Portal Glucose Infusion Exerts an Incretin Effect Associated With Changes in Pancreatic Neural Activity in Conscious Dogs

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We sought to determine whether an incretin effect could be observed when glucose was infused via the hepatic portal (Po) vein versus a peripheral (Pe) vein. Identical hyperglycemia (155 ± 7 and 154 ± 8 mg/dL, respectively) was produced in 2 groups (n = 9 each) of conscious dogs by Po or Pe glucose infusion. During glucose infusion, arterial plasma insulin levels increased by 28 ± 5 and 16 ± 3 μU/mL in Po and Pe, respectively (*P* < .05 between groups). Pancreatic insulin output increased by 10.4 ± 3.2 and 6.7 ± 2.3 mU/min in Po and Pe, respectively (*P* = .12 between groups). Arterial plasma glucagon levels and pancreatic glucagon output were similarly suppressed by Po and Pe glucose infusion. Pancreatic polypeptide (PP) output and norepinephrine (NE) spillover were measured as indices of pancreatic parasympathetic and sympathetic neural activity, respectively. During Pe, pancreatic PP output decreased from basal (Δ -4.8 ± 2.5 ng/min, *P* < .05), with no significant change in NE spillover (Δ +4.4 ± 4.0 ng/min). The PP output:NE spillover ratio decreased by 65% (*P* < .05), suggesting a shift toward a dominance of sympathetic tone. During Po, there were no significant changes in PP output (Δ -1.4 ± 3.1 ng/min) or NE spillover (Δ +1.6 ± 1.2 ng/min), and consequently there was no significant change in the PP output:NE spillover ratio. Thus, activation of the Po glucose signal appears to inhibit the shift toward sympathetic dominance that would otherwise result, thereby causing an incretin effect.

HYPERGLYCEMIA RESULTING from oral glucose consumption stimulates insulin secretion more than equivalent hyperglycemia achieved by peripheral glucose infusion.[1] This has been attributed in large part to the actions of gut hormones, such as glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP).[2] However, when 2 groups of dogs received identical (10 mg · kg^{-1} · min^{-1} for 3 hours) infusions of glucose via the hepatic portal (Po) or a peripheral (Pe) vein, arterial insulin concentrations tended to be higher during the first hour in dogs receiving the glucose via the Po route.[3] This suggested that insulin secretion might be enhanced by Po glucose infusion, as well as by oral glucose intake. It has also been recognized for some time that oral or Po glucose delivery promotes hepatic glucose uptake much more efficiently than does Pe-delivered glucose, even when similar glucose loads and hormone levels are maintained.[4] The differential effect of Po versus Pe glucose delivery is dependent on intact hepatic nerves.[5]

Consideration of these 2 parallel phenomena led us to hypothesize that Po glucose delivery would bring about an incretin effect. Further, we sought evidence that such a potential Po incretin effect could be neurally-mediated. We have recently developed the methodology required to access pancreatic venous blood and to monitor pancreatic blood flow in conscious, unstressed dogs.[6] Therefore, we were able to apply the arteriovenous difference technique to test our hypothesis under physiologic conditions, using pancreatic norepinephrine (NE) spillover as an index of pancreatic sympathetic activity[6,7] and net pancreatic polypeptide output as an index of pancreatic parasympathetic activity[8,9] and measuring major metabolites of glucose.

MATERIALS AND METHODS

Animals and Surgical Procedures

Experiments were peformed in 18 conscious 42-hour fasted purpose-bred adult dogs of mixed breed and either sex (21 to 28 kg). All animals were maintained on a diet of meat (Kal Kan, Vernon, CA) and chow (Purina Lab Canine Diet No. 5006; Purina Mills, St Louis, MO) composed of 34% protein, 14.5% fat, 46% carbohydrate, and 5.5% fiber, based on dry weight. The animals were housed in a facility that met American Association for Accreditation of Laboratory Animal Care guidelines, and the Vanderbilt University Medical Center Animal Care Committee approved the protocols.

After an overnight fast, each dog underwent a laparotomy under general anesthesia (15 mg/kg thiopental sodium intravenously, followed by 1% isoflurane in room air) with mechanical ventilation. The duodenum and the associated lobe of the pancreas were exposed, and the superior pancreaticoduodenal vein (SPDV) was ligated adjacent to the duodenum at the caudal extreme of the duodenal lobe of the pancreas. Silastic catheters (Dow Corning, Midland, MI) were placed in the SPDV for sampling of the pancreatic venous effluent as described previously[6] and into a splenic vein and a jejunal vein to permit infusion of glucose into the portal circulation.[10] A fourth catheter was placed in the left femoral artery (FA) to access peripheral arterial blood.[10]

To measure pancreatic venous blood flow, an ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the SPDV immediately caudal to the SPDV/Po vein juncture and approximately 1.5 cm rostral from the tip of the cannula. Catheters were filled with saline containing heparin (200 U/mL; Abbott Laboratories, North Chicago, IL) and knotted. After closure of the muscle layer, catheters and flow probe wires were placed in subcutaneous pockets and the skin incisions were closed.

