Insulin- and Glucagon-Independent Effects of Calcitonin Gene–Related Peptide in the Conscious Dog

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Calcitonin gene–related peptide (CGRP) causes vasodilation in many vascular beds, resulting in hypotension and tachycardia. The current studies were conducted in overnight-fasted conscious dogs to determine the effect of different CGRP dosages on carbohydrate metabolism and catecholamine release resulting from hemodynamic changes. During a pancreatic clamp, dogs received intraportal infusions of CGRP at 13, 26, and 52 (n = 3) or 52, 105, and 210 pmol · kg^{-1} · min^{-1} (n = 4; 60 minutes at each rate). Blood pressure decreased ($P < .05$) and the heart rate and hepatic blood flow (HBF) increased a maximum of 100% and 30%, respectively ($P < .05$). For the five CGRP infusion rates, arterial plasma epinephrine increased approximately 1.3-, 2.4-, 7.4-, 12-fold, and eightfold basal, respectively; norepinephrine increased about 2.3-, 3.3-, 4.1-, 4.6-, and 4.8-fold basal, respectively; and cortisol increased about twofold, 3.4-fold, fivefold, sixfold, and 6.2-fold basal, respectively. At CGRP infusion rates of 52 pmol · kg^{-1} · min^{-1} or higher, increases ($P < .05$) occurred for plasma glucose, endogenous glucose production (EndoR$_a$), and net hepatic uptake of gluconeogenic substrates (maximum change, 24 mg/dL, 1.3 mg · kg^{-1} · min^{-1}, and 9.9 μmol · kg^{-1} · min^{-1}, respectively). Arterial blood glycerol concentrations increased only a maximum of 30%. At the two highest CGRP infusion rates, glycerol returned to basal concentrations and arterial plasma nonesterified fatty acids (NEFAs) decreased. The increased net hepatic uptake of gluconeogenic substrates during CGRP infusion was sufficient to account for 49% to 58% of the increase in EndoR$_a$. CGRP has no apparent direct effects on hepatic carbohydrate metabolism, but the catecholamines, at levels similar to those observed during CGRP infusion, stimulate hepatic glycogenolysis. Therefore, some factor(s) other than CGRP, probably an increase in circulating catecholamine concentrations, would appear to be responsible for at least 42% to 51% of the increase in EndoR$_a$.

CALCITONIN GENE–RELATED PEPTIDE (CGRP), a neuropeptide produced by alternative processing of mRNA transcripts from the calcitonin gene, is widely distributed in the central and peripheral nervous systems, gastrointestinal tract, pancreas, and sensory and motor neurons of skeletal muscle.[1-4] CGRP has been shown to antagonize insulin activity in both skeletal muscle and liver.[5-7] Plasma glucose and insulin concentrations have been reported to increase significantly following CGRP injection,[8,9] and CGRP has been shown to decrease muscle glycogen synthesis both in vitro and in vivo.[7,10,11] Physiologic concentrations of CGRP inhibited the activation of muscle glycogen synthase and stimulated hepatic glucose production during euglycemic-hyperinsulinemic clamp studies in conscious rats.[5,6]

In addition to its metabolic effects, CGRP has well-recognized cardiovascular effects. It is a potent vasodilator in selected portions of the vasculature, including the liver, spleen, and heart,[12,13] and CGRP administration causes a decrease in blood pressure, increase in heart rate, and increase in the circulating level of catecholamines.[8,12,14,15] Rats injected with CGRP (5.67 nmol/kg) exhibited a twofold increase in the concentration of both plasma epinephrine and norepinephrine within 15 minutes.[9] No study has yet quantified the effects of CGRP and the catecholamine changes it induces in glucose metabolism in a model in which glucagon and insulin concentrations are prevented from changing. The aim of the current study was to quantify the metabolic effects of CGRP at infusion rates of 13 to 210 pmol · kg^{-1} · min^{-1} in the presence of basal insulin and glucagon concentrations, and to evaluate these effects in light of the concomitant catecholamine changes resulting from vasodilation and hypotension.

MATERIALS AND METHODS

Animals and Surgical Procedures

Experiments were performed on seven overnight-fasted conscious mongrel dogs (20 to 27 kg) of either sex fed as previously described.[16] The dogs were housed in a facility that meets American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Subcommittee.

Approximately 16 days before each experiment, a laparotomy was performed under general anesthesia, and Silastic (Dow Corning, Midland, MI) catheters for blood sampling were placed into a femoral artery, the portal vein, and the left common hepatic vein.[16] Doppler flow probes (Biomedical Instrumentation Laboratory, Baylor School of Medicine, Houston, TX) were positioned on the portal vein, distal to the catheter, and the hepatic artery to measure hepatic blood flow. Catheters for hormone infusion were positioned in a splenic and a jejunal vein.[16] After the catheters were inserted, they were filled with saline containing heparin (200 U/mL; Abbott Laboratories, North Chicago, IL), the free ends were knotted, and they were placed in subcutaneous pockets so that complete closure of the skin incision was possible.

On the day before the experiment, the leukocyte count and hematocrit were determined. Dogs were used for an experiment only if they had (1) a leukocyte count less than 18,000/μL, (2) a hematocrit greater than 36%, (3) a good appetite as evidenced by consumption of at least three fourths of the daily ration, and (4) normal stools.

On the day of the experiment, the subcutaneous ends of the catheters were freed through small skin incisions made under local anesthesia (2% lidocaine; Abbott). The contents of each catheter were aspirated, and the catheters were flushed with saline. Peripheral intravenous access was established in the left cephalic vein for infusion of indocyanine green dye (ICG) and tritiated glucose, and in the right cephalic vein for infusion of somatostatin. After these preparations, the

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dog was allowed to stand quietly in a Pavlov harness for 20 to 30 minutes before beginning the experiment.

