Pancreatic Noradrenergic Nerves Are Activated by Neuroglucopenia But Not by Hypotension or Hypoxia in the Dog

Evidence for Stress-specific and Regionally Selective Activation of the Sympathetic Nervous System

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Abstract

To determine if acute stress activates pancreatic noradrenergic nerves, pancreatic norepinephrine (NE) output (spillover) was measured in halothane-anesthetized dogs. Central neuroglucopenia, induced by intravenous 2-deoxy-D-glucose ([2-DG] 600 mg/kg + 13.5 mg/kg^{-1} per min^{-1}) increased pancreatic NE output from a baseline of 380±100 to 1,490±340 pg/min (Δ = +1,110±290 pg/min, $P < 0.01$). Surgical denervation of the pancreas reduced this response by 90% (Δ = +120±50 pg/min, $P < 0.01$ vs. intact innervation), suggesting that 2-DG activated pancreatic nerves by increasing the central sympathetic outflow to the pancreas rather than by acting directly on nerves within the pancreas itself. These experiments provide the first direct evidence of stress-induced activation of pancreatic noradrenergic nerves in vivo. In contrast, neither hemorrhagic hypotension (50 mmHg) nor hypoxia (6–8% O_2) increased pancreatic NE output (Δ = +80±110 and −20±60 pg/min, respectively, $P < 0.01$ vs. neuroglucopenia) despite both producing increases of arterial plasma NE and epinephrine similar to glucopenia. The activation of pancreatic noradrenergic nerves is thus stress specific. Furthermore, because both glucopenia and hypotension increased arterial NE, yet only glucopenia activated pancreatic nerves, it is suggested that a regionally selective pattern of sympathetic activation can be elicited by acute stress, a condition in which sympathetic activation has traditionally been thought to be generalized and nondiscrete.

Introduction

The presence of noradrenergic nerves in the pancreatic islets has been well documented (1, 2). The physiological importance of this noradrenergic input has been inferred from observations that electrical stimulation of sympathetic nerves inhibits insulin secretion (3, 4) and stimulates glucagon secretion (4, 5) and that similar patterns of islet hormone secretion occur during sympathoadrenal activation induced by acute stress (6). Despite such inferential evidence, there has been no direct demonstration that pancreatic noradrenergic nerves are, in fact, activated by acute stress.

A number of methods have been developed to assess noradrenergic activity in individual organs, the most direct being the measurement of local norepinephrine (NE)[1] spillover into an organ's venous effluent. However, because significant quantities of NE arrive via the arterial circulation, accurate assessment of net organ spillover requires knowledge of the extraction of arterial NE by that organ.

We have previously developed a method to measure pancreatic NE extraction (7), and thus pancreatic NE spillover. In this study, we first sought to validate this technique by measuring pancreatic NE output (spillover) before, during, and after direct activation of pancreatic noradrenergic nerves. To produce known activation, we electrically stimulated the thoracic splanchnic nerves of halothane-anesthetized dogs. As a further validation, we repeated the stimulations in dogs whose pancreata had been surgically denervated.

Next, to determine if acute stress activates pancreatic noradrenergic nerves, we measured pancreatic NE spillover during three types of stress known to produce sympathoadrenal activation: neuroglucopenia induced by the intravenous administration of 2-deoxy-D-glucose (2-DG) (8), hypotension induced by hemorrhage, and hypoxia induced by the partial replacement of inspired oxygen with nitrogen, all in halothane-anesthetized dogs. To assess the magnitude of the systemic sympathoadrenal activation produced by these stresses, we also measured arterial NE and epinephrine (EPI) levels before and during each stress.

To determine if the activation of pancreatic noradrenergic nerves during neuroglucopenia was due to increased sympathetic outflow to the pancreas, or to a direct effect of 2-DG on nerves within the pancreas itself, 2-DG was administered to dogs with surgically denervated pancreata. 2-DG also was infused directly into the superior pancreatic artery of dogs with intact pancreatic innervation. To address further whether the activation of pancreatic noradrenergic nerves is centrally mediated, a local glucopenia of the central nervous system was produced by administration of 2-DG and another glucopenia-inducing glucose analogue, 5-thioglucose (5-TG), into the lateral cerebral ventricles of halothane-anesthetized dogs.

Finally, to verify that increases of arterial plasma NE were because of the activation of systemic sympathetic nerves, rather than the release of NE from the adrenal medulla, as has been suggested (9, 10), the glucopenia and hypotension experiments were repeated in adrenal-deprived dogs.

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1. *Abbreviations used in this paper:* BTSNS, bilateral thoracic splanchnic nerve stimulation; EPI, epinephrine; FA, femoral artery; 5-TG, 5-thioglucose; HR, heart rate; MAP, mean arterial pressure; NE, norepinephrine; SPV, superior pancreatic vein; 2-DG, 2-deoxyglucose.

Methods

Animals and surgical procedures. After an overnight fast (~ 18 h), adult dogs of mixed breed (25–40 kg) were anesthetized with the ultrashortacting barbiturate, thiamylal sodium (Surital; Parke, Davis & Co., Morris Plains, NJ). Anesthesia was subsequently maintained with halothane (0.8%) administered from a calibrated vaporizer (Draeger, FRG) by mechanical ventilation in 100% oxygen. This anesthetic regimen was chosen because it has been demonstrated not to suppress sympathoadrenal activation produced by stress (11), as do some other anesthetics, e.g., pentobarbital (12, 13).

To access the pancreatic venous blood, a laparotomy was performed and an extracorporeal Silastic (Dow Corning Corp., Midland, MI) shunt containing a sampling port and an electromagnetic flow probe (In Vitro Metric Systems, Healdsburg, CA) was placed between the superior pancreatic vein (SPV) and the portal vein (4). To ensure that the NE measured in the pancreatic venous plasma was derived solely from the pancreas, the adjacent duodenum was surgically excluded (4). The femoral artery (FA) and vein were also cannulated for blood sampling and drug infusion.

