Characterization of Response of Circulating Glucagon to Intraduodenal and Intravenous Administration of Amino Acids

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ABSTRACT Studies were carried out to determine if hyperaminoacidemia stimulates the secretion of pancreatic glucagon, and, if so, to evaluate the effect of endogenous and exogenous pancreozymin and of hyperglycemia upon this response. The intravenous administration to 16 dogs of 1 g/kg of a 10 amino acid mixture over a 60 min period raised amino nitrogen to a mean level of 13.5 mg/100 ml; mean pancreaticoduodenal vein insulin rose from 84 to 459 μU/ml and glucagon from 1.1 to 2.7 mμg/ml. Further augmentation of both insulin and glucagon secretion was achieved during hyperaminoacidemia by infusing pancreozymin.

Since endogenous pancreozymin is known to be stimulated by amino acids in the gut, it seemed possible that intraduodenal loading of amino acids would elicit a greater insulin and glucagon response than could be explained by the accompanying hyperaminoacidemia. The intraduodenal administration of 1 g/kg of the amino acid mixture was followed by substantial hyperinsulinemia and hyperglucagonemia, which frequently anticipated the hyperaminoacidemia, and in many of the dogs the ratio of hormone rise to amino nitrogen rise was greater after intraduodenal than after the intravenous route of amino acid administration in the same animal. Intraduodenal administration of amino acids did not cause measurable release of intestinal glucagon-like immunoreactivity into the mesenteric vein plasma.

Hyperglycemia induced by constant glucose infusion prevented aminogenic hyperglucagonemia and even suppressed the augmenting action of pancreozymin; sudden termination of the infusion with continued amino acid infusion was associated with a striking rise in glucagon.

It is concluded (*a*) that hyperaminoacidemia stimulates pancreatic glucagon secretion, (*b*) that aminogenic hyperglucagonemia is augmented by the infusion of pancreozymin, (*c*) that intraduodenal administration of amino acids stimulates pancreatic glucagon secretion without measurable release of glucagon-like immunoreactivity into the mesenteric vein, and (*d*) that hyperglycemia prevents aminogenic hyperglucagonemia even during augmentation with pancreozymin. This conclusion suggests that the prevention of hypoglycemia during amino acid–induced insulin secretion may be an important function of glucagon.

Received for publication 20 May 1968.

INTRODUCTION

There is abundant experimental evidence which suggests that glucagon may be involved in the regulation of blood amino acid homeostasis. The administration of glucagon has been shown to lower plasma amino acids (1, 2), presumably by increasing their uptake by the liver (3), and to increase the incorporation of the carbon skeletons of certain amino acids into glucose (4, 5). Since recent studies with perfused rat livers indicate that this gluconeogenic effect occurs with physiologic

(6) as well as with pharmacologic increments of perfusing glucagon, it would not, therefore, be surprising if endogenous pancreatic glucagon secretion were stimulated by hyperaminoacidemia. If so, increased secretion of pancreatic glucagon during hyperaminoacidemia might account for the fact that, during aminogenic insulin secretion, blood glucose concentration does not fall to a degree commensurate with the observed level of hyperinsulinemia (7).

Assan, Rosselin, and Dolais (8) have already reported an apparent rise in peripheral plasma glucagon levels induced by hyperaminoacidemia; in 11 normal subjects given arginine by infusion a small increase averaging 0.33 mμg/ml was observed, and a rise averaging 0.25 mμg/ml was observed 50 min after the ingestion of 500 g of meat. Similar results were reported in four subjects by Fajans, Floyd, Knopf, and Conn (9). Our own group has reported striking rises in pancreaticoduodenal vein glucagon in 10 conscious dogs with indwelling catheters in the pancreaticoduodenal and mesenteric veins (10). The preparation was employed not only because measurements of glucagon in peripheral plasma may fail to detect small but physiologically important increases in pancreatic glucagon secretion (11), but also because such measurements cannot distinguish between pancreatogenous glucagon and enterogenous glucagon-like immunoreactivity (12–15), which probably comprises 70% or more of the total circulating immunoreactivity in the fasting state (16). During glucose absorption, for example, pancreatic glucagon secretion actually declines initially, but total immunoreactivity in the peripheral venous plasma rises; this rise is derived from gastrointestinal glucagon-like immunoreactivity (15).

In the present study, the triply catheterized dog was employed to permit simultaneous measurement of both pancreaticoduodenal vein glucagon and mesenteric vein glucagon-like immunoreactivity. In this system the proximity of sampling to the hormone source permits the detection of changes which may not be fully discernible in the peripheral blood and makes possible the differentiation between pancreatic glucagon and enteric glucagon-like immunoreactivity. The study was designed to verify the small apparent rise in "glucagon," previously reported to accompany hyperaminoacidemia, to determine if such a rise is derived from pancreatic glucagon, from enteric glucagon-like immunoreactivity, or from both, and to determine the effects of exogenous and endogenous pancreozymin and of hyperglycemia upon islet hormone responses to hyperaminoacidemia.

METHODS

All experiments were conducted in male mongrel dogs surgically prepared as follows. 2 days or more before each experiment, a dog was anesthetized with Nembutal, and the abdomen was opened by midline incision. A small glass *T* cannula, connected with Teflon tubing, was inserted into the superior pancreaticoduodenal vein at a distance of about 3 cm from its junction with the portal vein. The tubing was fixed at the duodenum with a suture and exteriorized. A second Teflon catheter was inserted through the left jugular vein with its opening reposing in the inferior vena cava between the heart and the hepatic vein. In some dogs a third catheter was passed through a mesenteric radicle into a major superior mesenteric vein. Until the time of the experiment, the pancreatic vein catheter was infused continuously at a constant rate with diluted heparin-saline solution so as to deliver 100 U of heparin in a volume of 20 ml/hr. Each animal received a daily intramuscular injection of 600,000 U of penicillin G. Dogs were fed on a Purina Chow diet, composed of 54% carbohydrate, 8% fat, and 24% protein.

Recovery from the surgical procedure was uneventful in the majority of dogs. Only dogs that appeared to be in good health were employed for experiments. The appearance of either diarrhea, loss of appetite, weight loss in excess of 1.5 kg, a white blood cell count above 30,000, or elevation of body temperature above 104°F was regarded as grounds for disqualification from the study. Dogs with a hematocrit of less than 35% were transfused on the day before the experiment. After completion of a series of experiments, each dog was sacrificed, and the position of the catheters and the patency of veins were checked. Dogs in which the pancreaticoduodenal vein was not patent were excluded from the study.

The amino acid mixture employed was identical in composition with that used by Floyd, Fajans, Conn, Knopf, and Rull (7):

	per cent		per cent
L-arginine	9.7	L-valine	12.0
L-lysine	15.1	L-histidine	4.9
L-phenylalanine	13.0	L-isoleucine	9.4
L-leucine	15.7	L-threonine	7.4
L-methionine	9.5	L-tryptophan	3.3

Blood specimens were obtained in syringes rinsed with a 10% solution of ethylenediaminetetraacetate (EDTA). Plasma was separated immediately and stored at −15 to −20°C for up to 30 days. Glucose concentration was measured by the ferricyanide method of Hoffman (17) using the Technicon AutoAnalyzer. Amino nitrogen con-

centration was determined by the method of Frame, Russel, and Wilhelmi (18). Insulin was measured by the radioimmunoassay of Yalow and Berson (19), and glucagon was assayed by the previously described radioimmunoassay (20) as recently modified (21). The antiglucagon antiserum employed was G128P, a serum which reacts with gastrointestinal glucagon-like immunoreactivity as well as with pancreatic glucagon.