Experimental Design

Each experiment consisted of an 80-minute tracer equilibration period (−120 to −40 minutes), a 40-minute control period (−40 to 0 minutes), and three successive 60-minute experimental periods (0 to 180 minutes) (Fig 1). A priming dose of [3-^{3}H]glucose (41.7 μCi; New England Nuclear, Boston, MA) was administered at −120 minutes. Continuous infusions of [3-^{3}H]glucose (0.34 μCi/min) and ICG (0.01 mg · kg^{-1} · min^{-1}; Becton Dickinson, Cockeysville, MD) were also started at −120 minutes and continued throughout the experiment. ICG was infused to allow confirmation of hepatic vein catheter placement, as well as to provide a method for measurement of hepatic blood flow in the event of Doppler probe failure. An infusion of somatostatin (0.8 μg · kg^{-1} · min^{-1}; Bachem, Torrance, CA) was started at −120 minutes to inhibit endogenous insulin and glucagon secretion, and intraportal replacement infusions of insulin and glucagon (250 μU · kg^{-1} · min^{-1} and 0.65 ng · kg^{-1} · min^{-1}, respectively; both from Eli Lilly, Indianapolis, IN) were started. The plasma glucose level was then monitored every 5 minutes, and the rate of insulin infusion was adjusted as necessary to maintain euglycemia. The final alteration in the insulin infusion rate was made at least 20 minutes before the start of the control period, and the rate (mean, 213 μU · kg^{-1} · min^{-1} for all experiments) remained unchanged thereafter. At 0 minutes, an intraportal infusion of human CGRP-1 (a gift from Eli Lilly) prepared with normal saline containing 3% of the dog's own plasma was begun. The CGRP infusion continued for three consecutive 60-minutes periods at a dose of 13, 26, and 52 (n = 3) or 52, 105, and 210 pmol · kg^{-1} · min^{-1} (n = 4).

CGRP was infused intraportally because the mesenteric organs are an especially important source of CGRP,[1,2] and portal vein CGRP concentrations are normally higher than those in the peripheral circulation. Basal arterial plasma CGRP concentrations in the rat have been reported to be approximately 36 ± 5 pmol/L, with portal vein concentrations of CGRP about 40% higher than those in the artery.[10] Infusion of CGRP into the portal vein also enabled us to determine whether CGRP may have a direct effect on the liver that would influence carbohydrate metabolism. It is difficult to interpret the significance of plasma CGRP concentrations, since CGRP is presumably released in a paracrine manner from neural terminals near specific blood vessels and tissues.[14] Nevertheless, peripheral venous CGRP infusion rates of 10 to 200 pmol · kg^{-1} · min^{-1} yielded plasma CGRP concentrations of approximately 120 to 1,700 pmol/L in the rat, dog, and human.[5,14,15,17] Therefore, the infusion rates we used should create a wide range of circulating CGRP concentrations.

Sample Collection and Processing

Blood samples were taken at 10-minute intervals during the control period, at 15 and 30 minutes after the start of each experimental period, and every 10 minutes thereafter for the remainder of each test period. Blood samples were appropriately treated for the respective assays immediately after collection and stored at −70°C at the end of the experiment for subsequent analysis.[16]

Plasma glucose, insulin, glucagon, cortisol, epinephrine, and norepinephrine concentrations, blood lactate, alanine, glycerol, and β-hydroxybutyrate (βOHB) concentrations, and plasma [^{3}H]glucose specific activity were determined as described previously.[18-20] Plasma nonesterified fatty acid (NEFA) levels were measured enzymatically.[19]

Calculations

Hepatic blood flow (HBF) was assessed by measuring the hepatic extraction of ICG and by Doppler flow cuff measurements.[19] The results obtained with the two methods were not significantly different. Doppler measurements make it possible to determine the relative proportion of HBF provided by the hepatic artery and the portal vein, and therefore, the calculations reported herein use Doppler-determined blood flows.

Net hepatic substrate balance (NHB) was calculated using the formula, $[H - (AQ_a + PQ_p)] \times HF$, where A, P, and H are the arterial, portal vein, and hepatic vein substrate concentrations, respectively; HF is the hepatic blood or plasma flow; and Q_a and Q_p are the proportion of HF accounted for by the hepatic artery and portal vein, respectively. The total NHB of gluconeogenic precursors was calculated as $NHB_{lactate} + NHB_{glycerol} + (NHB_{alanine} \times 2)$. Previous investigation in our laboratory has demonstrated that the total NHB of gluconeogenic amino acids is very close to the rate obtained by doubling the NHB of alanine.[21] Tracer-determined rates of glucose production (R_a) and utilization (R_d) were determined by equations for isotope dilution during a constant infusion of radioactive glucose ([3-^{3}H]glucose) as previously reported.[19]

Data are expressed as the mean ± SE. Statistical analyses were performed using ANOVA with repeated measures. Post hoc significance was determined with the Scheffé F test. A P value less than .05 was considered statistically significant.