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Protocol

Two days before study (and 8 to 12 days after the surgical procedures), blood was drawn to determine the leukocyte count and the hematocrit of each animal. The dog was studied only if it had a leukocyte count less than 18,000/μL, a hematocrit greater than 35%, a good appetite as evidenced by consumption of $\geq$ 3/4 of the daily food ration and normal stools.

On the morning of the study, catheters and flow probe leads were exteriorized from their subcutaneous pockets using local anesthesia (20 mg/mL lidocaine; Astra Pharmaceuticals, Worcester, MA). The contents of each catheter were aspirated, and the catheters were flushed with saline and subsequently used for blood sampling. An Angiocath (Deseret Medical, Becton Dickinson, Sandy, UT) was inserted into a cephalic vein for infusion of saline or glucose. Each dog was allowed to stand quietly in a Pavlov harness for 60 minutes before beginning the experiment.

After a 60-minute acclimation period (-90 to –30 minutes), there was a basal period (-30 to 0 minutes) in which blood samples were obtained simultaneously from the FA and the SPDV every 10 minutes. Glucose was then infused either via the Po vein (Po group, n = 9) or a cephalic vein (Pe group, n = 9) from 0 to 90 minutes. Glucose was infused at a rate of 8 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} in Po. In Pe, glucose was infused at a rate of 8 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} for the first 15 minutes, then the rate was decreased as necessary, based on periodic ($\approx$10 minutes) glucose measurements on small (0.2 mL) samples of FA blood, to maintain a steady-state hyperglycemia of approximately 150 mg/dL. Blood samples ($\approx$8 mL) were obtained from both the FA and SPDV at 120 minutes and every 10 minutes from 60 to 90 minutes.

Blood flow through the SPDV was recorded immediately prior to obtaining each blood sample. After completion of the experiment, animals were euthanized with an overdose of pentobarbital, and proper placement of the SPDV cannula and flow probe was confirmed during necropsy.

Analytical Procedures

Immediately after each blood sample was drawn, 3 mL of blood was placed in a tube with 60 μL of 0.2 mol/L glutathione (Sigma Chemical, St Louis, MO). This blood was vortexed and centrifuged, and the plasma was frozen at –70°C for subsequent epinephrine (EPI) and NE measurement by high-performance liquid chromatography (HPLC)[11] with interassay coefficient of variation (CV) of 7% and 5%, respectively. Alanine, ß-hydroxybutyrate (βOHB), glycerol, and lactate concentrations were determined on whole blood according to the methods of Lloyd et al[12] adapted to a Monarch 2000 centrifugal analyzer (Instrumentation Laboratories, Lexington, MA) as previously described.[3,13] Blood glycine, serine, and threonine were determined by HPLC separation.[14] A 20-μL aliquot of arterial blood was used immediately for duplicate measurement of hematocrit using capillary tubes. A total of 4 to 6 10-μL aliquots of plasma were immediately analyzed for glucose using the glucose-oxidase method in a glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma concentrations of immunoreactive insulin (IRI) and glucagon (IRG) were measured by double-antibody procedures[15,16] with interassay CVs of 7% and 5%, respectively. Plasma pancreatic polypeptide (PP) was measured by radioimmunoassay at Linco Research (St Charles, MO) (interassay CV, 12%). Plasma cortisol levels were measured using the Clinical Assays Gamma Coat radioimmunoassay with a CV of 6%. Plasma nonesterified fatty acids (NEFA) were determined colorimetrically with a commercial kit (Wako Chemicals, Richmond, VA) on the Monarch centrifugal analyzer. The levels of substrates were assessed during the basal and glucose infusion periods to ensure that they were not significantly different between groups and demonstrate that the 2 groups were metabolically similar. In addition, net hepatic uptake or release of some of these substrates is exquisitely sensitive to changes in pancreatic hormone secretion, and this, in turn, can alter circulating substrate concentrations. Differential changes in substrate concentrations, then, could provide further evidence of functional differences in the response to Po versus Pe glucose infusion.