In selected dogs, the pancreas was surgically denervated by continuing the duodenal exclusion to the level of the pyloric sphincter. Next, all of the mesenteric attachments to the pancreas were ligated and cut. Finally, the neural tissue surrounding the pancreatic blood vessels was dissected free, ligated, and severed.

To determine if the rise of NE measured in arterial plasma during stress was of neuronal origin, or derived from the adrenal medulla, as has been suggested by some reports (9, 10), other dogs were adrenal deprived; they either had both adrenals surgically removed or completely isolated by ligation.

In dogs whose thoracic splanchnic nerves were electrically stimulated, bilateral thoracotomies were performed at the seventh intercostal space. The splanchnic nerves were dissected free from the surrounding tissue along the dorsal ribcage, and bipolar electrodes (Harvard Apparatus Co., South Natick, MA) were placed on each nerve. The nerves were then severed anterior to the electrodes, the thoracotomy incisions were sutured closed, and the pleural cavity was evacuated.

In dogs in which neuroglucopenia was induced by the intracerebroventricular administration of glucopenic glucose analogues, a cranial midline incision was made and the muscles overlying the skull were dissected free. These muscles were then retracted and a 5-mm hole was drilled through the skull 40 mm anterior to the occipital crest and 9 mm lateral to the midline. An 18-gauge steel cannula was then inserted into the lateral ventricle (~ 30 mm deep). At the conclusion of the experiments, a fluorescent dye, (fluorescein isothiocyanate, Sigma Chemical Co., St. Louis, MO) was infused into the cannula. The dog was then killed, the brain was removed, and the placement of the cannula in the lateral ventricle was verified by observing an extensive staining of the cerebral ventricular system with an ultraviolet light source.

In dogs in which 2-DG was administered locally into the pancreas, a 22-gauge Teflon cannula (Abbott Inc., North Chicago, IL) was inserted into the superior pancreatic artery. Adequate perfusion of the pancreatic tissues was verified at the conclusion of the experiments by infusing a dye (sodium indigotindisulfonate; Hynson, Wescott, and Dunning, Baltimore, MD) into the pancreatic arterial cannula. A stabilization period of at least 60 min followed all surgical procedures before the experiments were begun.

Protocols. Because the pancreas extracts NE from arterial plasma (7), circulating NE makes only a small contribution to the NE measured in the pancreatic venous effluent. Therefore, to accurately calculate pancreatic NE output, a measurement of pancreatic NE extraction is needed. In these experiments, tritiated NE ([^{3}H]NE; New England Nuclear, Boston, MA) was administered intravenously as a bolus of 30 μCi followed by an infusion of 1.3 μCi/min and the pancreatic extraction of [^{3}H]NE was used as an ongoing index of the extraction of endogenous arterial NE. The structure of the [^{3}H]NE was: L-Ring-2,5,6,[^{3}H]NE and the specific activity was 40 Ci/mmol.

To validate our method of assessing pancreatic noradrenergic activity by measuring pancreatic NE spillover, the thoracic splanchnic nerves were electrically stimulated (bilateral thoracic splanchnic nerve stimulation [BTSNS]) for 10 min with square-wave pulses of 1 mS duration and 10 mA current at a frequency of 8 Hz. The stimulations were performed with a stimulator coupled to a stimulus isolation unit (models S-44 and PSIU6, respectively; Grass Instrument Co., Quincy, MA). Stimulation parameters were monitored with an oscilloscope. Paired baseline blood samples were drawn from the FA and from the pancreatic vein at 10 and 0 min before, at 5 and 10 min during, and at 5, 15, and 30 min after BTSNS. To determine if our technique for severing the nerves to the pancreas produced a complete denervation, BTSNS was repeated in dogs whose pancreata had been surgically isolated as described above.

To determine the adrenal, systemic, and pancreatic responses to different stresses, three stress states were tested: central neuroglucopenia, hemorrhagic hypotension, and hypoxia.

Neuroglucopenia was induced by the intravenous infusion of 2-DG (8), (U. S. Biochemical Corp., Cleveland, OH; 600 $mg \cdot kg^{-1}$ + 13.5 $mg \cdot kg^{-1} \cdot min^{-1}$). The long plasma half-life of 2-DG prevented the reversal of the glucopenic stress during the time course of the experiment. To determine if an increase of pancreatic NE output produced by 2-DG was the result of extrinsic activation of pancreatic noradrenergic nerves or to a local effect of glucopenia directly on nerves within the pancreas itself, 2-DG was also administered to dogs with surgically denervated pancreata.

Hemorrhagic hypotension was induced by rapidly removing blood (~ 20 ml/kg) from the FA until the mean arterial pressure (MAP) was 50 mmHg. MAP was maintained at 50 mmHg by further removal or reinfusion of blood. Hemorrhagic hypotension was reversed by rapidly reinfusing all of the blood removed during the hypotensive period.

Hypoxia was induced by reducing the inspired oxygen concentration to 6–8% of the inspired volume by replacing the oxygen with nitrogen. Hypoxia was reversed by reinstating the oxygen at 100% of the inspired volume.

To determine if central glucopenia induced by the intracerebroventricular injection of glucopenic glucose analogues activates pancreatic noradrenergic nerves, 2,000 mg 2-DG and 200 mg 5-TG, (U. S. Biochemical) were injected into the lateral ventricle in a volume of 6 ml of mock cerebrospinal fluid at a rate of 0.4 ml/min over 15 min.

Blood samples were drawn from the FA and from the pancreatic vein 15, 10, 5, and 0 min before, at 5, 15, 30, 35, 40, and 45 min after the onset of stress and 5, 15, and 30 min after the reversal of hypotension or hypoxia.

To determine if 2-DG directly activated pancreatic noradrenergic nerves, 20–100 mg/min 2-DG was infused into the superior pancreatic artery in saline at 0.9 ml/min for 30 min. Blood samples were drawn from the FA and from the pancreatic vein 10 min before and at 5, 10, 20, and 30 min during the pancreatic arterial 2-DG infusion.

