁻¹·min⁻¹) and minimal (0.1 ± 0.0 to 0.2 ± 0.1 mg·kg⁻¹·min⁻¹ and 0.1 ± 0.0 to 0.2 ± 0.1 mg·kg⁻¹·min⁻¹) gluconeogenic rates, remained unchanged in the Po-NE and control groups, respectively (Fig. 4).

Hepatic glycogenolytic rate. In response to norepinephrine infusion, hepatic glycogenolysis, calculated using the a-v difference data (Fig. 5), increased by 1.5 ± 0.5 mg·kg⁻¹·min⁻¹ (P < 0.05) within 15 min. In the control group hepatic glycogenolysis gradually fell, reaching 1.2 ± 0.4 mg·kg⁻¹·min⁻¹ (P < 0.05) by the end of the study (Fig. 5). The effect of norepinephrine on hepatic glycogenolysis (the difference between the glycogenolytic rate in the two groups) was, therefore, sustained over time (Δ1.5 and Δ1.3 mg·kg⁻¹·min⁻¹ initially and during the last 30 min of the test period, respectively; Fig. 5). When glycogenolysis was estimated using the tracer data, the pattern and magnitude of change were similar to those seen when the a-v difference data were used (Δ1.1 and Δ1.1 mg·kg⁻¹·min⁻¹ initially and during the last 30 min of the test period, respectively; Fig. 5).

**DISCUSSION**

The aim of the present study was to determine the effects of a selective increase in hepatic sinusoidal norepinephrine on hepatic glucose production. Because the arterial and portal levels of insulin and glucagon were clamped at basal values in both groups, the epinephrine and cortisol levels were unchanged in both groups, and the changes in the arterial glucose concentrations were matched in both groups, we were able to assess the effects of a selective increase in hepatic sinusoidal norepinephrine (from 139 ± 16 to 4,154 ± 559 pg/ml) on hepatic glucose metabolism. Given that the norepinephrine level was selectively increased within the hepatic sinusoids, we were able to separate the direct effects of the catecholamine on hepatic glucose production from the indirect effects that come about by virtue of its ability to increase the flow of gluconeogenic precursors and FFA from muscle and adipose tissue to the liver.
In response to the rise in hepatic sinusoidal norepinephrine, hepatic glucose production increased from $1.9 \pm 0.2$ to $3.5 \pm 0.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ by 15 min ($P < 0.05$) and then gradually fell over time. The efficient clearance of norepinephrine by the liver (>98%) prevented a significant increase in the arterial norepinephrine level ($188 \pm 24$ to $231 \pm 28 \text{ pg/ml}$). This in turn explains the absence of any lipolytic or glycogenolytic effects of norepinephrine on adipose tissue or muscle, respectively. It should be pointed out that portal infusion of norepinephrine may have increased the net hepatic fractional extraction of alanine and several other gluconeogenic amino acids slightly by the end of the study (even though the change did not reach significance). Because the arterial blood levels of those amino acids fell, any effect of this increase in fractional extraction on gluconeogenesis was offset by the decreasing plasma amino acid levels. Neither gluconeogenic efficiency nor the estimated gluconeogenic rate (maximal or minimal) changed during the 3 h of norepinephrine infusion. It seems unlikely that higher levels of norepinephrine would produce a direct gluconeogenic effect on the liver. In a recent study (6), we simultaneously infused both norepinephrine and epinephrine intraportally at very high rates for 90 min in the presence of pancreatic clamp, and even together they failed to have a direct gluconeogenic effect on the liver. Whether a longer elevation (in excess of 3 h) in the hepatic sinusoidal norepinephrine concentration would produce a meaningful gluconeogenic effect on the liver remains to be determined. Taken together, the above data suggest that the increase in glucose production caused by the selective release of norepinephrine from sympathetic nerve terminals within the liver would be solely attributable to an increase in hepatic glycogenolysis.