Calculations and Data Analysis

Pancreatic hormone output was calculated at each time point according to the following formula:

$$\text{Output} = ([\text{hormone}]_{\text{SPDV}} - [\text{hormone}]_{\text{FA}}) \times (1 - \text{hematocrit}) \times \text{blood flow}_{\text{SPDV}}.$$

Spillover of NE was calculated by the same principle; however, the pancreas extracts a significant portion (70% to 80%) of the NE arriving via the arterial circulation.[17] This extraction, representing neuronal reuptake and/or tissue degradation, must be taken into account to avoid overestimation of the arterial contribution to the pancreatic venous levels of NE and thus serious underestimation of NE spillover. It was previously shown that pancreatic extraction of EPI is virtually identical to NE extraction.[17] Because EPI is not synthesized in sympathetic nerves or normally released from the nerves, pancreatic extraction of EPI can be used to estimate pancreatic extraction of NE. Thus, pancreatic spillover of NE was calculated according to the following formula:

$$\text{Spillover} = ([\text{NE}]_{\text{SPDV}} - \text{arterial contribution to } [\text{NE}]_{\text{SPDV}}) \times (1 - \text{hematocrit}) \times \text{blood flow}_{\text{SPDV}}$$

in which arterial contribution to $[NE]_{SPDV} = [NE]_{FA} \times$ (1 - fractional extraction of NE) and fractional extraction of NE = fractional extraction of EPI = $([EPI]_{FA} - [EPI]_{SPDV})/[EPI]_{FA}$.

These calculations do not correct for neuronal reuptake of endogenously-released NE and therefore reflect net spillover rather than total release.

Data are presented as the mean $\pm$ SEM. Statistical analysis was performed using analysis of variance (ANOVA) for repeated measures. Statistical significance was set at $P < .05$.

RESULTS

Concentrations of Glucose and Other Substrates; EPI and Cortisol Concentrations

Basal arterial plasma glucose concentrations were virtually identical in the 2 groups (106 $\pm$ 2 and 105 $\pm$ 2 mg/dL in Po and Pe, respectively), and equivalent hyperglycemia (155 $\pm$ 7 and 154 $\pm$ 8 mg/dL during Po and Pe, respectively) was produced with the 2 routes of glucose infusion (Fig 1). Steady-state hyperglycemia was achieved with a constant Po infusion of glucose at 8 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}. When glucose was infused via a Pe vein, a rate of only 6.8 $\pm$ 0.4 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} was required.

The arterial plasma concentrations of EPI, cortisol, and NEFA and the blood concentrations of lactate, glycerol, βOHB, and gluconeogenic amino acids were not significantly different between groups during the basal period or during glucose infusion (Table 1). As one would expect, lipolysis was inhibited in both groups with a consequent decrease in blood levels of NEFA, glycerol, and βOHB. Similarly, blood amino acid concentrations decreased modestly in both groups.

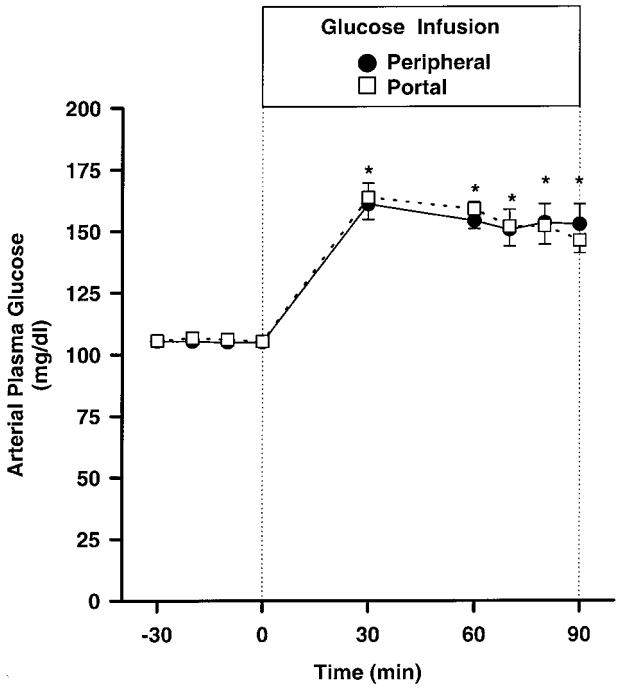

Fig 1. Arterial plasma glucose concentrations in 2 groups of 42-hour fasted conscious dogs, 1 group (Po, n = 9) receiving Po glucose infusion at 8 mg · kg^{-1} · min^{-1} and 1 group (Pe, n = 9) receiving Pe glucose at a rate that created the same glycemia as that observed in the Po group. *$P < .05$ *v* basal. There are no significant differences between the groups.