In all experiments, blood flow in the pancreatic venous shunt was monitored with an electromagnetic flowmeter (Zepeda Instruments, Seattle, WA). Hematocrit was determined at regular intervals throughout the experiments.

Assays and data analysis. Blood samples for catecholamine determinations were drawn into tubes containing EGTA and glutathione. Samples were placed on ice until centrifugation (20 min at 4°C). The plasma was then decanted and frozen at −20°C until assayed. Plasma NE and EPI were measured in duplicate with a highly sensitive and specific radioenzymatic assay (14). The intra- and interassay coefficients of variation for the plasma catecholamine assay in this laboratory are 6 and 12%, respectively. The concentration of plasma [^{3}H]NE was determined by liquid scintillation counting of radiolabeled NE after alumina extraction to separate NE from its metabolites (15).

The fractional extraction of [^{3}H]NE by the pancreas was calculated as: $[^3H]NE_{FA} - [^3H]NE_{SPV}/[^3H]NE_{FA}$. The contribution of arterial NE to that in pancreatic venous plasma was calculated as: $[NE]_{FA} \times (1$

– fractional extraction of [^{3}H]NE). Pancreatic NE output was calculated by the formula: {[NE]$_{SPV}$ – (arterial contribution)} × (SPV blood flow) × (1 –hematocrit).

The changes in arterial NE and EPI and of pancreatic NE output during BTSNS were calculated by subtracting the mean of the two baseline values from the mean of the 5- and 10-min values. The changes of arterial NE and EPI and of pancreatic NE output during stress were calculated by subtracting the mean of the four baseline values from the mean of the 30-, 35-, 40-, and 45-min values during the stresses. The changes of pancreatic NE output during the pancreatic arterial infusion of 2-DG were calculated by subtracting the mean of the baseline sample from the mean of the 20- and 30-min values during the infusion.

The data are expressed as mean±SE. Statistical comparisons of means within a group were made with a paired *t* test. Statistical comparisons of means of different groups were made with a two-sample *t* test. For multiple comparisons between more than two groups, analysis of variance was performed, followed by Dunnett's test.

Results

Responses to BTSNS. In dogs with intact pancreata, BTSNS increased arterial plasma NE levels from a baseline of 200±20 to 790±130 pg/ml (Δ = +600±130 pg/ml, $P < 0.0025$). Levels of NE in the pancreatic vein also increased from a baseline of 90±10 to 880±130 pg/ml (Δ = +800±130 pg/ml, $P < 0.0005$). The pancreatic extraction of [^{3}H]NE was 77±2% before, and 76±3% during BTSNS. These values, together with pancreatic venous blood flow and hematocrit, are given in Table I. Using these data, pancreatic NE output was calculated. NE output increased from a baseline of 160±20 to 2,310±700 pg/min during BTSNS (Δ = +2,150±690 pg/min, $P < 0.01$, Fig. 1). Arterial EPI also increased during BTSNS; from a baseline of 290±40 to 1,600±360 pg/ml (Δ = +1,310±400 pg/ml, $P < 0.01$, data not shown).

In dogs with surgically denervated pancreata, BTSNS increased arterial plasma NE from 290±50 to 1,110±150 pg/ml (Δ = +820±150 pg/ml, $P < 0.0005$), not significantly different than in dogs with intact pancreatic innervation. Pancreatic venous NE levels in the pancreatic-denervated dogs increased less than in the intact dogs; rising from 100±10 to 430±110 pg/ml (Δ = +330±100 pg/ml, $P < 0.01$). The pancreatic extraction of [^{3}H]NE and the hematocrit during BTSNS were similar to those in the intact dogs, however, the pancreatic venous blood flow appeared somewhat lower (Table I). In the pancreatic-denervated dogs, baseline pancreatic NE output was 90±10 pg/min and did not increase significantly in response to BTSNS (Δ = +110±70 pg/min, $P < 0.01$ vs. intact innervation, Fig. 1). Arterial EPI increased during BTSNS in the dogs with denervated pancreata from 460±90 pg/ml to 2,500±690 pg/ml (Δ = +2,040±700 pg/ml, $P < 0.025$), not significantly different from the increment in intact dogs (data not shown).

Responses to 2-DG-induced neuroglucopenia. The intravenous injection of 2-DG in dogs with intact pancreata increased arterial NE from 250±40 to 640±70 pg/ml (Δ = +380±60 pg/ml, $P < 0.0005$, Table I, see also Fig. 4). Pancreatic venous levels of NE increased to a similar degree during 2-DG (Δ = +360±100 pg/ml, Table I). Pancreatic extraction of [^{3}H]NE averaged 72±3% before and 71±3% during 2-DG. Pancreatic venous blood flow and hematocrit are presented in Table I. Thus pancreatic output of NE increased from 380±100 to 1,490±330 pg/min (Δ = +1,110±290 pg/min, $P < 0.01$) during central glucopenia in intact dogs (Table I, Fig. 2). Arterial EPI also increased in response to 2-DG (Δ = +750±170 pg/ml, $P < 0.005$, Table II, see also Fig. 5). In these animals, the MAP decreased slightly, by 15±5 mmHg and the heart rate (HR) increased by 30±17 bpm during the glucopenia.

In dogs with denervated pancreata, arterial plasma NE increased during 2-DG to a greater extent than in the intact dogs (i.e., by 770±170 pg/ml, $P < 0.005$), whereas SPV NE levels only rose by 220±40 pg/ml ($P < 0.0025$, Table I). The extraction of [^{3}H]NE, SPV blood flow, and hematocrit were similar to the corresponding values obtained in dogs with intact pancreata (Table I). Output of NE in dogs with denervated pancreata thus rose only by 120±50 pg/min during central glucopenia, a value only 10% of that in intact dogs ($P < 0.01$ vs. intact innervation, Fig. 2). Arterial EPI increased during 2-DG in dogs with denervated pancreata by an amount (740±200 pg/ml, $P < 0.01$) very similar to that in dogs with intact pancreata (data not shown).