Fig. 2. Arterial, portal, and hepatic sinusoidal plasma levels of norepinephrine during basal and test periods of groups in which norepinephrine (Po-NE) or saline + glucose (Con) were given in the presence of a pancreatic clamp in conscious 18-h-fasted dogs. Values are means ± SE. $P < 0.05$ vs. corresponding basal period.

Fig. 3. Net hepatic glucose output, tracer-determined endogenous glucose production (TDEGP), and arterial blood glucose during basal and test periods of groups in which norepinephrine (Po-NE) or saline + glucose (Con) were given in the presence of a pancreatic clamp in conscious 18-h-fasted dogs. Values are means ± SE. $P < 0.05$ vs. corresponding basal period.
Table 2. Tracer-determined glucose utilization and clearance during basal and test periods of groups in which norepinephrine or saline + glucose were given in the presence of a pancreatic clamp in conscious 18-h-fasted dogs

<table>
<thead>
<tr>
<th>Glucose utilization, mg kg⁻¹·min⁻¹</th>
<th>Basal Period, min</th>
<th>Test Period, min</th>
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<tr>
<td></td>
<td>−40 0</td>
<td>15 30 60 90 120 150 180</td>
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<tr>
<td>Control</td>
<td>2.5 ± 0.2 2.4 ± 0.2</td>
<td>2.6 ± 0.2 2.8 ± 0.1</td>
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<tr>
<td>Po-NE</td>
<td>2.6 ± 0.2 2.2 ± 0.1</td>
<td>2.5 ± 0.2 2.3 ± 0.2</td>
</tr>
<tr>
<td>Clearance, ml·kg⁻¹·min⁻¹</td>
<td>Control</td>
<td>2.4 ± 0.2 2.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Po-NE</td>
<td>2.4 ± 0.2 2.1 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. corresponding basal period.

Because our data do not support a direct gluconeogenic action of norepinephrine on the liver, the question arises as to whether a direct gluconeogenic effect of the catecholamine is seen in the perfused liver of the 24-h-fasted (glycogen-depleted) rat (34). The reason that the catecholamine can stimulate gluconeogenesis directly at the liver in vitro but not in vivo is unclear, but several possibilities must be considered. First, in the present study all of the basal neural and hormonal signals impacting on the liver were present, whereas in the in vitro studies they were absent. Second, the distribution of adrenergic receptor subtypes in rat liver is different from that in dog liver (2, 12, 25, 36). Additionally, a 24-h-fasted rat liver is devoid of glycogen, whereas the liver of the overnight-fasted dog is not (5, 6, 34, 37, 38). Because the gluconeogenic effect of the catecholamine is seen in the perfused fasted rat liver but not in the perfused fed rat liver (1, 2, 31, 34), it is possible that the glycogenolytic effect of norepinephrine may have a suppressive effect on its gluconeogenic action. Finally, to see the gluconeogenic effect of norepinephrine in the perfused rat liver, very high levels (5–10 mM) of gluconeogenic precursors (i.e., lactate, alanine, glycerol) had to be included in the perfusate (34). Because in the present study norepinephrine was administered intraportally, the load of gluconeogenic precursors reaching the liver remained at basal values. This may mean that a direct effect of the catecholamine on gluconeogenesis within the liver can only be manifest if there is an elevated load of gluconeogenic precursors reaching the liver. Each or all of the above suggestions could explain why there was no increase in gluconeogenesis during portal norepinephrine infusion in the current study, whereas such has been reported to occur in vitro. Whether a direct gluconeogenic effect of norepinephrine on the liver would become apparent in the presence of an increase in the load of gluconeogenic precursors or FFA reaching the liver remains to be determined.

Previous studies in the human (32, 35) and the dog (8, 9) showed that norepinephrine given via a limb vein increased hepatic glucose production mainly by stimulating gluconeogenesis. Because norepinephrine was delivered peripherally in those studies, it dramatically increased the supply of alanine, lactate, and glycerol.