When introduced into the assays in the concentrations encountered in plasma, the amino acid mixture did not affect either the glucagon or insulin radioimmunoassays.[1]

RESULTS

Effect of amino acid infusion. The 10 amino acid mixture was infused at a constant rate of 17 mg/kg of body weight per min for 60 min (a total dose of 1 g/kg) in a group of 16 dogs. Mean amino nitrogen concentration rose to a peak of 13.5 mg/100 ml (SEM ± 0.6). The mean pancreaticoduodenal vein insulin level rose promptly from a base line value of 84 μU/ml (SEM ± 22) to a peak level of 459 μU/ml (SEM ± 66). Mean glucagon concentration in the pancreaticoduodenal vein rose simultaneously in somewhat parallel fashion, from a base line level of 1.1 mμg/ml (SEM ± 0.1) to a peak value of 2.7 mμg/ml (SEM ± 0.4). The mean blood glucose concentration rose 4 mg/100 ml initially and subsequently declined 3 mg/100 ml (Fig. 1 *a*). The individual results are recorded in Table I.

Since the levels of amino nitrogen in the foregoing experiments were considerably above the 5.4–10.4 mg/100 ml range reported by Floyd et al. (7) to occur in man after a large protein meal, observations at more physiological levels of hyperaminoacidemia were made in another group of six dogs by reducing the infusion rate to 6.7 mg/min per kg of the amino acid mixture for a 75 min period (a total dose of 0.5 g/kg). Qualitatively

[1] Schalch has suggested that the amino acids interfere with the immunoassay of glucagon in vitro, and that the reported rise in glucagon after amino acid infusion may be an artifact (personal communication). To determine the in vitro effect of the amino acid mixture upon the glucagon assay, the mixture was assayed in varying concentrations. No interference with the glucagon assay was observed at concentrations below 2%. Since, in these experiments, the highest plasma concentration of amino nitrogen measured was 26 mg/100 ml, which corresponds to only 260 mg/100 ml of amino acids, it seems improbable that hyperaminoacidemia could have influenced the glucagon measurements reported here.

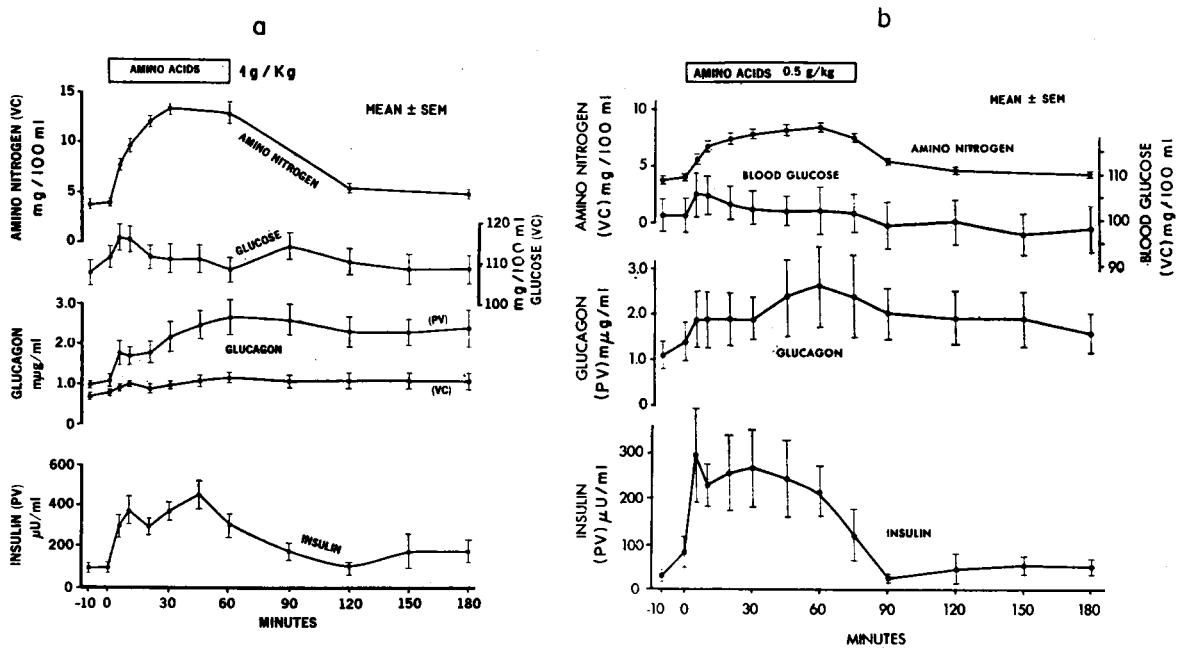

FIGURE 1 The effect of intravenous infusion of amino acid mixture upon pancreaticoduodenal vein (*PV*) concentration of insulin and glucagon. Panel *a* shows experiments in which amino acids were infused at a rate of 17 mg/kg per min for 60 min for a total dose of 1 g/kg per 60 min. Panel *b* shows the effects of their infusion at a rate of 6.7 mg/kg per min for 75 min for a total dose of 0.5 g/kg. The concentration of amino nitrogen and glucose were measured in inferior vena caval (*VC*) plasma.

TABLE I

Effects of Intravenous Administration of Amino Acids (1 g/kg) upon Blood Glucose, Glucagon, and Insulin