In dogs infused with 2-DG and 5-TG in the lateral cerebral ventricles, baseline arterial plasma NE increased by 710±130 pg/ml, ($P < 0.005$), and SPV plasma NE levels increased by 590±120 pg/ml, ($P < 0.005$). Pancreatic venous blood flow and hematocrit from these dogs are shown in Table I. Thus, pancreatic NE output increased by 850±190 pg/min, ($P < 0.01$) in response to the central administration of 2-DG and 5-TG, not significantly different from the response to the intravenous administration of 2-DG (Table I).

In six dogs, 2-DG was infused locally into the superior pancreatic artery at rates calculated to exceed systemic arterial

Table I. NE Responses to Nerve Stimulation and Stress

	Δ NE_{FA}	Δ NE_{SPV}	% NE ext.	BF_{SPV}	1 – Hct	Δ Pancreatic NE output
	pg/ml			*ml/min*		*pg/min*
BTSNS (n = 8)	+600±130	+800±130	76±3	6.0±0.8	0.50±0.01	+2,150±700
BTSNS (denervated) (n = 8)	+820±150	+330±100	73±2	3.4±0.5	0.50±0.02	+110±70
Neuroglucopenia (intravenous 2-DG) (n = 6)	+380±60	+360±100	71±3	7.0±1.1	0.58±0.02	+1,110±290
Neuroglucopenia (2-DG + 5-TG, ICV) (n = 5)	+710±130	+590±120	75±3	4.3±0.2	0.52±0.01	+850±190
Neuroglucopenia (denervated) (n = 6)	+770±170	+220±40	72±1	8.7±1.8	0.60±0.02	+120±50
Hypotension (n = 6)	+420±70	+130±80	86±2	2.7±0.5	0.56±0.01	+80±110
Hypoxia (n = 5)	+590±310	+110±50	73±2	7.6±0.4	0.53±0.03	–20±60

Values are means±SE. BF, blood flow; Ext, extraction; Hct, hematocrit; ICV, intracerebroventricular.

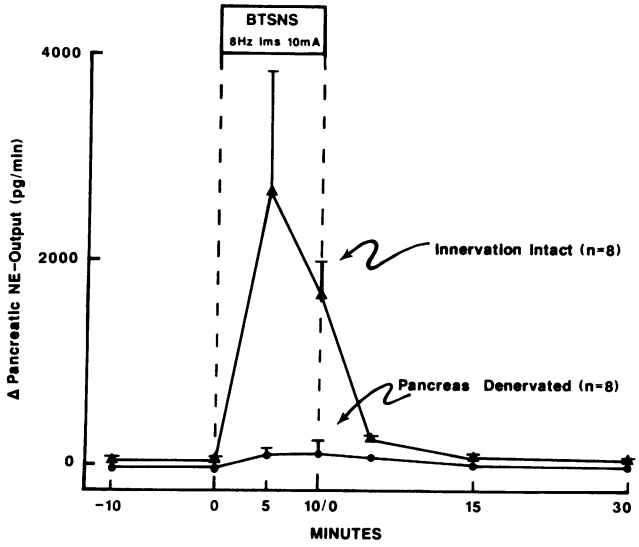

Figure 1. The change of pancreatic NE output in response to BTSNS (8 Hz, 1 mS, 10 mA) in dogs with intact pancreatic innervation and in dogs with denervated pancreata.

levels during IV 2-DG administration. This local infusion was calculated to produce pancreatic arterial plasma 2-DG levels of 1,580±490 mg/dl. Intravenous 2-DG administration only produced arterial plasma 2-DG levels of 270±20 mg/dl. Pancreatic NE output increased only marginally during the pancreatic arterial 2-DG infusion (Δ = +50±20 pg/min, $P < 0.0025$ vs. intravenous 2-DG).

In adrenal-deprived dogs, neuroglucopenia induced by intravenous 2-DG produced a larger increase of arterial plasma NE than in dogs with intact adrenals (Δ = +950±120 pg/ml, $P < 0.005$ vs. dogs with intact adrenals). Arterial EPI did not increase in response to neuroglucopenia in the adrenal-deprived animals (Δ = −10±20 pg/ml, $P < 0.005$ vs. intact adrenals, Table II).

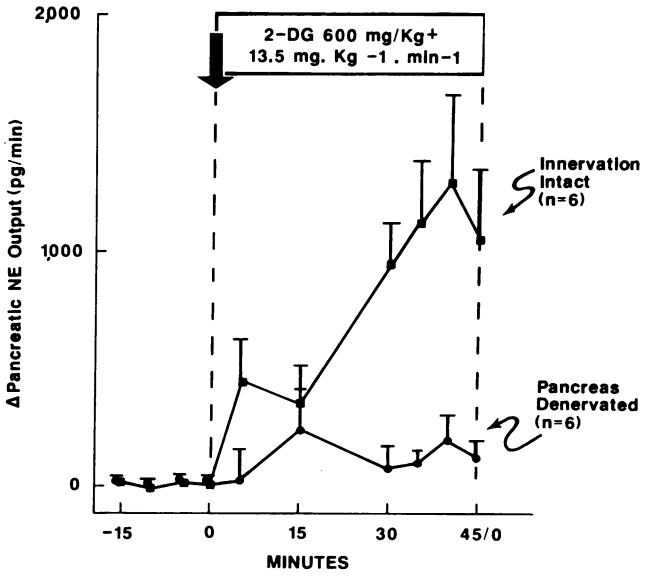

Figure 2. The change of pancreatic NE output in response to 2-DG-induced neuroglucopenia in dogs with intact pancreatic innervation and in dogs with denervated pancreata.