Table 3. Arterial blood or plasma levels as well as net hepatic balance and fractional extraction of alanine, lactate, glycerol, FFA, and BOHB during basal and test periods of groups in which norepinephrine or saline + glucose were given in the presence of a pancreatic clamp in conscious 18-h-fasted dogs

<table>
<thead>
<tr>
<th>Blood or Plasma Level, µmol/l</th>
<th>Net Hepatic Balance, µmol·kg⁻¹·min⁻¹</th>
<th>Net Hepatic Fractional Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal period</td>
<td>Test period</td>
</tr>
<tr>
<td></td>
<td>−40-0 min 0-180 min</td>
<td></td>
</tr>
<tr>
<td>Alanine Control</td>
<td>443 ± 75 474 ± 97</td>
<td>−2.2 ± 0.7 −3.1 ± 0.8</td>
</tr>
<tr>
<td>Po-NE</td>
<td>346 ± 56 334 ± 53</td>
<td>−2.8 ± 0.7 −3.6 ± 0.6</td>
</tr>
<tr>
<td>Lactate Control</td>
<td>825 ± 180 801 ± 147</td>
<td>8.9 ± 3.8 8.8 ± 3.2</td>
</tr>
<tr>
<td>Po-NE</td>
<td>634 ± 116 691 ± 106</td>
<td>8.9 ± 3.8 8.8 ± 3.2</td>
</tr>
<tr>
<td>Glycerol Control</td>
<td>79 ± 14 72 ± 15</td>
<td>−1.4 ± 0.4 −1.3 ± 0.5</td>
</tr>
<tr>
<td>Po-NE</td>
<td>65 ± 12 75 ± 10</td>
<td>−1.0 ± 0.3 −1.2 ± 0.2</td>
</tr>
<tr>
<td>Plasma FFA Control</td>
<td>833 ± 192 717 ± 101</td>
<td>−2.4 ± 0.7 −2.8 ± 0.8</td>
</tr>
<tr>
<td>Po-NE</td>
<td>679 ± 125 712 ± 113</td>
<td>−2.7 ± 0.7 −2.8 ± 0.7</td>
</tr>
<tr>
<td>BOHB Control</td>
<td>19 ± 6 17 ± 4</td>
<td>0.8 ± 0.2 0.7 ± 0.2</td>
</tr>
<tr>
<td>Po-NE</td>
<td>19 ± 4 20 ± 4</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

Values (means ± SE) are calculated according to samples taken at −40, 0, 15, 30, 60, 90, 120, 150, and 180 min during basal and test periods. Negative or positive numbers mean net hepatic uptake or output, respectively. FFA, free fatty acid; BOHB, β-hydroxybutyrate.
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Table 4. Arterial blood levels, net hepatic balances, and fractional extraction of glutamate, glutamine, glycine, serine, and threonine during basal period and the last hour of test periods of groups in which norepinephrine or saline + glucose were given in the presence of a pancreatic clamp in conscious 18-h-fasted dogs

<table>
<thead>
<tr>
<th></th>
<th>Arterial Blood Level, µmol/l</th>
<th>Net Hepatic Balance, µmol·kg⁻¹·min⁻¹</th>
<th>Net Hepatic Fractional Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal period</td>
<td>Test period</td>
<td>Basal period</td>
</tr>
<tr>
<td>Glutamate</td>
<td>40-0 min</td>
<td>120-180 min</td>
<td>40-0 min</td>
</tr>
<tr>
<td>Control</td>
<td>71 ± 4</td>
<td>60 ± 5</td>
<td>0.01 ± 0.07</td>
</tr>
<tr>
<td>Po-Ne</td>
<td>80 ± 5</td>
<td>72 ± 4</td>
<td>0.00 ± 0.09</td>
</tr>
<tr>
<td>Glutamine</td>
<td>737 ± 63</td>
<td>696 ± 67</td>
<td>0.71 ± 0.74</td>
</tr>
<tr>
<td>Po-Ne</td>
<td>735 ± 126</td>
<td>672 ± 125</td>
<td>0.43 ± 0.67</td>
</tr>
<tr>
<td>Glycine</td>
<td>250 ± 39</td>
<td>207 ± 47</td>
<td>-0.91 ± 0.23</td>
</tr>
<tr>
<td>Po-Ne</td>
<td>254 ± 33</td>
<td>187 ± 23*</td>
<td>-1.32 ± 0.21</td>
</tr>
<tr>
<td>Serine</td>
<td>157 ± 25</td>
<td>132 ± 24</td>
<td>-0.82 ± 0.21</td>
</tr>
<tr>
<td>Po-Ne</td>
<td>138 ± 20</td>
<td>112 ± 15</td>
<td>-0.65 ± 0.16</td>
</tr>
<tr>
<td>Threonine</td>
<td>210 ± 33</td>
<td>191 ± 36</td>
<td>-0.31 ± 0.14</td>
</tr>
<tr>
<td>Po-Ne</td>
<td>214 ± 19</td>
<td>201 ± 24</td>
<td>-0.24 ± 0.12</td>
</tr>
</tbody>
</table>