Dog No.	Measurement	Source	Minutes after start of infusion: Control period −10	Control period 0	Infusion period (17 mg/kg per min) 5	10	20	30	45	60	90	120	150	180
			min											
2–07	Amino *N* (*mg/100 ml*)	VC	4.4	4.2	8.0	10.5	12.5	18.0		15.5		5.8		4.2
	Glucose (*mg/100 ml*)	VC	109	100	102	105	103	101	107	100	110	105	105	115
	Glucagon (*mμg/ml*)	PV	0.8	0.7	1.2	1.6	3.6	1.3	1.9	4.4	1.6	1.0	1.0	1.9
	Glucagon	VC	0.5		0.8	0.8	0.8	1.0	1.1	1.0	0.9	0.8	0.7	0.8
	Insulin (*μU/ml*)	PV	79	55	286	253	228	198	248	203	79	40	60	60
2–11	Amino *N*	VC		3.6	5.8	5.6	9.5	10.5		12.0		5.0		5.0
	Glucose	VC	121	127	126	120	113	109	109	121	127	116	117	124
	Glucagon	PV	1.0	1.4	1.8	1.0	1.0	1.4	1.4	1.5	2.2	2.0	2.6	1.4
	Glucagon	VC	0.6	0.7	0.9	0.8	0.7	0.8	0.8	1.1	0.8	0.7	0.9	0.9
	Insulin	PV	20	99	208	248	471	407	258	298	40	25	179	164
2–12	Amino *N*	VC	5.2	5.2	8.6	10.5	13.0	16.0		19.5		6.4		5.6
	Glucose	VC	98	97	101	101	98	101	98	100	108	110	104	109
	Glucagon	PV	1.3	1.1	1.4	1.6	1.1	1.2	1.5	2.2	1.9	1.3	1.3	1.0
	Glucagon	VC	0.6	0.8	0.6	0.8	0.9	0.8	0.7	1.2	0.8	0.8	0.7	0.7
	Insulin	PV	193	131	179	188	322	744	392	794	179	278	114	278
2–15	Amino *N*	VC	4.4	4.0	7.2	9.5	13.0	10.0		12.5		4.8		4.8
	Glucose	VC	99	101	99	100	100	90	85	84	91	91	86	88
	Glucagon	PV	0.9	1.2	1.3	1.1	0.9	1.0	1.5	2.4	1.9	1.8	1.8	1.4
	Glucagon	VC	0.4	0.9	0.4	0.5	0.5	0.5	0.5	0.9	0.6	0.7	0.7	0.6
	Insulin	PV	164	99	179	496	332	397	451	491	99	89	20	27
2–32	Amino *N*	VC	2.7	4.0	9.0	10.6	13.5	15.5		11.0		5.6		5.6
	Glucose	VC	98	100	108	104	102	102	96	90	103	102	93	93
	Glucagon	PV	1.0	0.8	0.8	1.1	1.4	1.5	1.8	2.2	2.8	2.8	3.2	3.2
	Glucagon	VC	0.9	0.9	0.9	1.2	1.2	1.0	1.8	1.2	1.2	1.2	2.0	1.4
	Insulin	PV	79	60	193	228	164	293	595	134	382	193	188	159
2–33	Amino *N*	VC	3.6	3.6	9.2	10.5	11.0	15.0		12.5		5.0		4.3
	Glucose	VC	100	108	114	106	102	100	106	96	104	104	106	106
	Glucagon	PV	1.4	1.3	1.4	1.3	1.4	2.0	2.6	2.4	2.8	1.8	1.8	1.8
	Glucagon	VC	0.6	0.7	0.7	0.7	0.8	0.8	0.9	1.0	1.1	0.8	0.7	0.8
	Insulin	PV	99	47	144	694	352	546	1042	64	17	17	184	134
2–35	Amino *N*	VC	4.2	4.2	8.2	11.5	13.0	13.0		14.0		5.6		5.0
	Glucose	VC	109	108	111	104	110	112	122	114	112	110	103	97
	Glucagon	PV	0.7	0.8	1.7	1.8	1.0	2.7	2.8	2.4	2.4	1.8	3.0	4.0
	Glucagon	VC	0.5	0.6	0.9	1.0	1.0	0.8	1.2	1.8	0.8	0.8	0.8	1.0
	Insulin	PV	55	32	317	79	213	308	446	74	74	60	312	456
2–36	Amino *N*	VC	4.2	4.4	8.0	8.4	14.0	13.5		14.0		5.8		5.6
	Glucose	VC	106	110	113	112	109	108	94	94	110	95	95	94
	Glucagon	PV	0.7	0.4		0.8	1.0	1.4	4.0	1.6	4.2	3.4	2.6	1.4
	Glucagon	VC	0.4	0.3	0.6	0.5	0.4	0.5	0.5	0.6	0.8	0.6	0.4	0.4
	Insulin	PV	238	114		193	362	441	694	99	238	25	74	119
2–69	Amino *N*	VC	3.6	3.8	6.8	8.0	8.0	9.5		12.5		5.0		4.6
	Glucose	VC	110	119	119	121	117	120	124	127	129	120	109	110
	Glucagon	PV	1.7	2.6	3.0	3.4	3.2	4.4	5.2	3.9	5.2	4.0	4.6	6.2
	Glucagon	VC	1.4	1.4	1.7	1.7	0.9	1.9	2.1	1.9	2.8	3.6	3.6	3.8
	Insulin	PV	35	55	117	327	149	129	382	618	461	45	30	940
2–70	Amino *N*	VC	3.4	3.4	7.8	9.0	11.5	12.5		12.5		4.8		4.4
	Glucose	VC	104	114	125	118	114	119	116	112	118	115	109	103
	Glucagon	PV	0.7	0.7	0.7	0.8	0.9	1.1	1.4	1.4	1.3	1.5	1.7	1.5
	Glucagon	VC	0.7	0.7	0.8	0.8	0.8	1.0	0.9	0.9	0.9	0.8	0.9	0.8
	Insulin	PV	65	50	140	205	190	200	260	145	245	90	45	50
2–71	Amino *N*	VC	3.4	3.4	6.6	7.5	11.0	11.0		14.0		5.4		4.6
	Glucose	VC	114	121	132	132	130	140	134	117	124	124	135	133
	Glucagon	PV	1.0	1.0	1.8	1.4	1.8	1.4	1.4	2.4	1.8	1.0	1.6	1.8
	Glucagon	VC	1.0	1.1	1.4	1.2	1.2	1.2	1.2	1.4	1.2	1.2	1.4	1.4
	Insulin	PV	8	5	110	125	345	260	180	495	80	15	10	15

TABLE I—(*Concluded*)