Responses to hemorrhagic hypotension. During hemorrhagic hypotension in dogs with intact adrenals, arterial plasma NE levels rose by 420±70 pg/ml ($P < 0.005$, Table I, see also Fig. 4) and SPV NE levels by 130±80 pg/ml (Table I). From these data together with pancreatic extraction of [^{3}H]NE, blood flow, and hematocrit (Table I), pancreatic NE spillover was calculated and found to remain unchanged (Δ = +80±110 pg/min, Fig. 3). Pancreatic vein blood flow decreased significantly during hemorrhagic hypotension (Δ = −3.0±0.5 ml/min, $P < 0.0005$), resulting in a greater degree of NE extraction by the pancreas (Table I). Arterial EPI levels increased markedly during hypotension in dogs with intact adrenals (Δ = +1,620±510 pg/ml, $P < 0.025$, see also Fig. 5, Table II). The decrease of MAP during hypotension averaged −51±3 mmHg. HR increased by 21±9 bpm.

In adrenal-deprived dogs, hypotension did not influence arterial EPI levels, but it increased arterial plasma NE by an amount similar to that in the dogs with intact adrenals (Δ = +330±110 pg/ml, $P < 0.05$, Table II).

Hemorrhagic hypotension produced a substantial decrease of plasma NE clearance (Δ = −41±2%, $P < 0.0005$). It thus is likely that increases of arterial NE observed during hemorrhagic hypotension overestimate increases of systemic noradrenergic outflow. However, the whole body NE appearance rate in both groups of hemorrhaged animals, intact and adrenal deprived, increased significantly (Δ = +210±50 ng/min, $P < 0.025$ and +220±70 ng/min, $P < 0.05$, respectively, data not shown), demonstrating the activation of systemic noradrenergic nerves. NE clearance did not decrease during either glucopenia or hypoxia. Plasma NE clearance and appearance rates were measured by the isotope dilution technique (15).

Responses to hypoxia. During hypoxia, arterial NE levels increased by 590±310 pg/ml (Fig. 4, Table I), and SPV NE levels increased by only 110±50 pg/ml (Table I). These values, together with extraction of [^{3}H]NE, SPV blood flow, and hematocrit are shown in Table I. Thus, during hypoxia, pancreatic NE spillover did not change significantly (Δ = −20±60 pg/min, Fig. 3). Arterial EPI levels increased by 1,490±810 pg/ml during hypoxia, similar to the increments that occurred during other stresses (Fig. 5). Neither MAP (Δ = +11±9 mmHg) or HR (Δ = −7±8 bpm) changed significantly during hypoxia. Hypoxia experiments were not repeated in adrenal-deprived dogs.

Discussion

The technique developed and applied in this study to measure pancreatic NE spillover is unique in that it provides a direct measurement of pancreatic noradrenergic activity in situ. An important component of this technique is the estimation of the fractional extraction of arterial NE by the pancreas. Because the pancreas extracts ~ 70% of arterial NE (7), the use of uncorrected arteriovenous differences of NE across the pancreas would markedly underestimate pancreatic noradrenergic activity. For example, during neuroglucopenia, the increase of arterial NE actually exceeds that in the pancreatic vein. Using arteriovenous differences alone, one could wrongly conclude that pancreatic noradrenergic nerves were not activated. However, the extraction of [^{3}H]NE indicates that most of the arte-

Table II. Comparison of the Adrenal Medullary and Systemic Noradrenergic Reponses to Stress in Intact and Adrenal-deprived Dogs

	Neuroglucopenia (n = 6)	Neuroglucopenia (adrenal, deprived) (n = 4)	Hemorrhagic hypotension (n = 6)	Hemorrhagic hypotension (adrenal deprived) (n = 4)
Δ Plasma EPI_{FA} (*pg/ml*)	+750±170	−10±20	+1,620±510	+40±30
Δ Plasma NE_{FA} (*pg/ml*)	+380±60	+950±120	+420±70	+330±110

Values are means±SE.

rial NE never reaches the pancreatic venous plasma, and therefore should not be subtracted from the pancreatic venous NE level when calculating NE output. Esler et al. have used similar techniques to measure organ-specific noradrenergic activity in man (16), and have recently reviewed methods for assessing regional sympathetic neural activity (17).

Despite the logical basis of the spillover calculations, we sought to experimentally validate the technique by measuring NE output from the pancreas before and during the sympathetic activation produced by BTSNS in halothane-anesthetized dogs with intact and surgically denervated pancreata. BTSNS produced a rapid and large increase of pancreatic NE output. Pancreatic NE output rapidly returned to control levels after the period of nerve stimulation. Because the pancreatic NE output response was eliminated by prior surgical denervation of the pancreas, while in both groups of dogs BTSNS produced substantial increases of both arterial NE and EPI, it seems clear that this technique provides a reliable and specific index of the activity of noradrenergic nerves innervating the pancreas.

Having validated the technique for measuring pancreatic NE output during BTSNS, we next sought to determine if pancreatic noradrenergic nerves are activated during stress. We measured pancreatic NE output and arterial plasma catecholamines before and during three types of stress known to produce sympathoadrenal activation: central neuroglucopenia induced by intravenous 2-DG (8), hypotension induced by hemorrhage, and hypoxia induced by the partial replacement of the inspired oxygen with nitrogen.

Neuroglucopenia produced a large increase of pancreatic NE output, indicating that this stress activated pancreatic noradrenergic nerves. This increase was somewhat smaller and more delayed than the response to BTSNS. However, electrical stimulation of both major nerve tracts might be expected to produce near-maximal neurotransmitter release. The pancreatic noradrenergic activation produced by neuroglucopenia was accompanied by a moderate increase of arterial plasma NE. This increase was also present in adrenal-deprived animals, suggesting that nonpancreatic noradrenergic nerves are also activated in response to neuroglucopenia. This conclusion contrasts with that of another study, in which urinary NE excretion in adrenalectomized rats did not increase during neuroglucopenia (18). A large increase of arterial EPI confirmed adrenal medullary activation by neuroglucopenia in this study. As expected, the arterial EPI response to neuroglucopenia was abolished by adrenal deprivation.