RESULTS

Basal values for animals receiving the two different infusion regimens were not different, with the exception of epinephrine (Table 1). Therefore, basal data for the seven animals were combined, except in the case of epinephrine. To compensate for the difference in basal values, epinephrine data were expressed as the change from basal (Fig 2). Similarly, results for animals

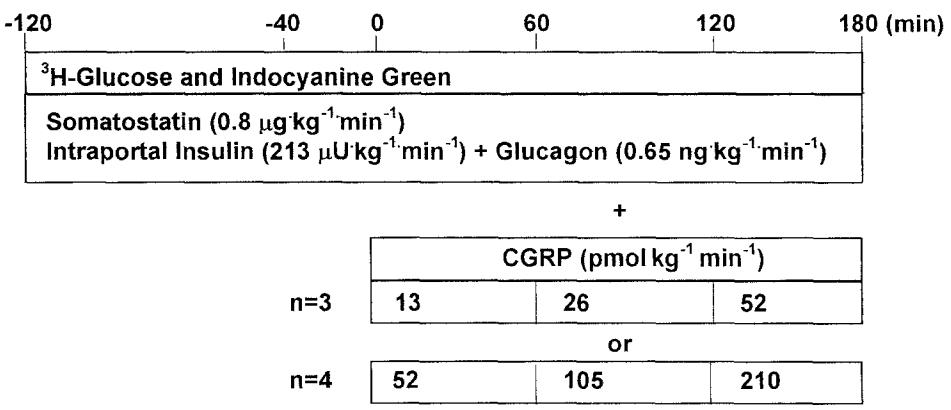

Fig 1. Study design.

Table 1. Basal Parameters in Dogs Receiving CGRP at 13, 26, and 52 pmol · kg^{-1} · min^{-1} (low CGRP) and 52, 105, and 210 pmol · kg^{-1} · min^{-1} (high CGRP)

Parameter	Low CGRP (n = 3)	High CGRP (n = 4)
Arterial plasma glucose (mg/dL)	108 ± 6	108 ± 6
NHGO (mg · kg^{-1} · min^{-1})	2.4 ± 0.04	2.4 ± 0.3
Arterial blood lactate (μmol/L)	655 ± 12	593 ± 46
Net hepatic lactate output (μmol · kg^{-1} · min^{-1})	1.9 ± 4.8	2.9 ± 6.4
Arterial blood alanine (μmol/L)	284 ± 22	277 ± 22
Net hepatic alanine uptake (μmol · kg^{-1} · min^{-1})	2.2 ± 0 1	2.6 ± 0.5
Arterial blood glycerol (μmol/L)	100 ± 29	85 ± 16
Net hepatic glycerol uptake (μmol · kg^{-1} · min^{-1})	1.8 ± 0.6	1.6 ± 0.4
Arterial plasma NEFA (μmol/L)	921 ± 280	853 ± 150
Net hepatic NEFA uptake (μmol · kg^{-1} · min^{-1})	4.0 ± 0.7	3.0 ± 0.3
Insulin (μU/mL)	8 ± 1	7 ± 1
Glucagon (pg/mL)	62 ± 10	52 ± 6
Epinephrine (pg/mL)	47 ± 15*	117 ± 16*
Norepinephrine (pg/mL)	130 ± 36	175 ± 57
Cortisol (μg/dL)	2.9 ± 0.8	2.3 ± 0.2
HBF (mL · kg^{-1} · min^{-1})	24 ± 1	25 ± 3

NOTE. Values are the mean ± SEM.
*$P < .05$ for difference between the 2 dosage regimens.

that received CGRP at 52 pmol · kg^{-1} · min^{-1} during the first hour of infusion did not differ from the results for animals receiving CGRP at that rate during the last hour of the infusion period. Therefore, data obtained during infusion of CGRP at 52 pmol · kg^{-1} · min^{-1} were combined for all seven dogs.

Hormone Levels

Arterial plasma insulin levels throughout the basal and CGRP infusion periods were 8 to 10 μU/mL (Table 2). Arterial plasma glucagon was 56 ± 6 pg/mL during the basal period and 61 to 70 pg/mL during CGRP infusion (Table 2; NS *v* basal). Epinephrine increased with each increase in CGRP infusion up to 105 pmol · kg^{-1} · min^{-1} (maximal increase over basal, 873 ± 216 pg/mL, $P < .05$ *v* basal; Fig 2). Epinephrine increased slightly less at the highest CGRP infusion rate, but the mean increase in epinephrine observed at that CGRP infusion rate (518 ± 203 pg/mL) was not significantly different from the maximal change observed. Norepinephrine also increased in a dose-dependent manner with increases in the CGRP infusion rate; the maximal norepinephrine concentration observed was 734 ± 176 pg/mL ($P < .05$ *v* basal). The arterial plasma cortisol concentration was 2.5 ± 0.4 μg/dL during the basal period and increased during CGRP infusion in a dose-dependent manner, reaching a maximum of 15.9 ± 1.7 μg/dL at the highest infusion rate.

Glucose Metabolism

EndoR_a increased in a dose-dependent manner from 2.7 ± 0.2 (basal) to a peak of 3.9 ± 0.4 mg · kg^{-1} · min^{-1} ($P < .05$) with infusion of CGRP at 52 pmol · kg^{-1} · min^{-1}. Similarly, R_d