Dog No.	Measurement	Source	Control period		Minutes after start of infusion: Infusion period (17 mg/kg per min)									
			−10	0	5	10	20	30	45	60	90	120	150	180
			min											
2–81	Amino *N*	VC	4.6	4.6	8.0	10.0	12.0	12.5		13.0		5.0		4.0
	Glucose	VC	120	118	125	128	131	126	126	125	137	141	136	132
	Glucagon	PV	0.8	1.2	5.2	2.4	3.2	4.0	5.6	7.6	5.4	6.6	4.6	6.6
	Glucagon	VC	0.6	0.8	1.0	1.1	1.2	1.4	1.6	1.6	1.2	1.4	1.0	1.2
	Insulin	PV	130	380	875	855	770	785	600	660	550	430	1460	135
2–82	Amino *N*	VC	4.0	4.2		13.0	16.5	16.5		18.0		5.6		4.8
	Glucose	VC	120	119	117	117	112	110	110	112	119	112	112	105
	Glucagon	PV	1.6	1.9	1.3	3.6	2.0	6.0	3.6	4.4	4.8	3.6	3.6	3.4
	Glucagon	VC	0.9	0.8	1.0	1.2	1.0	1.0	1.4	1.4	1.6	1.4	1.2	1.2
	Insulin	PV	203	84	764	779	308	283	908	278	60	20	15	218
2–84	Amino *N*	VC	3.6	3.8	9.6	11.0	14.0	16.0		12.8		6.0		4.6
	Glucose	VC	119	122	139	146	130	126	133	125	117	122	119	125
	Glucagon	PV	0.4	0.7	1.5	1.8	1.7	1.4	1.6	1.2	1.0	0.8	0.8	0.8
	Glucagon	VC	0.2	0.6	1.0	1.0	1.0	1.2	0.8	1.2	1.0	0.8	1.0	0.6
	Insulin	PV	119	84	397	769	238	278	491	99	99	20	60	30
2–85	Amino *N*	VC	3.6	4.0	8.1	10.5	12.5	14.5		17.0		5.3		5.0
	Glucose	VC	95	98	102	107	107	109	109	112	91	90	91	90
	Glucagon	PV	1.2	1.3	2.6	2.4	2.7	3.0	1.6	2.4	1.6	1.4	1.2	1.0
	Glucagon	VC	0.8	1.1	1.6	1.4	1.6	1.6	1.3	1.6	1.2	0.6	1.2	1.2
	Insulin	PV	8	30	190	155	105	345	50	240	30	5	10	10
2–86	Amino *N*	VC	3.2	3.8	6.8	9.0	10.5	11.8		11.8		5.3		5.0
	Glucose	VC	124	127	132	135	113	118	114	104	129	118	118	116
	Glucagon	PV	0.8	1.0	1.2	1.4	1.3	1.5	2.1	1.0	1.4	1.6	1.0	1.2
	Glucagon	VC	0.6	0.5	0.6	0.7	0.5	0.7	0.5	0.6	0.6	0.6	0.5	0.5
	Insulin	PV	70	35	315	445	175	265	345	30	60	20	70	60
Mean ± SEM														
	Amino N	VC	3.9	4.0	7.8	9.7	12.2	13.5		13.0		5.4		4.9
	±SEM		0.16	0.11	0.27	0.44	0.50	0.64		1.06		0.12		0.12
	Glucose	VC	107.9	111.8	116.6	116.0	111.9	111.9	111.4	108.3	114.3	110.9	108.6	108.8
	± SEM		3.0	2.6	3.1	3.4	2.7	3.1	3.5	3.3	3.3	3.3	3.6	3.6
	P value		NS		<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Glucagon	PV	1.0	1.1	1.8	1.7	1.8	2.2	2.5	2.7	2.6	2.3	2.3	2.4
	± SEM		0.09	0.13	0.29	0.21	0.23	0.36	0.35	0.42	0.36	0.38	0.31	0.45
	P value		NS		NS	<0.001	<0.025	<0.005	<0.001	<0.005	<0.001	<0.01	<0.001	<0.01
	Glucagon	VC	0.7	0.8	0.9	1.0	0.9	1.0	1.1	1.2	1.1	1.1	1.1	1.1
	±SEM		0.07	0.07	0.09	0.08	0.08	0.09	0.12	0.10	0.13	0.18	0.19	0.20
	P value		NS		<0.025	<0.005	NS	<0.005	<0.005	<0.005	<0.005	NS	NS	NS
	Insulin	PV	97.8	83.8	293.1	377.4	295.3	367.4	458.9	295.1	168.3	85.8	176.9	178.4
	±SEM		17.8	21.6	59.2	65.2	40.0	46.4	65.5	60.4	41.0	29.4	88.1	58.5
	P value		NS		<0.005	<0.001	<0.001	<0.001	<0.001	<0.005	<0.025	NS	NS	NS

VC, vena cava; PV, pancreaticoduodenal vein.

similar results were obtained, although the increase in glucagon was not statistically significant. Mean insulin concentration rose to an early peak of 296 μU/ml, while mean glucagon rose concomitantly to 2.6 mμg/ml. Mean plasma glucose rose 5 mg/100 ml initially and later fell 4 mg/100 ml (Fig. 1 *b*). The results of all experiments are presented in Table II.

Effects of intraduodenal amino acid administration. A 1.0 g/kg quantity of the amino acid mixture was administered intraduodenally over a 45 min period to the same group of dogs whose response to the 1.0 g/kg intravenous load appeared in Table I. This was followed by an elevation of mean vena caval amino nitrogen to a peak level of 10.7 mg/100 ml. The mean pancreaticoduodenal insulin concentration rose to a peak of 345 μU/ml (SEM ± 86) and glucagon rose to a peak of 2.1 mμg/ml (SEM ± 0.3) (Fig. 2). The results of individual intraduodenal experiments are recorded in Table III.

The source of apparent hyperglucagonemia accompanying amino acid absorption. It has recently been shown that the apparent rise in gluca-

TABLE II

Effect of Intravenous Amino Acids (0.5 g/kg) on Glucose, Glucagon, and Insulin

Dog No.	Measurement*	Source	Minutes after start of infusion: Control period −10	Control period 0	Infusion period (6.7 mg/kg per min) 5	10	20	30	45	60	75	90	120	150	180
			min												
3-25	Amino *N*	VC	3.7	4.0	5.9	7.3	7.8	8.5	9.3	9.5	8.5	5.4	4.6		4.3
	Glucose	VC	92	89	91	91	91	91	97	91	94	82	91	91	91
	Glucagon	PV	1.5	1.6	2.6	2.3	2.9	3.0	2.8	2.9	2.7	2.6	2.2	2.3	2.1
	Insulin	PV	26	20	70	126	160	150	136	80	400	10	20	60	30
3-30	Amino *N*	VC	4.3	4.2	5.6	6.0	7.3	8.5	8.3	8.5	8.0	6.2	5.0		4.0
	Glucose	VC	109	112	126	118	112	109	110	113	113	106	106	104	102
	Glucagon	PV	2.0	2.8	4.4	4.2	3.2	3.0	6.0	6.8	6.2	3.8	4.4	4.4	3.4
	Insulin	PV	26	106	546	150	100	100	146	250	110	0	10	160	70
3-31	Amino *N*	VC	3.8	3.7	5.3	7.0	8.5	8.5	8.0	7.5	6.0	4.6	4.2		3.4
	Glucose	VC	94	94	98	98	98	98	100	102	99	106	106	99	92
	Glucagon	PV	1.5	2.4	2.2	2.8	3.2	2.5	2.6	3.0	3.2	3.2	2.6	2.4	2.0
	Insulin	PV	10	140	290	310	430	370	136	160	0	10	220	30	100
3-51	Amino *N*	VC	3.0	2.8	3.4	4.9	6.0	6.5	6.3	8.5	7.5	4.6	4.2		4.0
	Glucose	VC	113	110	114	116	115	116	113	118	114	110	106	111	112
	Glucagon	PV	0	0	0.1	0.2	0.2	0.5	0.6	0.6	0.6	0.7	0.6	0.1	0.6
	Insulin	PV	0	0	40	100	80	140	100	200	110	30	20	20	20
3-52	Amino *N*	VC	4.0	4.1	5.8	6.4	6.0	6.5	8.0	7.5	6.5	5.3	4.6	4.6	4.2
	Glucose	VC	102	106	106	106	106	108	104	105	103	107	112	100	112
	Glucagon	PV	0.6	0.6	0.8	0.6	0.6	0.9	1.0	0.9	0.6	0.8	0.9	1.0	0.9
	Insulin	PV	20	20	170	380	200	180	280	130	20	40	20	40	60
3-54	Amino *N*	VC		5.0	7.4	8.3	9.0	8.5	8.5	8.3	7.8	5.7	5.7	5.3	5.1
	Glucose	VC	98	98	102	107	104	97	92	86	90	83	80	80	84
	Glucagon	PV	1.0	1.0	1.5	1.0	1.1	1.2	1.3	1.1	0.8	1.0	0.8	1.1	0.8
	Insulin	PV	100	220	660	300	580	640	660	480	60	40	0	20	30
Mean ± SEM															
	Amino *N*	VC	3.8	4.0	5.6	6.7	7.4	7.8	8.1	8.3	7.4	5.3	4.7		4.2
	± SEM		0.2	0.3	0.5	0.5	0.5	0.4	0.4	0.3	0.4	0.3	0.3		0.3
	Glucose	VC	101.3	101.5	106.2	106.0	104.3	103.2	10.27	102.5	102.1	99.0	1002.	97.5	98.8
	± SEM		3.6	3.8	5.1	4.2	3.8	3.9	3.3	5.4	4.4	5.3	5.0	4.4	4.9
	Glucagon	PV	1.1	1.4	1.9	1.9	1.9	1.9	2.4	2.6	2.4	2.0	1.9	1.9	1.6
	± SEM		0.29	0.44	0.62	0.62	0.56	0.46	0.85	0.89	0.90	0.56	0.59	0.62	0.44
	P value				NS	NS	NS	NS	NS	NS	NS	<0.025	NS	NS	NS
	Insulin	PV	30.3	84.3	296	228	258	263	243	317	117	21.6	48.3	55.0	51.6
	± SEM		14.5	35.3	104.5	47.6	82.4	84.9	87.2	57.7	59.6	7.1	34.5	21.9	12.5
	P value				<0.05	<0.05	<0.025	NS	NS	<0.05	NS	NS	NS	NS	NS