Although stress-induced activation of the noradrenergic input to the pancreas has often been postulated (19, 20), previously there has been no direct measurement of increased pancreatic noradrenergic activity during acute stress in vivo. However, other studies have indirectly suggested that stress may activate pancreatic noradrenergic nerves in vivo. Jarhult and Holst found that unloading the carotid baroreceptors (simulated hypotension) in adrenalectomized cats produced

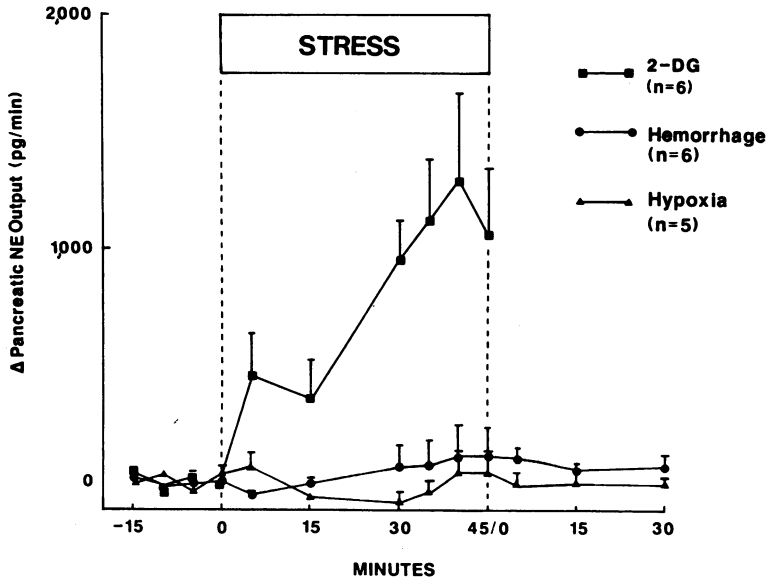

Figure 3. The change of pancreatic NE output in response to 2-DG-induced neuroglucopenia, hemorrhagic hypotension, and hypoxia.

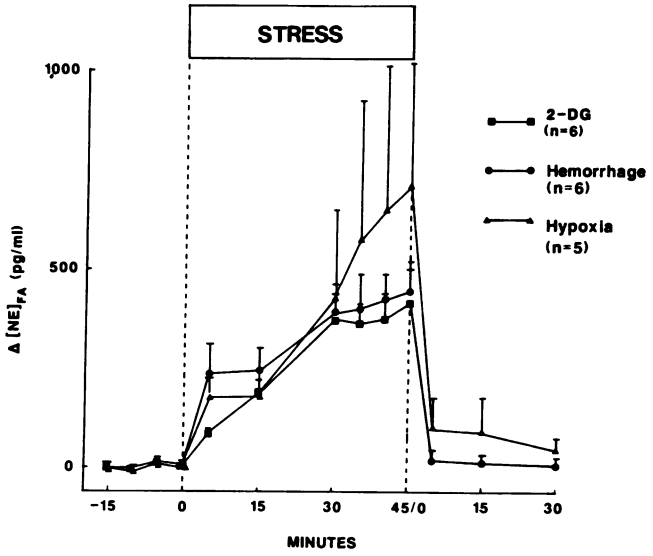

Figure 4. The change of femoral arterial NE in response to 2-DG-induced neuroglucopenia, hemorrhagic hypotension, and hypoxia.

sympathomimetic effects on pancreatic hormone secretion (21). Increased NE turnover also has been demonstrated in the pancreata of cold-exposed rats, suggesting that chronic stress of this type may increase pancreatic noradrenergic activity (22).

Stress-induced increases of pancreatic noradrenergic activity have also been indirectly suggested through in vitro experimentation. For example, the activity of intrinsic pancreatic noradrenergic nerves apparently increases with abrupt lowering of ambient glucose levels. This is inferred from reports that the enhanced glucagon release, which results from lowering perfusate glucose levels in isolated canine (23) or rat (24) pancreata, is blocked by the alpha-adrenergic antagonist, phentolamine. More direct evidence is provided by a report that perfusion of isolated canine pancreata with an extremely low-glucose media releases large amounts of NE (25). These reports suggest that severe local glucopenia may directly activate noradrenergic nerves in the isolated pancreas in vitro. In contrast, this study demonstrates that the activation of pancreatic noradrenergic nerves by glucopenia is largely eliminated by pancreatic denervation. Thus, in vivo, glucopenia appears to activate a central mechanism that in turn increases the sympathetic outflow to the pancreas. This conclusion is further supported by our demonstration that central glucopenia, induced by the cerebral ventricular administration of glucopenic agents, also increases pancreatic norepinephrine output and that the local pancreatic administration of 2-DG does not.

In contrast to the increase of pancreatic NE output observed during glucopenia, neither hypotension nor hypoxia increased pancreatic NE output. This stress-specific activation occurred despite similar degrees of surgical stress in all three experiments. Therefore, it is unlikely that the presence of surgical stress contributed to the specificity of the activation. Surgical stress may contribute to the increases of systemic catecholamines seen during all three stresses. However, the increases of both arterial NE and EPI during hypotension and hypoxia were similar to those observed during neuroglucopenia. The ability of stress to activate pancreatic noradrenergic nerves thus may depend more on the type of stress than its intensity. These findings further suggest that there may be a variety of sympathoadrenal responses that are centrally orchestrated to elicit a homeostatic response appropriate for the type of stress.