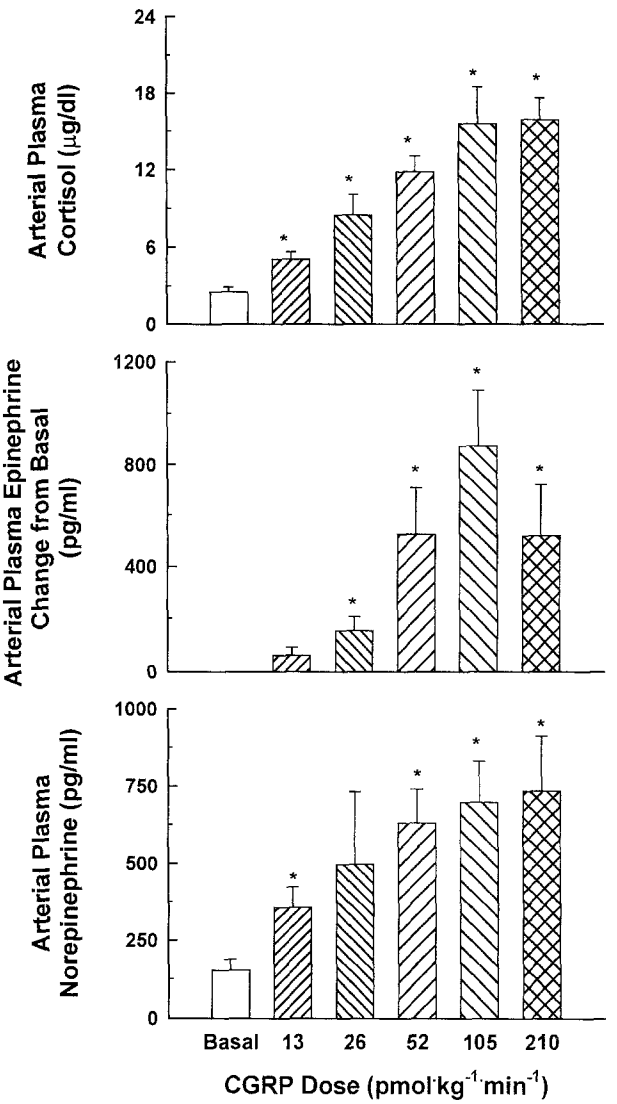

Fig 2. Arterial plasma cortisol, epinephrine, and norepinephrine levels during the basal period (n = 7) and during CGRP infusion at a dose of 13 (n = 3), 26 (n = 3), 52 (n = 7), 105 (n = 4), or 210 (n = 4) pmol · kg^{-1} · min^{-1} in overnight-fasted conscious dogs in which insulin and glucagon were replaced intraportally at basal rates during somatostatin infusion. Data for epinephrine are expressed as the change from basal because basal values differed in the dogs receiving the 2 treatment regimens. The data for each dosage represent the mean of 2 sampling points. *$P < .05$ *v* basal.

Table 2. Arterial Plasma Insulin and Glucagon Concentrations in Overnight-Fasted Conscious Dogs in the Basal State and During a Continuous CGRP Infusion

Hormone	Basal Condition (n = 7)	CGRP Infusion Rate (pmol · kg^{-1} min^{-1}) 13 (n = 3)	26 (n = 3)	52 (n = 7)	105 (n = 4)	210 (n = 4)
Insulin (μU/mL)	8 ± 1	10 ± 1	9 ± 1	10 ± 1	10 ± 1	8 ± 1
Glucagon (pg/mL)	56 ± 6	61 ± 16	68 ± 10	67 ± 11	70 ± 14	68 ± 4

NOTE. There were no significant differences *v* basal values.

increased from 2.6 ± 0.2 to 3.3 ± 0.5 mg · kg^{-1} · min^{-1} ($P < .05$) at the same CGRP infusion rate. The slight mismatch between R_a and R_d was associated with an increase in plasma glucose from 108 ± 4 (basal) to 127 ± 9 mg/dL ($P < .05$; Fig 3). Net hepatic glucose output (NHGO) also showed a tendency to increase with CGRP infusion rates of at least 26 pmol · kg^{-1} · min^{-1}, but only at an infusion rate of 105 pmol · kg^{-1} · min^{-1} was NHGO significantly increased over the

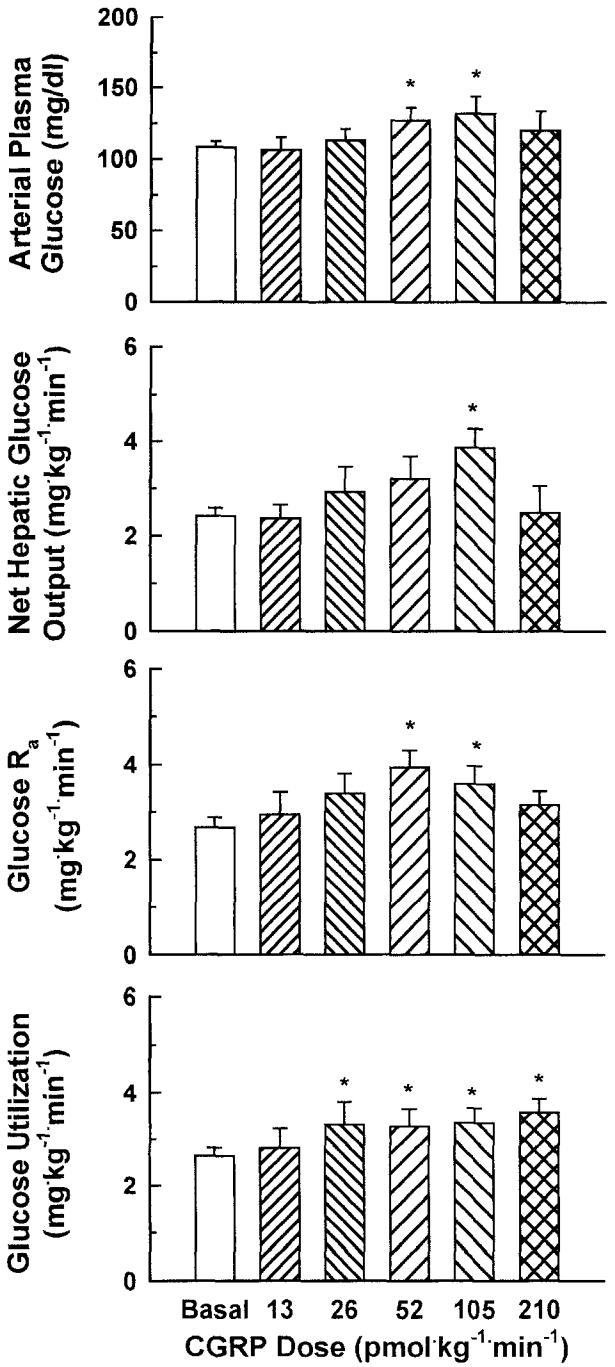

Fig 3. Arterial plasma glucose, tracer-determined glucose R_a, glucose utilization, and NHGO. Tracer-determined data are the mean of 3 sampling points at each dosage. *$P < .05$ *v* basal.