VC, vena cava; PV, pancreaticoduodenal vein.

* Amino *N* (mg/100 ml); Glucose (mg/100 ml); Glucagon (mμg/ml); Insulin (μU/ml).

gon after glucose loading first reported by Samols, Marri, Tyler, and Marks (22) and by Lawrence (23) occurs in the absence of the pancreas and is probably derived from glucagon-like immunoreactivity present in the gastrointestinal tract (15). To examine the possibility of an intestinal contribution to the apparent rise in plasma glucagon which follows intraduodenal amino acid loading, mesenteric venous plasma collected during absorption of a 1.0 g/kg amino acid load was assayed for glucagon. The results, illustrated in Fig. 3, reveal no significant measurable rise in mesenteric vein glucagon-like immunoreactivity; statistically significant increments above the value at zero time occurred only in the pancreaticoduodenal vein plasma and indicated that the hyperglucagonemia was largely, if not entirely, pancreatogenous.

Effect of pancreozymin on islet hormone response to hyperaminoacidemia. Since the islet response observed during hyperaminoacidemia was qualitatively identical with that which follows infusion of pancreozymin (21), a hormone released during amino acid absorption (24), it seemed of interest to determine if pancreozymin was capable of augmenting the secretion of insulin and glucagon during their stimulation by hyperaminoacidemia.

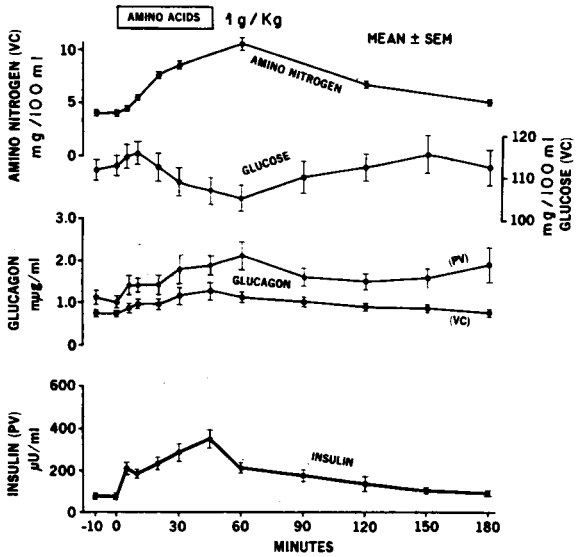

FIGURE 2 The effect of intraduodenal administration of amino acid mixture (1 g/kg) upon glucagon and insulin concentration in the pancreaticoduodenal vein plasma.

Highly purified exogenous pancreozymin,[2] infused at a rate of 8 Harper-Raper Units/min, caused in each of six experiments an immediate and sharp rise in the pancreaticoduodenal vein concentration of both hormones, already elevated by the hyperaminoacidemia. The most dramatic example is shown in Fig. 4, and the results of all such experiments are recorded in Table IV.

Comparison of intravenous and intraduodenal routes of amino acid loading. The possibility that islet hormone response to ingested amino acids might be augmented by endogenous pancreozymin was tested by comparing the islet hormone response to intraduodenal amino acid loading with that observed during intravenous amino acid infusion, each dog serving as his own control. The hormone response after the intraduodenal administration of 1 g/kg over a 45 min period (Table III) did not differ significantly from that observed after the intravenous administration of 1.0 g/kg over a period of 60 min (Table I). Comparison of the areas under the insulin and glucagon curves, or of the ratios obtained by dividing these areas of hormone response by the area under the amino nitrogen curve, also failed to yield a statistically significant difference (Table VI A).

It seemed possible that the lack of a significant difference might reflect a fault of the experimental design. First, the intraduodenal tubes had been placed in a caudal direction, and sufficient exposure of the upper duodenum to the amino acids might not have occurred; second, the 7.5 pH of the amino acids might not have been low enough to evoke a maximum discharge of intestinal hormones; and finally the unphysiologically high amino acid levels induced by the 1 g/kg dose might have concealed a relatively small degree of enterohumoral augmentation. For these reasons the same six dogs, whose response to 0.5 g/kg (6.7 mg/min) of amino acids given intravenously appears in Table II, were given 0.5 g/kg dose of amino acids adjusted to pH 4, the postprandial

[2] Obtained through the kindness of Professor Erik Jorpes and Viktor Mutt, Karolinska Institute, Stockholm, Sweden.

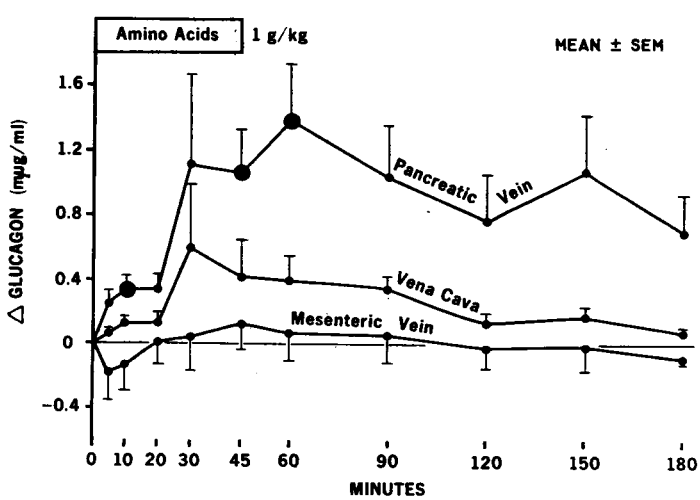

FIGURE 3 Mean increments of glucagon in the pancreaticoduodenal, inferior vena caval, and mesenteric vein plasma after the intraduodenal administrataion of 1 g/kg of amino acid mixture in a group of eight dogs. A heavy dot on the mean curve indicates that this point differs significantly ($P < 0.01$) from the mean value at zero time.