Insulin and Glucagon Concentrations and Pancreatic Output

The arterial plasma insulin levels increased significantly from basal in both groups (from 10 ± 2 to 38 ± 6 μU/mL during Po glucose infusion and from 7 ± 1 to 23 ± 2 μU/mL during Pe glucose infusion [last 30 minutes]; Fig 2A). The arterial insulin concentrations were significantly greater (increment from basal ≅75% larger) during Po than during Pe glucose infusion ($P < .05$). Pancreatic insulin output changed in proportion to the arterial levels (Fig 2B), but the increment (last 30 minutes of study minus basal period) in output (Δ +11.1 ± 3.2 *v* +6.7 ± 2.3 mU/min for Po and Pe, respectively) was not statistically greater in Po versus Pe ($P = .12$) as a result of the large variance in the individual secretion rates.

Hyperglycemia elicited a significant suppression of arterial plasma glucagon and pancreatic glucagon output in both the Pe and Po groups (Fig 3). Neither glucagon concentrations nor glucagon output was significantly different between groups at any time.

Indices of Autonomic Function

Pancreatic PP output (an index of parasympathetic nervous activity) in the basal state was similar in the Po and Pe groups. It did not change significantly when glucose was infused intraportally (Δ -1.4 ± 3.1 ng/min; Fig 4), but was suppressed during Pe glucose infusion (Δ -4.8 ± 2.5 ng/min, $P < .05$ *v* basal; Fig 5). The decrement in pancreatic PP output in Pe was not large enough to be significantly different from the rate in Po ($P = .20$).

Pancreatic NE spillover (an index of sympathetic neural activity) was also similar in the basal state in the 2 groups, and it did not change appreciably during Po glucose infusion (Δ +1.6 ± 1.2 ng/min; Fig 4). There was a tendency for pancreatic NE spillover to increase (Δ 4.4 ± 4.0 ng/min) during Pe glucose infusion, but this did not reach statistical significance ($P = .20$). Likewise, NE spillover was not significantly different between the 2 groups during glucose infusion ($P = .22$).

The ratio of pancreatic PP output to pancreatic NE spillover can be considered an index of pancreatic autonomic balance. The higher this ratio is, the more parasympathetic activity dominates the neural tone and conversely, the lower the ratio, the more sympathetic activity dominates neural tone. In the basal state this autonomic ratio was not statistically different in the 2 groups (2.6 ± 1.1 in Po and 1.3 ± 0.3 in Pe; Fig 4). The ratio did not change significantly during Po glucose infusion (mean for 60 to 90 minutes, 1.7 ± 0.5, or a 35% decrease from basal, $P = .24$). However, Pe glucose infusion resulted in a significant (65%) decrease in the PP output:NE spillover ratio

Table 1. Arterial Concentrations of Hormones and Substrates in 42 Hour Fasted Conscious Dogs in the Basal State and During Hyperglycemia Induced by Infusion of Glucose via the Hepatic Po or a Pe Vein

	Portal		Peripheral	
	Basal	Hyperglycemia	Basal	Hyperglycemia
Plasma epinephrine (pg/mL)	106 ± 16	118 ± 19	110 ± 15	111 ± 20
Plasma cortisol (μg/dL)	1.4 ± 0.2	3.0 ± 0.5*	2.4 ± 0.7	2.9 ± 0.8
Blood lactate (μmol/L)	758 ± 98	899 ± 76	761 ± 89	767 ± 65
Plasma NEFA (μmol/L)	810 ± 156	247 ± 82*	827 ± 84	247 ± 35*
Blood βOHB (μmol/L)	25 ± 7	8 ± 2*	35 ± 9	10 ± 2*
Blood glycerol (μmol/L)	69 ± 9	34 ± 7*	73 ± 6	34 ± 2*
Blood serine (μmol/L)	135 ± 12	118 ± 12	163 ± 12	133 ± 10*
Blood glycine (μmol/L)	200 ± 12	169 ± 18	235 ± 22	184 ± 15*
Blood threonine (μmol/L)	269 ± 31	217 ± 23*	297 ± 17	243 ± 20*
Blood alanine (μmol/L)	325 ± 14	293 ± 12*	344 ± 20	308 ± 9

NOTE. Basal values are the mean ± SE of 4 sampling times between −30 and 0 minutes (n = 9/group). Values during hyperglycemia are the mean ± SE of 4 sampling times between 60 to 90 minutes.

Abbreviations: NEFA, nonesterified fatty acids; βOHB, β-hydroxybutyrate.

*$P < .05$ *v* basal. There are no significant differences between the groups.