Values (means ± SE) are calculated according to samples take at -40, 0, 30, 60, 120, 150, and 180 min during basal and test periods. Negative or positive numbers mean net hepatic uptake or output, respectively. *P < 0.05 vs. corresponding basal period.

reaching the liver as a result of its glycogenolytic and lipolytic effects on muscle and adipose tissue, respectively. The data of Connolly et al. (8) suggested, in fact, that hepatic glycogenolysis was reduced by norepinephrine when its glycogenolytic functions were manifest. In our recent study (5) we showed that the glycogenolytic effect of epinephrine on the liver was significantly suppressed when its effect on glycogenolytic precursor supply was present. This suggests that the glycogenolytic suppression observed by Connolly et al. was due to a suppressive effect resulting from the peripheral actions of norepinephrine to increase the supply of glycogenolytic precursors and FFA reaching the liver. Indeed, in the present study, in the absence of its peripheral effects norepinephrine had a significant glycogenolytic effect on the liver.

The infusion rate of norepinephrine used in the current study elevated hepatic sinusoidal levels of the catecholamine to 4,154 ± 559 pg/ml. This level was chosen in an attempt to mimic synaptic cleft norepinephrine levels seen during moderate stress (i.e., mild hypoglycemia or heavy exercise). Estimates of norepinephrine levels occurring within synapses have been made (21) by blocking reuptake and degradation of norepinephrine at the same time as measuring the plasma level of norepinephrine required to cause a 20-mmHg pressor response. That work suggested that plasma norepinephrine levels of 3,500–4,000 pg/ml must be achieved to elevate synaptic cleft norepinephrine levels to those present during hypotensive stress. One should bear in mind, however, that in response to more extreme stress (i.e., deep hypoglycemia, hemorrhagic shock) the norepinephrine levels at nerve terminals within the liver could reach levels in excess of 10,000 pg/ml (35). Furthermore, a recent study in the human (17) showed that norepinephrine can reach levels as much as 7,000 pg/ml in plasma during exhaustive exercise, meaning that even higher levels must exist at nerve terminals within the liver. Clearly, the norepinephrine levels used in the present study are consistent with those that must occur around hepatocytes during certain stressful situations.