TABLE III

Effects of Intraduodenal Amino Acids (1 g/kg) upon Blood Glucose, Glucagon, and Insulin

Dog No.	Measurement	Source	Control period		Minutes after start of infusion: Intraduodenal infusion period (22 mg/kg per min)									
			−10	0	5	10	20	30	45	60	90	120	150	180
			min											
2–07	Amino *N* (*mg/100 ml*)	VC		3.8	4.4	4.6	6.0	7.5		10.0		6.6		
	Amino *N*	MV	3.6	5.0	6.5	10.5		8.5		18.0		9.5		5.4
	Glucose (*mg/100 ml*)	VC	108	103	105	115	100	80	86	89	98	93	82	83
	Glucagon (*mμg/ml*)	PV	2.6	1.4	1.8	1.8	1.1	6.2	3.0	3.6	4.0	3.8	4.2	3.4
	Glucagon	VC	1.0	1.0	1.3	1.3	1.0	4.2	3.0	1.4	1.8	1.0	1.5	1.2
	Glucagon	MV	1.4	1.8	1.0	1.0	1.3	1.0	1.4	1.4	1.6	1.5	1.6	1.7
	Insulin (*μU/ml*)	PV	69	154	119	164	99	243	40	79	60	45	47	47
2–11	Amino *N*	VC	3.4	3.4	4.0	5.8	7.0	6.5		5.0		4.4		3.6
	Amino *N*	MV	4.4	3.4	10.5	15.0	15.0	17.0		7.0		7.0		4.4
	Glucose	VC	114	119	129	128	110	123	124	123	134	118	122	
	Glucagon	PV	0.6	0.8	0.8	0.9	0.8	1.0	1.0	0.9	0.9	1.0	2.0	1.1
	Glucagon	VC	0.4	0.5	0.7	0.5	0.5	0.5	0.6	0.5	0.6	0.7	0.6	0.6
	Glucagon	MV	0.6	0.6	0.7	0.6	0.8	0.8	0.7	1.0	0.7	0.6	0.8	0.7
	Insulin	PV	17	139	129	109	377	99	174	104	34	84	129	149
2–12	Amino *N*	VC	4.2	4.4	5.2	7.2	9.5	10.0		10.5		7.4		6.4
	Amino *N*	MV	4.6	3.0	10.5	18.2	18.2	20.0		20.0		11.0		9.2
	Glucose	VC	110	109	110	115	109	107	112	109	118	114	116	117
	Glucagon	PV	1.9	1.1	1.7	1.9	1.7	2.4	3.2	4.5	3.4	2.2	3.4	2.4
	Glucagon	VC	0.7	0.7	0.8	0.9	0.9	1.5	1.0	1.9	1.0	0.8	0.8	0.8
	Glucagon	MV	0.6	1.7	0.9	0.9	1.0	0.9	1.2	0.9	0.7	1.1	1.4	1.4
	Insulin	PV	164	139	268	372	417	317	233	60	208	1240	45	347
2–15	Amino *N*	VC	5.2	5.0	5.0	7.0	9.0	11.0		12.5		7.2		6.2
	Amino *N*	MV	4.0	3.4	7.0	12.0	11.0	12.0		20.0		12.2		3.2
	Glucose	VC	106	107	105	102	100	91	84	78	82	86	87	81
	Glucagon	PV	0.6	0.7	1.1	1.0	1.3	1.9	2.8	2.6	1.6	1.7	1.2	1.2
	Glucagon	VC	0.3	0.4	0.4	0.6	0.8	0.8	1.1	1.0	1.0	0.6	0.5	0.6
	Glucagon	MV	0.5	0.5	0.7	0.7	1.0	1.4	1.4	1.1	0.6		0.3	0.2
	Insulin	PV	174	42	193	238	268	357	476	278	40	20	17	119
2–32	Amino *N*	VC	4.6	4.6	4.8	5.8	8.5	9.5		11.0		9.0		6.2
	Amino *N*	MV		5.6	7.0	12.0	15.0	19.0		21.0		12.5		7.2
	Glucose	VC	110	117	115	114	113	113	109	107	104	107	113	113
	Glucagon	PV	0.9	0.8	0.8	1.1	1.3	1.1	1.1	1.6	1.4	1.2	1.0	1.2
	Glucagon	VC	0.8	0.8	0.8	1.0	0.8	0.8	0.8	1.4	1.2	1.0	0.8	0.8
	Glucagon	MV	0.8	0.9	0.8	1.1	1.0	1.0	1.1	1.2	1.2	1.2	0.8	0.8
	Insulin	PV	40	47	79	570	74	129	193	134	124	17	144	55
2–33	Amino *N*	VC	4.0	4.2		4.8	6.0	7.5		12.5		7.0		4.8
	Amino *N*	MV	3.6	5.2	5.5	8.0	8.0	11.0		22.0		9.5		5.8
	Glucose	VC	117	116	117	117	120	120	116	114	112	118	126	125
	Glucagon	PV	0.8	0.6	0.8	1.0	0.9	0.9	1.1	1.3	1.6	1.3	1.0	1.0
	Glucagon	VC	0.4	0.6	0.6	0.8	0.8	0.8	0.6	0.6	0.8	0.6	0.6	0.6
	Glucagon	MV	0.5	0.4	0.5	0.5	0.6	0.6	0.8	0.8	0.9	0.6	0.7	0.4
	Insulin	PV	84	74	30	154	744	213	188	466	466	139	283	84
2–35	Amino *N*	VC	3.8	3.8	3.8	4.2	6.5	7.5		9.5		6.8		5.8
	Amino *N*	MV	4.6	4.6	6.5	14.0	15.0	19.0		23.0		10.0		9.2
	Glucose	VC	104	108	112	112	108	106	107	102	104	107	104	99
	Glucagon	PV	0.6	0.6	0.6	0.9	1.2	1.7	1.8	2.0	1.0	1.2	1.0	1.0
	Glucagon	VC	0.5	0.5	0.6	0.5	0.8	0.8	0.8	0.8	0.8	1.0	0.8	0.6
	Glucagon	MV	0.6	0.6	0.6	0.6	0.8	0.9	0.9	0.8	1.2	0.6	0.8	0.6
	Insulin	PV	40	89	188	169	94	198	794	79	293	60	144	69

VC, vena cava; MV, mesenteric vein; PV, pancreaticoduodenal vein.

* *P* values, against each zero time sample.

‡ Mean of eight experiments.