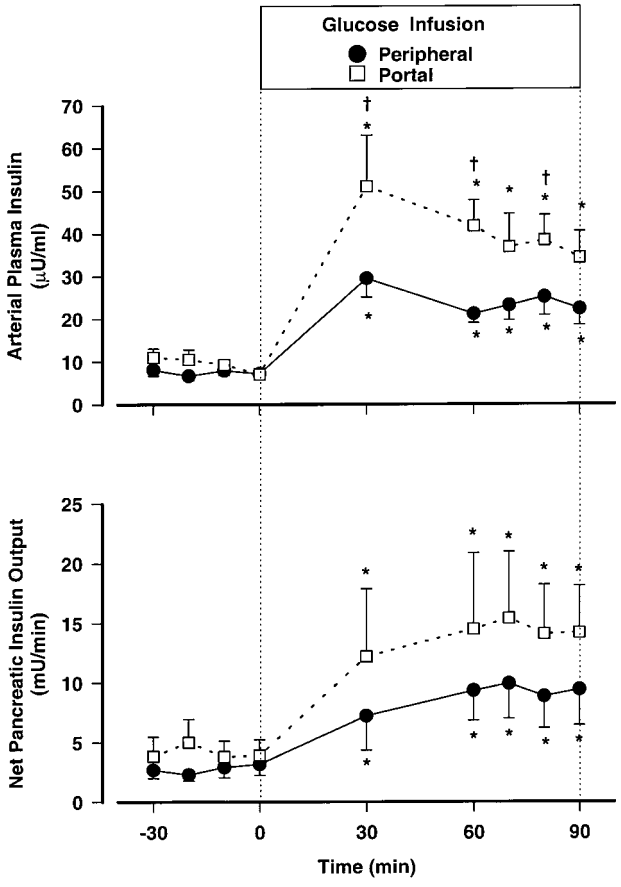

Fig 2. Arterial plasma insulin concentrations and net pancreatic insulin output in 2 groups of 42-hour fasted conscious dogs, 1 group (Po, n = 9) receiving Po glucose infusion at 8 mg · kg^{-1} · min^{-1} and 1 group (Pe, n = 9) receiving Pe glucose at a rate that created the same glycemia as that observed in the Po group. *$P < .05$ *v* basal. †$P < .05$ between groups.

to 0.4 ± 0.2 during 60 to 90 minutes ($P < .05$), a value that was significantly lower than the ratio in the Po group. These data indicate that Pe glucose infusion was associated with a shift to sympathetic nerve dominance that would limit insulin secretion. This did not occur in the presence of Po glucose infusion, thus potentially explaining the larger increment in insulin when glucose was delivered portally.

DISCUSSION

The route of glucose administration significantly influences net hepatic glucose uptake and nutrient disposition.[4,10,18-20] The liver extracts glucose more efficiently when glucose enters via the Po vein than when glucose is infused by a Pe vein, even when insulin is kept equal in the 2 circumstances, and this effect is dependent on intact hepatic innervation.[5] Thus, the Po signal has been defined as the pathway activated (presumably by hepatic/portal glucose sensors) when hepatic Po vein glucose levels exceed those in the artery. Moreover, activation of the Po signal apparently influences other metabolically-important organs, acting to reduce Pe insulin sensitivity at the level of muscle and, conceivably, adipose tissue.[3,10,13] In view of the developing hypothesis that the hepatic glucose sensors may coordinate an integrated metabolic response to physiologic nutrient ingestion via neurally-mediated mechanisms,[21] it seemed appropriate to question whether pancreatic hormone secretion may represent another arm of such integrated metabolic control.

It has been known for nearly 70 years that oral glucose is more effective in stimulating insulin release than is intravenous glucose.[22] Gut hormones, such as GLP-1 and GIP have been strongly implicated in the humoral incretin effect.[23,24] However, less consideration has been given to the possible role of the autonomic nervous system and of hepatoportal glucose sensors to observations made in the whole animal or in humans. Abundant evidence indicates that the sympathetic and parasympathetic nervous systems are involved in the regulation of pancreatic hormone secretion (see Ahren et al[31] for review). For these reasons, we undertook the current studies to determine if infusion of glucose via the Po vein (which would mimic the effect of a high-carbohydrate meal, but bypass any oral or