In the present study, a 30-fold elevation in the hepatic sinusoidal plasma norepinephrine increased NHGO (the difference between the two groups) by 1.6 mg·kg⁻¹·min⁻¹ within 15 min. This is similar to the increment caused by a 10-fold increase in hepatic sinusoidal plasma epinephrine (1.9 mg·kg⁻¹·min⁻¹) (5). Because the basal norepinephrine level is approximately threefold that of the basal epinephrine level, the potency of epinephrine is, on a molar basis, about 3.3 times as potent as that of norepinephrine. Because the direct effects of the two catecholamines on the liver both reflect alterations that cause glycogenolysis, one has to explain their different potencies by some other mechanism. One possible explanation is that binding affinities of the two hormones for the adrenergic receptor are different. Another possibility is that the two catecholamines stimulate hepatic glycogenolysis via different adrenergic receptors. It has been shown in vitro that epinephrine (10, 23) and norepinephrine (10, 12) stimulate hepatic glucose production primarily through β₂-adrenergic receptors, which increase the level of cAMP, and through β₁-adrenergic receptors, which increase the level of Ca²⁺ in the cytosol, respectively. Early in vitro studies (7, 12, 29) showed that the stimulation of hepatic glucose production by norepinephrine, acting through β₁-adrenergic receptors, is smaller in magnitude than that caused by equimolar epinephrine acting through β₂-adrenergic receptors. An in vitro study using dog hepatocytes (36) showed that the effect of epinephrine on glucose output was inhibited by 77 and 27% in the presence of propranolol and phentolamine, respectively. These data suggest that, in the dog, epinephrine...
acts primarily via β-adrenergic receptors. Because the major type of adrenergic receptor in dog liver is β2 (23, 25), and because β2-receptors are much less sensitive to norepinephrine than to epinephrine (7, 10, 16), the effect of norepinephrine on hepatic glucose production in the current study is probably mediated through α-adrenergic receptors.

In the present study the effect of norepinephrine on hepatic glucose production, as indicated by the difference between the changes in tracer-determined glucose production in the two groups, remained essentially unchanged over time whether the tracer or a-v difference data are examined (Fig. 3). It should be noted that the use of the tracer method to estimate the magnitude of the norepinephrine effect results in a slight overestimate of hepatic glucose release because glucose produced by the kidney is included (see MATERIALS AND METHODS). The error was probably equal in the two groups and is unlikely to have changed over time, so that it would have no impact on the calculated effect of norepinephrine (i.e., the difference between glucose production in the two groups). The use of a-v difference data would, on the other hand, lead to a small underestimate of hepatic glucose release in both groups, but the error would be slightly greater (=0.2 to 0.3 mg·kg⁻¹·min⁻¹; see MATERIALS AND METHODS) in the control group because of the effect of hyperglycemia per se on hepatic glucose uptake. This would exaggerate the difference between the two groups and would explain why there was a slightly greater effect of norepinephrine on hepatic glucose production when the latter was calculated using a-v data. The sustained effect (Figs. 3 and 5) of norepinephrine on hepatic glucose production (glycogenolysis) is in contrast to the waning effect of epinephrine or glucagon reported in earlier publications (6, 26, 38, 39). It has been shown in vitro (4, 10, 24) that the effects of the latter two hormones on hepatic glucose production are transient, reflecting a spike decline pattern of intracellular cAMP production. An early study in the dog (3) showed that a selective fourfold increase in glucagon (insulin was kept constant at basal) increased tracer-determined hepatic glucose production by 4.8 mg·kg⁻¹·min⁻¹ within 30 min but that
the magnitude of the increase fell to 2.8 mg·kg⁻¹·min⁻¹ by 3 h. Similarly, in a recent study (5) we showed that a selective 10-fold increase in liver sinusoidal epinephrine (i.e., insulin and glucagon were kept constant and basal) increased tracer-determined hepatic glucose production by 1.9 mg·kg⁻¹·min⁻¹ within 30 min but that the effect fell to 0.8 mg·kg⁻¹·min⁻¹ by 3 h. The time dependence of glucagon’s and epinephrine’s action was also clearly evident when α-β difference data were examined. The persistent effect of norepinephrine on hepatic glucose production and glycogenolysis in the present study could reflect the fact that the initial rise in glucose production caused by norepinephrine was somewhat less than that seen with epinephrine and glucagon. Alternatively, it could be taken to further support the concept that the action of norepinephrine on the liver is mediated by a different intracellular mechanism than epinephrine or glucagon.

In conclusion, the direct effect of norepinephrine on hepatic glucose production is attributable to a stimulation of glycogenolysis. The catecholamine has little, if any, direct gluconeogenic effect on the liver in the absence of its ability to increase the gluconeogenic precursor load to the liver. Compared with the previously determined effects of epinephrine or glucagon on hepatic glucose production, the effect of norepinephrine is less potent but more sustained.

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