A physiologic role for this stress-specific activation of pancreatic noradrenergic nerves might be inferred from the known function of the endocrine pancreas to regulate plasma glucose levels and the known effects of sympathetic activation on pancreatic hormone release. Electrical stimulation of sympathetic nerves inhibits insulin secretion (3, 4) and stimulates glucagon secretion (4, 5). Decreases of plasma insulin levels and increased plasma glucagon levels would be expected to increase plasma glucose levels by increasing hepatic glucose production and reducing insulin-mediated glucose utilization (26). In-

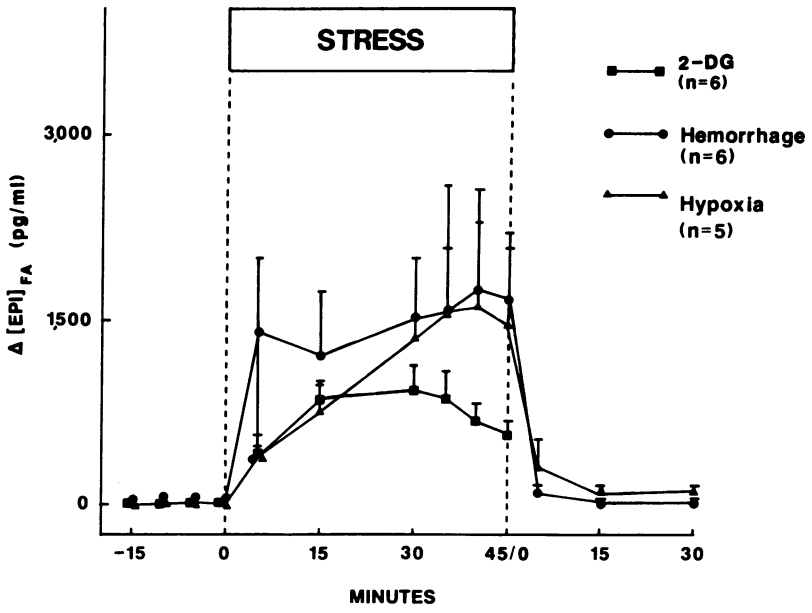

Figure 5. The change of femoral arterial EPI in response to 2-DG-induced neuroglucopenia, hemorrhagic hypotension, and hypoxia.

deed, impaired insulin secretion and increased glucagon secretion have been observed in some studies examining glucopenia (27, 28). Although it is tempting to speculate that the central activation of pancreatic noradrenergic nerves contributes to the counterregulatory response for defending plasma glucose during periods of glucopenia, two cautions are indicated. First, a role for the local pancreatic nerves in mediating these islet hormone responses remains to be experimentally demonstrated. Second, the NE output we measured was from the entire right lobe of the pancreas, and not solely from the endocrine tissue of that lobe. Therefore, these measurements do not necessarily reflect increased noradrenergic input to the islets during glucopenic stress.

In addition to the concept of stress specificity of sympathetic activation, the data from this study suggest a related concept of regionally selective activation of the sympathetic nervous system. For example, arterial NE increased during hemorrhagic hypotension, whereas pancreatic NE output did not. Because the increase of arterial NE observed during hypotension was unaffected by adrenal denervation, this increase of NE likely reflects neuronal NE release rather than the release of NE from the adrenal medulla (9, 10). Nonpancreatic noradrenergic nerves thus were activated by hypotension, despite its failure to increase pancreatic noradrenergic activity.

Our demonstration of a differential pattern in the noradrenergic response during hypotension (regional selectivity) contrasts with the more traditional view that acute stresses produce a generalized activation of the sympathetic nervous system (29). However, other studies have also questioned the traditional view. Young and Landsberg have demonstrated a dissociation between tissue NE turnover and adrenal medullary activation during several stress states (30). In addition, a differential activation of the sympathetic nerves to skin or muscle has been demonstrated during exercise or mental calculation (31, 32). This study is unique, however, in suggesting regional selectivity in the activation of the sympathetic nerves during acute homeostatically disruptive stress.

In summary, we have directly demonstrated that neuroglucopenia activates pancreatic noradrenergic nerves in vivo, whereas hemorrhagic hypotension and hypoxia do not. When viewed with the peripheral adrenergic responses, these data provide an important demonstration of stress-specific and regionally selective activation of the sympathetic nervous system in acute stress.

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References

1. Miller, R. E. 1981. Pancreatic neuroendocrinology: peripheral neural mechanisms in the regulation of the islets of langherhans. *Endocr. Rev.* 4:471–494.

2. Ahren, B., G. J. Taborsky, Jr., and D. Porte, Jr. 1986. Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. *Diabetologia.* 29:827–836.

3. Porte, D., Jr. 1973. Neural regulation of insulin secretion in the dog. *J. Clin. Invest.* 52:210–214.

4. Ahren, B., R. C. Veith, and G. J. Taborsky, Jr. 1987. Sympathetic nerve stimulation vs. pancreatic norepinephrine infusion in the dog. 1. Effects on basal insulin and glucagon release. *Endocrinology.* 121:323–331.

5. Marliss, E. B., L. Girardier, J. Seydoux, C. B. Wollheim, Y. Kanazawa, L. Orci, A. E. Renold, and D. Porte, Jr. 1973. Glucagon secretion induced by pancreatic nerve stimulation in the dog. *J. Clin. Invest.* 52:1246–1249.

6. Porte, D., Jr., and S. C. Woods. 1983. Neural regulation of islet hormones and its role in stress hyperglycemia. *In* Diabetes Mellitus Theory and Practice. Ellenburg, M. and H. Rifkin, editors. Medical Examination Publishing Co., Inc., New York. 267–294.

7. Ahren, B., B. E. Dunning, P. J. Havel, R. C. Veith, and G. J. Taborsky, Jr. 1988. Extraction of epinephrine and norepinephrine by the dog pancreas *in vivo*. *Metab. Clin. Exp.* 37:68–73.

8. Brown, J. 1962. Effects of 2-deoxyglucose on carbohydrate metabolism: review of the literature and studies in the rat. *Metab. Clin. Exp.* 11:1098–1112.

9. Millar, R. A., E. B. Keener, and B. G. Benfey. 1958. Plasma adrenaline and noradrenaline after phenoxybenzamine administration, and during hemorrhagic hypotension, in normal and adrenalectomized dogs. *Br. J. Pharmacol.* 14:9–13.