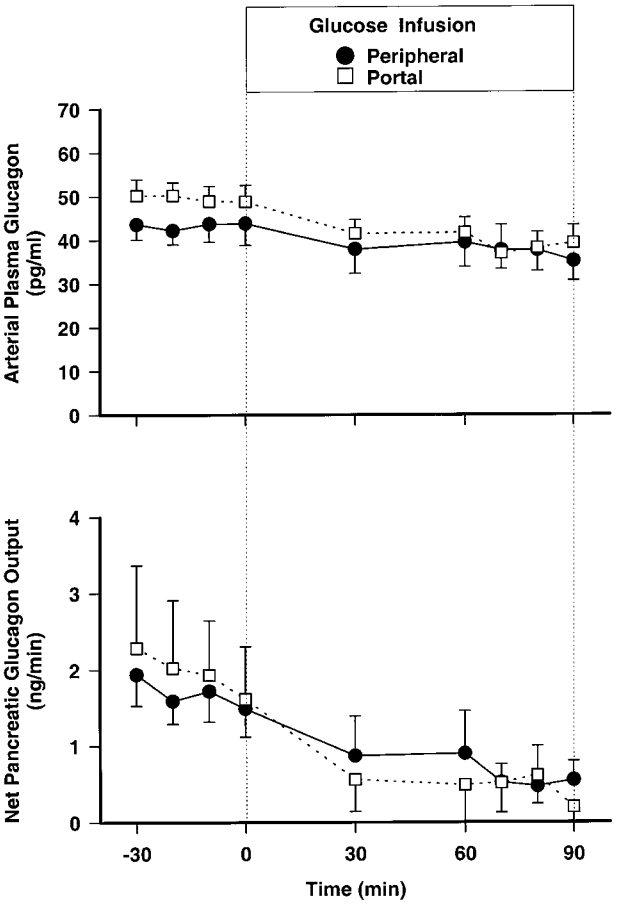

Fig 3. Arterial plasma glucagon concentrations and net pancreatic glucagon output in 2 groups of 42-hour fasted conscious dogs, 1 group (Po, n = 9) receiving Po glucose infusion at 8 mg · kg^{-1} · min^{-1} and 1 group (Pe, n = 9) receiving Pe glucose at a rate that created the same glycemia as that observed in the Po group. There are no significant differences between the groups

gastrointestinal factors) would exert more profound effects on pancreatic hormone secretion than equivalent hyperglycemia achieved by Pe glucose infusion.

When arterial glucose levels were matched during hyperglycemia produced by Po or Pe glucose infusion, we observed that the glucose-induced increment (change from baseline) in arterial plasma insulin levels was 75% higher during Po versus Pe glucose infusion. The augmented increment in circulating insulin in Po appeared to reflect secretion, because pancreatic insulin output was approximately 40% greater in the Po group. Unfortunately, owing to high variability in the basal secretion rate among the animals, the increase in secretion did not quite reach statistical significance. We are not able to describe the kinetics of the initial change in insulin secretion that occurred with the onset of glucose infusion, because our first sampling time in the infusion period occurred at 30 minutes. Steady-state conditions improve the reliability of the arteriovenous difference technique, and thus we chose to focus on the latter. Because the difference in insulin concentrations observed with Pe versus Po glucose delivery was maintained throughout the