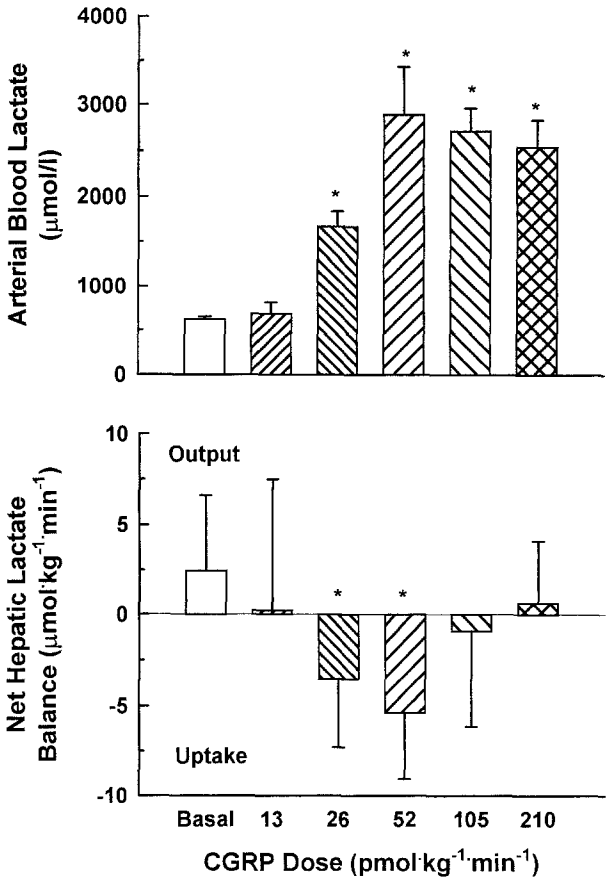

Fig 4. Arterial blood lactate and net hepatic lactate balance. *$P < .05$ *v* basal.

basal value (3.9 ± 0.4 *v* 2.4 ± 0.2 mg · kg^{-1} · min^{-1}). At the highest infusion rate, neither $EndoR_a$ nor NHGO were significantly changed from basal.

Gluconeogenic Precursor Metabolism

Arterial blood lactate increased from 620 ± 31 (basal) to 1,670 ± 165 μmol/L with infusion of CGRP at 26 pmol · kg^{-1} · min^{-1} ($P < .05$) and further increased to 2,893 ± 543 μmol/L with infusion of CGRP at 52 pmol · kg^{-1} · min^{-1} ($P < .05$ *v* 26 pmol · kg^{-1} · min^{-1}; Fig 4). The lactate concentration remained unchanged at higher CGRP infusion rates. Net hepatic lactate release occurred at a rate of 2.5 ± 4.2 μmol · kg^{-1} · min^{-1} during the control period, but net hepatic lactate uptake was observed soon after initiation of the CGRP infusion. Net hepatic uptake of lactate peaked at 5.4 ± 3.7 μmol · kg^{-1} · min^{-1} with the 52-pmol · kg^{-1} · min^{-1} CGRP rate (change, 7.8 ± 4.1 μmol · kg^{-1} · min^{-1} *v* basal, $P < .05$).

Arterial blood alanine increased from 280 ± 16 μmol/L in the basal period to a peak of 401 ± 41 μmol/L with a CGRP infusion rate of 105 pmol · kg^{-1} · min^{-1} ($P < .05$). Net hepatic alanine uptake remained at basal levels (2.5 ± 0.3 μmol · kg^{-1} · min^{-1}) at CGRP infusion rates of 13 and 26 pmol · kg^{-1} · min^{-1}, but then increased in a dose-dependent manner (maximal rate, 4.1 ± 0.7 μmol · kg^{-1} · min^{-1}, $P < .05$) (Fig 5).