10. Khalil, Z., P. D. Marley, and B. G. Livett. 1986. Elevation in plasma catecholamines in response to insulin stress is under both neuronal and nonneuronal control. *Endocrinology.* 119:159–167.

11. Havel, P. J., D. E. Flatness, J. B. Halter, J. D. Best, R. C. Veith, and G. J. Taborsky Jr. 1987. Halothane anesthesia does not suppress sympathetic activation produced by neuroglucopenia. *Am. J. Physiol.* 252 (*Endocrinol. Metab.* 15):E667–E672.

12. Taborsky, G. J., Jr., J. B. Halter, D. Baum, J. D. Best, and D. Porte, Jr. 1984. Pentobarbital anesthesia suppresses basal and 2-deoxy-D-glucose stimulated plasma catecholamines. *Am. J. Physiol.* 247 (*Regulatory Integrative Comp. Physiol.* 16):R905–R910.

13. Zimpfer, M., W. T. Manders, A. C. Barger, and S. F. Vatner. 1982. Pentobarbital alters compensatory neural and humoral mechanisms in response to hemorrhage. *Am. J. Physiol.* 243 (*Heart Circ. Physiol.* 12):H713–H721.

14. Evans, M. I., J. B. Halter, and D. Porte, Jr. 1978. Comparison of double- and single-isotope enzymatic derivative methods for measuring catecholamines in human plasma. *Clin. Chem.* 24:567–570.

15. Best, J. D., G. J. Taborsky, Jr., D. E. Flatness, and J. B. Halter. 1984. Effect of pentobarbital anesthesia on plasma norepinephrine kinetics in the dog. *Endocrinology.* 115:853–857.

16. Esler, M., G. Jennings, P. Korner, P. Blombery, N. Sacharias, and P. Leonard. 1984. Measurement of total and organ-specific norepinephrine kinetics in humans. *Am. J. Physiol.* 247 (*Endocrinol. Metab.* 10):E21–E28.

17. Esler, M., G. Jennings, P. Korner, I. Willett, F. Dudley, G. Hasking, W. Anderson, and G. Lambert. 1988. Assessment of human sympathetic nervous system activity from measurements of norepinephrine turnover. *Hypertension (Dallas).* 11:3–20.

18. Rappaport, E. B., J. B. Young, and L. Landsberg. 1982. Effects of 2-deoxy-D-glucose on the cardiac sympathetic nerves and the adrenal medulla in the rat: further evidence for a dissociation of the sympathetic nervous system and adrenal medullary response. *Endocrinology.* 110:650–656.

19. Gerich, J. E., and M. Lorenzi. 1978. The role of the autonomic nervous system and somatostatin in the control of insulin and glucagon secretion. *In* Frontiers in Neuroendocrinology. Vol. 5. W. F. Ganong and L. Martini, editors. Raven Press, New York. 265–288.

20. Smith, P. H., S. C. Woods, and D. Porte, Jr. 1979. Control of the endocrine pancreas by the autonomic nervous system and related neural factors. *In* Integrative Functions of the Autonomic Nervous System. C. McC. Brooks, editor. Elsevier Science Publishers, Amsterdam. 84–97.

21. Jarhult, J., and J. J. Holst. 1978. Reflex adrenergic control of

the endocrine pancreas evoked by unloading of carotid baroceptors in cats. *Acta Physiol. Scand.* 104:188–202.

22. Young, J. B., and L. Landsberg. 1979. Effect of diet and cold exposure on norepinephrine turnover in pancreas and liver. *Am. J. Physiol:* 236 (*Endocrinol. Metab.* 5):E524–E533.

23. Iversen, J. 1973. Adrenergic receptors and the secretion of glucagon and insulin from the isolated, perfused canine pancreas. *J. Clin. Invest.* 52:2102–2115.

24. Hisatomi A., H. Maruyama, L. Orci, M. Vasko, and R. H. Unger. 1985. Adrenergically mediated intrapancreatic control of the glucagon response to glucopenia in the isolated rat pancreas. *J. Clin. Invest.* 75:420–426.

25. Christensen, N. J., and J. Iversen. 1973. Release of large amounts of noradrenaline from the isolated perfused canine pancreas during glucose deprivation. *Diabetologia.* 9:396–399.

26. Cherrington, A. D., J. L. Chiasson, J. E. Liljenquist, A. S. Jennings, V. Keller, and W. W. Lacy. 1976. The role of insulin and glucagon in the regulation of basal glucose production in the postabsorbtive dog. *J. Clin. Invest.* 58:1407–1418.

27. Frohman, L. A., E. E. Muller, and D. Cocchi. 1973. Central nervous system mediated inhibition of insulin secretion due to 2-deoxyglucose. *Horm. Metab. Res.* 5:21–26.

28. Frohman, L. A., and K. Nagai. 1976. Central nervous system mediated stimulation of glucagon secretion in the dog following 2-deoxyglucose. *Metab. Clin. Exp.* (Suppl. 1) 25:1449–1452.

29. Folkow, B. 1984. Plasma catecholamines as markers for sympatho-adrenal activity in man. *Acta Physiol. Scand.* (Suppl.) 527:7–9.

30. Young, J. B., R. M. Rosa, and L. Landsberg. 1984. Dissociation of the sympathetic nervous system and adrenal medullary responses. *Am. J. Physiol.* 247 (*Endocrinol. Metab.* 10):E35–E40.

31. Wallin, B. G. 1974. Regional control of sympathetic outflow in human skin and muscle nerves. *In* Central rythymic and regulation. W. Umpach and H. P. Koepchen, editors. Hippokrates-Verlag GMBH, Stuttgart. 190–195.

32. Hjemdahl P., U. Freyschuss, A. Juhlin-Dannfelt, and B. Linde. 1984. Differentiated sympathetic activation during mental stress evoked by the Stroop test. *Acta Physiol. Scand.* (Suppl.) 527:25–29.