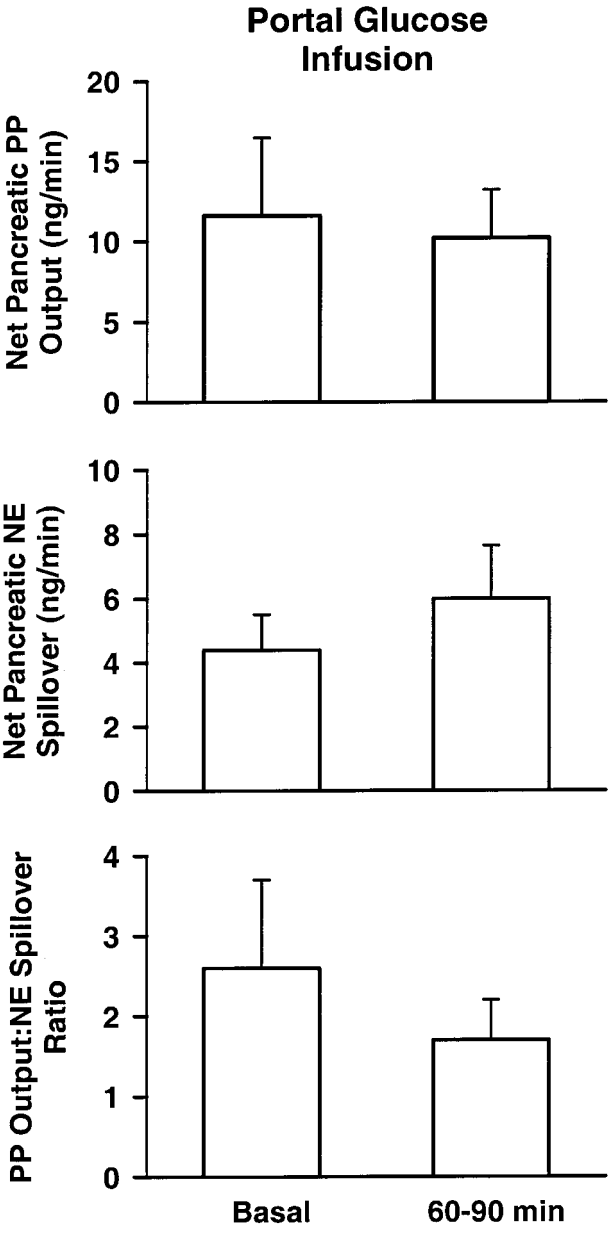

Fig 4. Net pancreatic PP output and NE spillover and ratio of PP output:NE spillover in 42-hour fasted conscious dogs receiving Po glucose infusion at 8 mg · kg^{-1} · min^{-1}. There are no significant changes over time. Data are mean ± SE; n = 9/group.

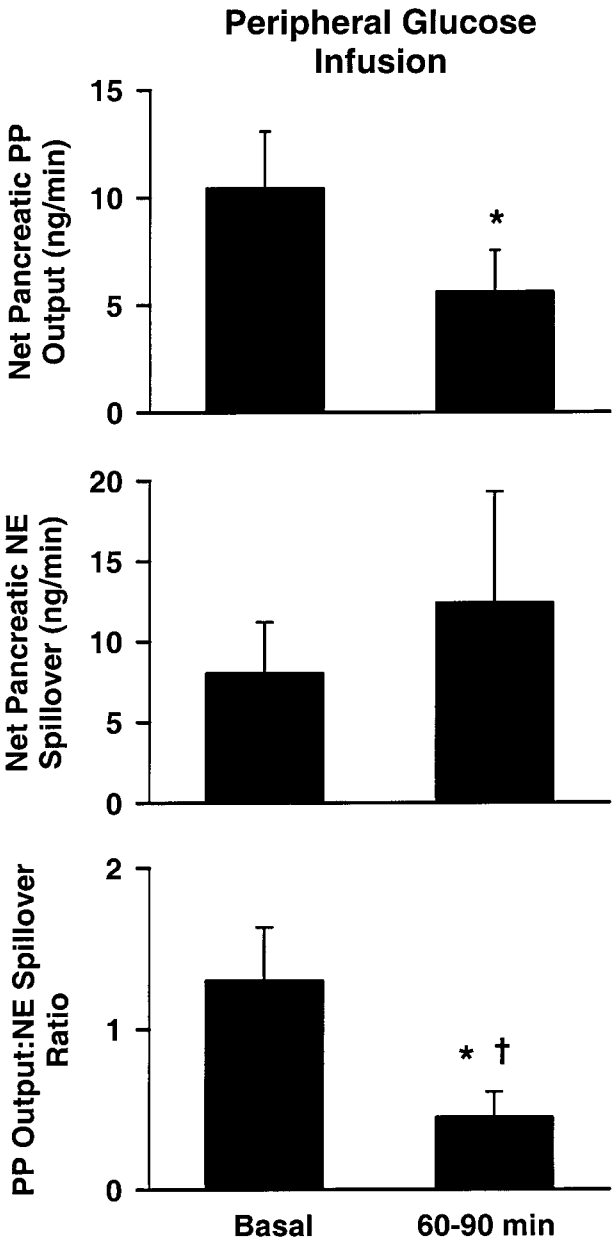

Fig 5. Net pancreatic PP output and NE spillover and ratio of PP output:NE spillover in 42-hour fasted conscious dogs receiving Pe glucose at a rate that created the same glycemia as that observed in the Po group. Data are mean ± SE. n = 9/group *$P < .05$ *v* basal. †$P < .05$ *v* Po.

glucose infusion period, it would appear that whatever the mechanism, it was maintained.

Glycemic levels were essentially identical in the 2 groups, but the glucose infusion rate in the Po group was 1.2 mg · kg^{-1} · min^{-1} (18%) greater than that required in the Pe group. This supports our finding of increased insulin levels in the Po glucose infusion group. It is well established that the Po glucose signal enhances net hepatic glucose uptake (NHGU)[4,10,18,20] and suppresses extrahepatic (primarily skeletal muscle) glucose uptake.[13,25,26] Thus, if insulin had not increased, one would have expected these 2 effects to offset, and glucose uptake in the 2 groups should have been equal. The increase in the glucose requirement in the Po group indicates that NHGU and/or extrahepatic glucose uptake must have increased. This enhancement could have resulted from the increased insulin levels and/or an increase in the Po glucose load. In a previous study, we infused glucose at 10 mg · kg^{-1} · min^{-1} into the Po vein or a Pe vein.[3] In that study, there was a significant, but transient, enhancement of arterial plasma insulin concentrations by Po glucose delivery. The lack of a sustained enhancement of insulin concentrations in that study may help to explain why the arterial glucose concentrations were not significantly different with the 2 routes of delivery.[3]