Blood glycerol increased from 91 ± 16 (basal) to peak values

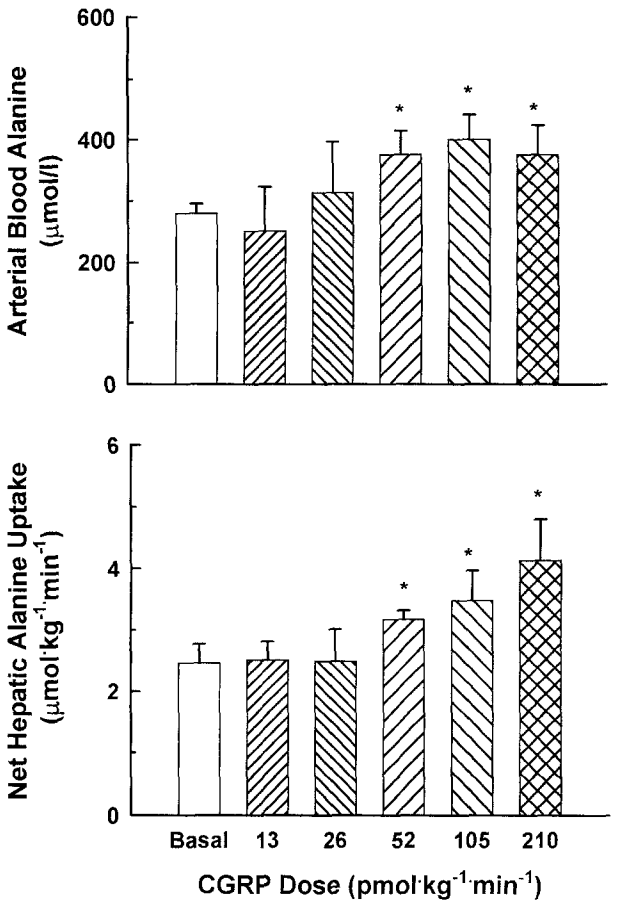

Fig 5. Arterial blood alanine and net hepatic alanine uptake. *$P <$ .05 *v* basal.

of 121 ± 19 μmol/L with infusion of CGRP at 52 pmol · kg^{-1} · min^{-1} ($P < .05$; Table 3). Glycerol gradually returned to basal levels with higher doses of CGRP. Net hepatic glycerol uptake increased significantly with low but not with high CGRP infusion rates, from 1.7 ± 0.3 (basal) to a peak of 3.0 ± 0.6 μmol · kg^{-1} · min^{-1} with CGRP 52 pmol · kg^{-1} · min^{-1} ($P < .05$).

NEFA and Ketone Metabolism

A significant decrease in arterial plasma NEFA occurred at the two highest rates of CGRP infusion. Net hepatic uptake of NEFA did not change during CGRP infusion. CGRP infusion did not cause any significant changes in the blood concentration or net hepatic production of βOHB (Table 3).

Table 4. Range of Values for MAP (mm Hg) and Heart Rate (beats per minute) at Each CGRP Dosage Level

Parameter	Basal Condition	CGRP Infusion Rate (pmol kg^{-1} min^{-1}) 13	26	52	105	210
MAP	83-113	64-99	47-51*	50-91*	65-91*	57-74*
HR	85-150	150-170	160-190*	150-215*	170-240*	230-235*

NOTE. See Table 2.
*$P <$ .05 *v* basal.

Cardiovascular Parameters and HBF

A significant ($P < .05$) decline in systolic, diastolic, and mean arterial blood pressure (MAP) was observed with CGRP infusion (Table 4). Conversely, the heart rate increased in a dose-dependent manner from a basal rate of 119 ± 21 to 232 ± 4 beats per minute ($P < .05$; Table 4). Maximal changes in blood pressure were found with the 52-pmol · kg^{-1} · min^{-1} infusion rate, but the heart rate was directly proportional to the increase in the CGRP infusion rate ($R^2 = .54$, $P < .001$).

HBP increased about 25% to 30% in all dogs at the lowest CGRP infusion rate (Fig 6). HBF failed to increase further with additional increases in CGRP. Most of the increase occurred as a result of the elevation in hepatic arterial flow, which increased almost twofold ($P < .05$ *v* basal). As with total hepatic flow, the increase in arterial flow was present at the lowest CGRP infusion rate.

DISCUSSION

This is the first report regarding the effect of CGRP in a model in which it is possible to measure simultaneously the substrate balance across the liver and the circulating concentration of catecholamines in the presence of fixed basal concentrations of insulin and glucagon. Under these conditions, CGRP was associated with mild hyperglycemia (maximum change [Δ] in plasma glucose, 24 mg/dL), an increase in $EndoR_a$ (maximum Δ, 1.3 mg · kg^{-1} · min^{-1}), and an increase in net hepatic uptake of gluconeogenic substrates (maximum Δ, 9.9 μmol · kg^{-1} · min^{-1}). The maximal response in these parameters occurred at CGRP infusion rates of 52 to 105 pmol · kg^{-1} · min^{-1}. It is evident that peripheral release of gluconeogenic precursors was enhanced during CGRP infusion in the conscious dog, as previously shown in the rat,[8] because the circulating concentrations increased even in the face of

Table 3. Arterial Concentrations (μmol/L) and NHB (uptake or output, μmol · kg^{-1} · min^{-1}) of Glycerol, NEFA, and βOHB in Overnight-Fasted Conscious Dogs in the Basal State and During a Continuous CGRP Infusion

Substrate	Basal Condition	CGRP Infusion Rate (pmol kg^{-1} min^{-1}) 13	26	52	105	210
Blood glycerol	91 ± 16	119 ± 30	114 ± 20	121 ± 19*	87 ± 19	96 ± 14
Glycerol uptake	1.7 ± 0.3	2.8 ± 0.9*	2.4 ± 1.0	3.0 ± 0.6*	1.8 ± 0.4	2.2 ± 0.2
Plasma NEFA	882 ± 144	1,018 ± 235	822 ± 107	750 ± 143	544 ± 62*	506 ± 55*
NEFA uptake	3.4 ± 0.6	3.5 ± 0.2	2.8 ± 0.8	2.8 ± 0.3	1.8 ± 0.6	2.0 ± 0.8
Blood βOHB	19 ± 4	18 ± 5	18 ± 5	21 ± 2	19 ± 2	18 ± 3
βOHB output	0 8 ± 0.2	1.0 ± 0.2	0.8 ± 0.03	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.1

NOTE. See Table 2.
*$P <$ .05 *v* basal.