TABLE III—(*Continued*)

Dog No.	Measurement	Source	Control period		Minutes after start of infusion: Intraduodenal infusion period (22 mg/kg per min)									
			−10	0	5	10	20	30	45	60	90	120	150	180
			min											
2–36	Amino *N*	VC	4.0	4.0	4.0	5.2	7.5	7.7		12.0		7.2		5.4
	Amino *N*	MV	4.0	4.2		11.0	18.0	18.0		19.0		8.5		4.6
	Glucose	VC	112	110	109	114	114	112	110	106	107	112	118	119
	Glucagon	PV	0.4	0.7	1.1	0.8	0.5	0.4	1.0	1.2	1.0	0.4	1.4	0.8
	Glucagon	VC	0.2	0.5	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.8	0.4
	Glucagon	MV	0.2	0.2		0.4	0.3	0.4	0.4	0.2	0.2	0.4	0.2	0.2
	Insulin	PV	104	119	104	64	79	159	466	208	99	94	94	99
2–69	Amino *N*	VC	4.0	3.0	5.8	6.2	10.5	12.0		17.0		5.4		5.2
	Amino *N*	MV	4.0	4.0	12.5	16.0	19.0	2.15		26.0		6.0		5.4
	Glucose	VC	113	114	130	120	123	120	114	117	118	129	139	137
	Glucagon	PV	2.4	3.0	4.4	3.5	4.7	4.2	4.0	4.2	2.0	2.1	2.0	2.2
	Glucagon	VC	1.7	1.6	2.1	1.9	2.1	1.8	3.2	2.3	1.9	1.5	1.5	1.7
	Insulin	PV	145	100	245	230	445	1350	1250	500	25	50	125	123
2–70	Amino *N*	VC	3.6	3.8	3.6	5.0	6.5	8.5		9.5		5.4		5.4
	Amino *N*	MV	4.2	4.0	5.5	8.5	12.0	14.0		15.5		5.0		7.8
	Glucose	VC	100	102	105	107	103	101	100	98	110	117	114	105
	Glucagon	PV	0.7	0.7	0.6	0.8	0.7	0.9	1.0	0.9	0.9	1.0	0.8	0.8
	Glucagon	VC	0.7	0.8	0.8	0.8	0.8	0.9	1.0	0.9	0.7	0.8	0.6	0.6
	Insulin	PV	130	55	175	60	85	210	215	290	35	95	90	60
2–71	Amino *N*	VC	4.2	4.4	4.6	5.4	7.0	7.5		8.5		5.6		5.2
	Amino *N*	MV	6.2	6.2		14.5	16.0	16.5		16.0		5.0		6.0
	Glucose	VC	128	129	124	124	129	125	121	126	129	137	133	137
	Glucagon	PV	1.2	1.4	1.4	1.4	1.4	1.3	1.4	0.6	1.2	1.2	1.0	1.2
	Glucagon	VC	1.0	1.0	1.0	1.2	1.2	1.2	1.3	1.2	1.2	1.0	1.2	0.8
	Insulin	PV	25	15	77	25	50	45	35	120	20	20	10	20
2–81	Amino *N*	VC	4.2	3.8	4.2	6.4	9.0	10.0		11.5		6.2		4.8
	Amino *N*	MV	5.4	4.4	6.0	11.0	14.0	17.0		22.5		9.0		6.4
	Glucose	VC	125	123	127	124	122	116	114	117	136	130	149	127
	Glutagon	PV	1.1	1.0	1.1	1.2	1.3	1.4	1.1	1.4	1.0	1.0	1.0	
	Glucagon	VC	0.8	0.6	0.8	0.8	0.8	0.8	0.8	1.0	0.4	0.8	0.6	0.6
	Insulin	PV	95	90	380	190	330	570	825	625	765	210	270	90
2–82	Animo *N*	VC	3.8	3.8	3.8	4.4	5.5	7.0		9.0		7.4		5.8
	Amino *N*	MV	5.0	4.8	12.5	15.5	18.5	19.0		17.0		8.5		8.0
	Glucose	VC	113	108	107	106	100	94	95	93	97	104	112	110
	Glucagon	PV	1.2	1.1	1.2	1.6	1.4	1.5	2.2	4.2	1.4	1.6	2.4	1.6
	Glucagon	VC	0.9	0.9	0.8	1.0	1.2	1.2	1.7	1.4	1.4	1.2	1.2	0.8
	Insulin	PV	20	79	99	193	74	114	208	60	30	20	30	60
2–84	Amino *N*	VC	4.0	4.2	4.0	6.0	8.5	9.3		12.5		7.2		5.3
	Amino *N*	MV	4.2	4.0	4.5	12.5	13.0	14.5		14.3		7.8		5.9
	Glucose	VC	118	125	131	135	140	128	118	106	122	112	114	122
	Glucagon	PV	0.8	0.8	1.1	1.3	1.2	1.4	1.7	1.6	1.4	1.2	1.0	1.2
	Glucagon	VC	0.6	0.5	0.6	1.0	1.0	1.0	1.3	p.6	1.0	0.8	0.8	1.0
	Insulin	PV	60	45	918	288	203	406	278	129	491	50	69	60
2–85	Amino *N*	VC	3.9	3.8	5.0	6.0	6.8	6.8		8.0		8.0		
	Amino *N*	MV	3.9	4.0	8.3	8.5	10.0	10.5		9.0		7.8		4.4
	Glucose	VC	91	91	92	96	93	90	90	88	92	98	96	100
	Glucagon	PV	0.9	0.8	1.2	1.4	1.1	1.3	1.5	1.1	2.1	1.0	1.0	1.0
	Glucagon	VC	0.9	1.0	1.3	1.7	1.3	1.3	1.3	1.1	1.1	1.0	0.9	0.5
	Insulin	PV	13	23	60	40	70	10	35	10	20	20	30	60
2–86	Amino *N*	VC	3.5	3.8	4.7	5.8	8.0	8.5		11.0		7.2		5.3
	Amino *N*	MV	4.0	3.9	10.0	15.0	16.5	16.5		17.5		8.5		6.0
	Glucose	VC	117	119	118	115	114	106	108	105	100	114	116	110
	Glucagon	PV	1.0	1.0	2.1	1.2	1.7	1.9	1.9	2.3	1.6	1.3	1.4	1.3
	Glucagon	VC	0.6	0.5	0.7	0.8	0.9	1.0	1.1	1.2	0.9	0.9	0.9	0.7
	Insulin	PV	65	50	240	25	225	105	125	240	40	79	40	70

TABLE III—(*Concluded*)