Arterial levels of glucagon were modestly, but detectably, suppressed during hyperglycemia regardless of the route of glucose infusion. As we have reported recently,[6] suppression of glucagon secretion is more readily perceived when measuring glucagon output than arterial glucagon levels. It is likely that glucagon secretion in both groups decreased as a result of hyperinsulinemia per se, because insulin is thought to strongly influence glucagon secretion via a local paracrine effect.[27] The higher insulin levels in the Po group might have been expected to decrease glucagon secretion further in that group than in Pe, but the increased suppression would have been hard to detect, given the almost complete suppression already present.

Data from nerve recordings obtained during Po glucose administration strongly suggest that the afferent portion of the portal signal is mediated by a reduction of hepatic vagal afferent activity.[28] The afferent firing rate in the hepatic branch of the vagus nerve is inversely correlated with the Po vein glucose concentration.[28] It appears that the efferent limb (or 1 of the efferent limbs) of the portal signal involves effects on the pancreas. Nagase et al[29] observed that the efferent firing rate in the pancreatic branch of the vagus nerve increased in response to intraportal glucose injection, an effect that was ablated by sectioning the hepatic branch of the vagus nerve. After Po glucose injection, the jugular vein plasma insulin concentrations were significantly reduced in the hepatic-vagotomized rats compared with sham-vagotomized controls.[29] In contrast, hepatic vagotomy did not alter plasma insulin concentrations after glucose injection into the jugular vein.[29] This suggests that afferent signals from glucose-sensitive cells in the hepatoportal region are conveyed to the brain via the hepatic branch of the vagus, and that this results in a change in efferent signaling from the brain to the pancreas via the vagus. Alternatively, vagal efferent fibers have been found to pass directly from the hepatic branch of the vagus to the pancreas in the rat,[30] suggesting that there might be a direct pathway for stimulation of the pancreatic response to the presence of glucose in the Po vein. Here we sought to confirm in a conscious dog model that neural signaling to the pancreas is altered by Po versus Pe glucose delivery. Toward this end, we measured pancreatic PP output, indicative of efferent pancreatic vagal activity, and net pancreatic NE spillover, indicative of efferent pancreatic sympathetic neural activity. Consistent with our recent report,[6] hyperglycemia elicited by Pe glucose infusion was accompanied by a decrease of pancreatic PP output, suggesting withdrawal of pancreatic vagal tone. No such reduction of PP output was observed during Po glucose infusion; if anything PP output trended upward late in the hyperglycemic period. It appears, therefore, that there was no withdrawal of pancreatic vagal tone during hyperglycemia produced by Po glucose infusion. This suggests the possibility that ongoing vagal activity was supporting insulin secretion.

In both groups of dogs, there was significant pancreatic NE spillover in the basal state (5 to 8 ng/min), suggesting that pancreatic sympathetic tone may act to chronically modulate (restrain) insulin secretion. In both groups, there was a tendency for this index of sympathetic tone to increase with hyperglycemia. Nevertheless, there was no statistically significant change in pancreatic NE spillover in either group, and the groups did not differ significantly at any time.

For reasons put forth above, the ratio of pancreatic PP output to pancreatic NE spillover may be considered an indication of pancreatic autonomic balance, although the units are arbitrary and the ratio is, perhaps, inappropriate to compare between animals. Within animals, however, we believe that this ratio has value. Clearly, as Pe glucose infusion progressed, autonomic balance favored the sympathetic nervous system, which would act to restrain insulin release. No such change of autonomic balance was observed during Po glucose infusion, suggesting maintenance of a more parasympathetic balance. While our data do not rule out involvement of humoral factors in the hormonal responses observed, they do point to a role for the ANS in control of pancreatic hormone secretion during hyperglycemia.

In summary, although many questions are raised by the studies described herein, a few issues have been resolved. Pe glucose administration increases insulin levels, but at a fixed arterial glucose level, Po glucose administration augments the response. Pe glucose administration suppresses the pancreatic output of PP, suggesting withdrawal of pancreatic vagal tone, but this does not occur when glucose is infused via the Po vein, mimicking the effect of food intake. We conclude, therefore, that a portion of the well-known incretin effect (of oral *v* IV glucose administration to augment insulin secretion) reflects activation of hepatic glucose sensors that communicate via autonomic nerves to the central nervous system (CNS), and we suggest that the hepatoportal region plays a more important role in the integrated metabolic and hormonal response to food ingestion than previously appreciated.

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