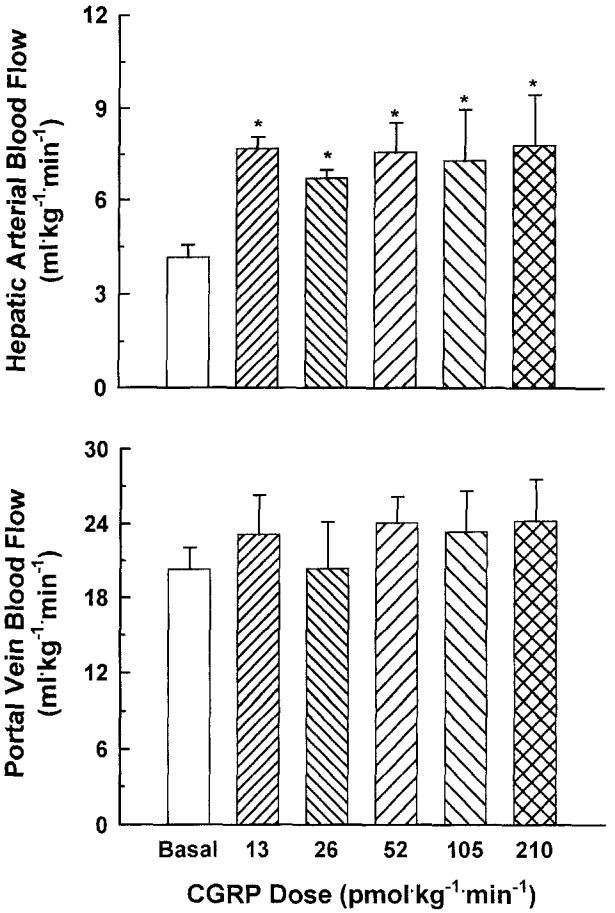

Fig 6. Arterial and portal blood flow. *$P < .05$ *v* basal.

increased net hepatic uptake of the precursors. Both the increase in circulating gluconeogenic substrates and the acceleration of HBF (~30%, resulting from an increase in hepatic artery flow as previously reported[22]) contributed to an increase in the load of gluconeogenic precursors reaching the liver. However, at the CGRP 52- and 105-pmol · kg^{-1} · min^{-1} infusion rates (the two infusion rates at which $EndoR_a$ was significantly elevated over basal), the enhancement of net hepatic substrate uptake was only sufficient to account for 58% and 49%, respectively, of the carbon required for the increase in $EndoR_a$. The rate of NHGO was significantly elevated over basal only at the CGRP 105-pmol · kg^{-1} · min^{-1} infusion rate, and the increase in net hepatic gluconeogenic precursor uptake at that infusion rate could account for only 35% of the change in NHGO. This indicates that a direct effect on the liver, as well as an indirect effect (ie, increased substrate supply), explained the increases in $EndoR_a$ and NHGO during CGRP infusion.

We have previously reported results from a group of six dogs studied under conditions identical to those in Fig 1, except that saline was infused instead of CGRP.[18] This group[18] serves as a time control for the current experiment. In the control group, there were no significant changes in plasma glucose or gluconeogenic substrate concentrations, $EndoR_a$, or net hepatic uptake of gluconeogenic precursors.[18] Thus, the changes in glucose metabolism observed during the current studies were clearly associated with CGRP infusion and were not merely a result of prolonged exposure to infusion of somatostatin, insulin, and glucagon. However, these results do not necessarily indicate that the effects of CGRP on carbohydrate metabolism in vivo are direct. Indeed, the results are also consistent with the effects resulting from the increases observed in circulating catecholamine and cortisol concentrations, which resulted from the cardiovascular changes occurring during CGRP infusion.[23] CGRP decreases vascular resistance in many organs, including the liver and skeletal muscle, in the rat and dog.[22,24] The hypotension and tachycardia occurring in the present investigation were consistent with previous reports of CGRP effects in the rat, dog, and human.[12,14,15] Thus, the possibility exists that cortisol and/or catecholamines were responsible for the changes in glucose metabolism observed in response to CGRP.

Both CGRP and the closely related peptide amylin increase cyclic adenosine monophosphate (cAMP) and glycogen phosphorylase activity in isolated skeletal muscle.[25] These changes are accompanied by stimulation of glycogenolysis and lactate release.[11,25] It is not clear whether the hyperlactatemic effects of CGRP are direct or result from cross-reactivity between CGRP and amylin receptors. CGRP concentrations have been reported to increase after glucose ingestion,[26] but an amylin-selective antagonist was able to completely block the hindlimb release of lactate in response to intravenous glucose infusion in rats.[27] It is therefore possible that CGRP was responsible for enhancing gluconeogenic precursor supply to the liver in the current study. Nevertheless, as we have previously stated, at least 42% to 51% of the increase in $EndoR_a$ observed with CGRP infusion rates of 52 and 105 pmol · kg^{-1} · min^{-1} must have resulted from hepatic glycogenolysis. Neither amylin nor CGRP have been found to exert any direct effect on hepatic glucose metabolism, including glycogen synthesis or mass, glycogen phosphorylase activity, glucose output, lactate uptake, gluconeogenesis from lactate, or tyrosine aminotransferase activity.[28,29] Therefore, these data suggest that CGRP itself was insufficient to produce the total increase in $EndoR_a$.

Cortisol concentrations increased fourfold at the 52-pmol · kg^{-1} · min^{-1} CGRP infusion rate and sixfold at the two higher infusion rates, although they did not change in the control group.[18] An isolated fourfold basal increase in circulating cortisol in the conscious dog caused no significant changes in either the circulating level or NHB of lactate, glycerol, βOHB, or NEFA[18] (NEFA data are unpublished). However, after 3 hours of cortisol infusion, net hepatic alanine uptake and fractional extraction increased about 50% and 38%, respectively, and the rate of gluconeogenic conversion of alanine to glucose increased 100%.[18] Despite the increased uptake of alanine by the liver, circulating alanine concentrations were stable, indicating an increase in the supply of alanine from nonhepatic tissues. The rate of hepatic glycogenolysis apparently changed reciprocally with the rate of gluconeogenesis, because glucose R_a did not increase during cortisol infusion.[18] Although hypercortisolemia may have been responsible for part of the eventual increase in the arterial alanine concentration and net hepatic uptake of alanine during CGRP infusion in the present study, by itself, it cannot account for the increase in

hepatic glucose production associated with CGRP administration.

We recently examined the direct and indirect effects of epinephrine on hepatic glucose production in conscious dogs.[19] In these studies, insulin, glucagon, and norepinephrine remained at basal levels. Epinephrine was infused into either the peripheral or hepatic portal circulation at rates chosen so that the hepatic sinusoidal epinephrine concentration was the same with the two routes of delivery. During peripheral epinephrine infusion, arterial epinephrine levels were 1,064 ± 144 pg/mL, similar to the peak value obtained in the current study (990 ± 222 pg/mL).[19] $EndoR_a$ increased 2.9 mg · kg^{-1} · min^{-1} during peripheral epinephrine infusion (not different *v* $EndoR_a$ change observed during CGRP infusion), with gluconeogenesis accounting for approximately 60% of the increase and glycogenolysis for the remainder.[19] The increase in gluconeogenesis was accounted for by mobilization of gluconeogenic substrates from the muscle and adipose tissue. During portal epinephrine infusion, arterial epinephrine concentrations did not change from basal. $EndoR_a$ increased in response to portal epinephrine delivery, with enhancement of glycogenolysis accounting for all of the increase.[19] Thus, epinephrine's direct effect on the liver (at least over the 3-hour study period) was limited to glycogenolysis, and its gluconeogenic effects resulted from its action in peripheral tissues. With these combined hepatic and peripheral actions, epinephrine is capable of increasing $EndoR_a$ to a similar extent as observed during CGRP infusion.[19]

We have also examined the effects of a selective increase in norepinephrine on hepatic glucose metabolism.[20] Infusion of norepinephrine into the portal vein to create 30- to 40-fold basal portal concentrations without an increase in arterial norepinephrine increased the net hepatic glucose production in the dog about 1.3 mg · kg^{-1} · min^{-1} without altering the gluconeogenic rate significantly.[20] On the other hand, peripheral infusion of norepinephrine to elevate the arterial norepinephrine concentration to about 30-fold basal increased net hepatic glucose production to the same extent (~1.3 mg · kg^{-1} · min^{-1}) via a marked elevation of hepatic gluconeogenesis.[16,30] The increase in gluconeogenesis was due to an increase in the supply of gluconeogenic precursors to the liver. A 10-fold increase in arterial norepinephrine, similar to the magnitude of increase evident during CGRP infusion, did not increase hepatic glucose production in the conscious dog.[16] In fact, both $EndoR_a$ and NHGO declined during the 3-hour norepinephrine infusion period, despite increased release of lactate and glycerol from peripheral tissues and increased net hepatic uptake of these substrates.[16] A decrease in the rate of hepatic glycogenolysis was apparently responsible for the decrease in $EndoR_a$ and NHGO.[16] Norepinephrine concentrations within the synapse during CGRP administration were probably much higher than those observed in the peripheral circulation, and thus, it remains possible that norepinephrine played a larger role than was observed during peripheral norepinephrine infusion. Nevertheless, from the data available, it is unlikely that norepinephrine at the levels observed during CGRP infusion was responsible for stimulating hepatic glycogenolysis sufficiently to produce the changes that occurred in EndoRa and NHGO. However, norepinephrine may have acted in conjunction with epinephrine to stimulate the provision of gluconeogenic precursors to the liver.

There was little lipolysis evident during CGRP infusion. Glycerol increased a maximum of only 30%, and returned to basal values with the two highest CGRP infusion rates. Circulating NEFA concentrations did not increase significantly with any dosage of CGRP; indeed, they declined significantly at the two highest CGRP infusion rates. In light of the significant increases in catecholamine concentrations observed in the current studies, a marked lipolytic response would have been expected.[16,19,31] An antilipolytic effect of CGRP has not been previously reported and would be a surprising finding, since CGRP has been reported to increase cAMP levels in a variety of tissues,[32-34] as do catecholamines. Amylin administration has no apparent effect on lipolysis.[35,36] On the other hand, blood flow to adipose tissue decreases more than the blood flow to most other vascular beds in response to hypovolemic insults such as hemorrhage, and hypoperfusion of adipose tissue curtails the release of lipolytic products.[37-39] Lactate is also believed to be antilipolytic.[40,41] Thus, hyperlactatemia or hypoperfusion secondary to hypotension appear to be a more likely explanation for the blunted lipolytic response than a direct effect of CGRP.

In conclusion, CGRP infusion was associated with significant increases in $EndoR_a$, approximately half of which could be explained by an increase in the net hepatic uptake of gluconeogenic substrates and half of which must have resulted from intrahepatic events (eg, enhanced glycogenolysis). The increase in the mobilization of gluconeogenic substrates during CGRP infusion could have been a result of the action of CGRP per se, cortisol, and/or the catecholamines. Because CGRP has not been found to have any effect on hepatic carbohydrate metabolism, the catecholamines (particularly epinephrine) appear most likely responsible for the enhancement of hepatic glycogenolysis. Future studies (eg, examination of CGRP effects in a model with hypotension prevented) can address in a direct manner the question of whether the effects of CGRP on carbohydrate metabolism are secondary to changes in catecholamine release.

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