Dog No.	Measurement	Source	Control period −10	Control period 0	Intraduodenal infusion period (22 mg/kg per min) Minutes after start of infusion 5	10	20	30	45	60	90	120	150	180
			min											
Mean ± SEM*														
	Amino *N*	VC	4.0	4.0	4.5	5.6	7.6	8.6		10.7		6.8		5.4
	± SEM		0.11	0.12	0.16	0.22	0.36	0.40		0.66		0.23		0.19
	Amino *N*	MV	4.4	4.4	8.1	12.7	14.5	15.9		18.0		8.6		6.2
	± SEM		0.18	0.21	0.71	0.75	0.82	0.94		1.24		0.56		0.43
	Glucose	VC	111.6	112.5	114.8	115.3	112.4	108.3	106.8	104.9	110.2	112.3	115.1	112.3
	± SEM		2.3	2.4	2.8	2.5	3.9	3.5	3.1	3.3	3.8	3.3	4.4	4.4
	P value				NS	NS	NS	NS	<0.01	<0.01	NS	NS	NS	NS
	Glucagon	PV	1.1	1.0	1.4	1.4	1.4	1.8	1.9	2.1	1.6	1.5	1.6	1.9
	± SEM		0.16	0.15	0.23	0.17	0.24	0.36	0.23	0.33	0.22	0.19	0.24	0.45
	P value				<0.01	<0.005	<0.01	<0.02	<0.001	<0.02	<0.02	<0.05	<0.05	NS
	Glucagon	VC	0.7	0.7	0.9	1.0	1.0	1.2	1.3	1.2	1.0	0.9	0.9	0.8
	± SEM		0.09	0.08	0.10	0.10	0.10	0.22	0.20	0.13	0.11	0.08	0.08	0.08
	P value				<0.05	<0.001	<0.001	<0.05	>0.01	>0.001	<0.01	<0.01	<0.02	NS
	Glucagon	MV	0.7	0.8	0.7	0.7	0.9	0.9	1.0	0.9	0.9	0.9	0.8	0.8
	± SEM†		0.12	0.21	0.06	0.09	0.11	0.10	0.12	0.13	0.15	0.15	0.17	0.19
	P value				NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Insulin	PV	77.8	78.8	206.5	180.7	227.1	282.8	345.3	211.4	172.5	139.6	104.2	94.2
	± SEM		13.3	10.7	52.7	35.8	48.5	79.7	85.5	44.9	55.3	74.5	24.8	18.7
	P value				<0.05	<0.02	<0.01	<0.02	<0.01	<0.02	NS	NS	NS	NS

pH of gastric contents in dogs, through an intraduodenal tube which pointed cephalad and terminated in the upper portion of the duodenum. The mean level of amino nitrogen rose to a peak of 7.4 mg/100 ml. The mean insulin rose from a base line level of 57 to a peak of 148 μU/ml, and the mean glucagon level rose from 1.9 to 2.8 mμg/ml, even though in two of six dogs glucagon failed to rise at all. These results are recorded in Table V, and the mean response is shown in Fig. 5. Despite the seemingly brisker initial hormone response relative to the amino nitrogen level, the results did not differ to a statistically significant degree from the results of the intravenous experiments (Table VI B).

Despite the lack of statistically significant evidence of enhancement of the hormonal response with intraduodenal administration, with the large intraduodenal load (1 g/kg), the glucagon response per mg/100 ml rise in amino nitrogen to this route of administration exceeded the response to intravenous loading in 11 of 16 dogs, and the insulin response was greater in 6 of 16. With the smaller amino acid load (0.5 g/kg), the glucagon response to intraduodenal administration was greater in all six comparisons, and the insulin response was greater in three of six comparisons. The ratios of hormone rise to amino nitrogen rise are recorded for all experiments in Tables VI A and B. The frequent occurrence of a seemingly greater hormonal response to intraduodenally administered amino acid at a lower amino nitrogen level makes it difficult to exclude augmentation from the gut. Furthermore, it should be pointed out that the abrupt rise in mean insulin concentration 5 min after the intraduodenal administration of amino acids and the similarly rapid increase in mean glucagon concentration at 5 and 10 min anticipate the appearance of hyperaminoacidemia (Figs. 2 and 5) and also are compatible with enterohumoral stimulation of the islets.

Effect of hyperglycemia on aminogenic hyperglucagonemia. It has been reported previously that hyperglycemia suppresses glucagon secretion both in the fasting state (15) and during insulin hypoglycemia (25), but the effect of hyperglycemia upon aminogenic hyperglucagonemia has not been tested. Therefore, in a group of five dogs, glucose was infused at a constant rate of 200 mg/min, for at least 90 min before and during the intravenous infusion of amino acids, administered at a rate of 100 mg/min. The pancreaticoduodenal vein concentration of glucagon, which had declined during hyperglycemia from a mean

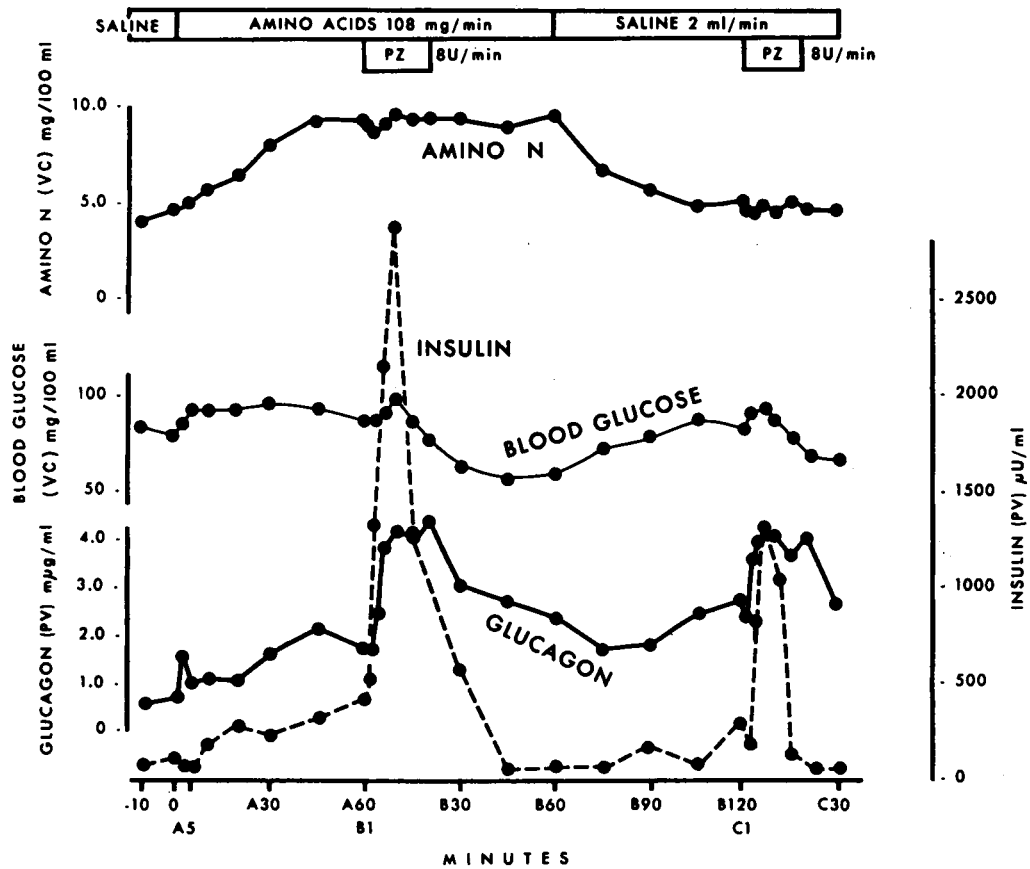

FIGURE 4 The effect of pancreozymin infusion (*PZ*) upon the levels of pancreaticoduodenal vein insulin and glucagon during hyperaminoacidemia.

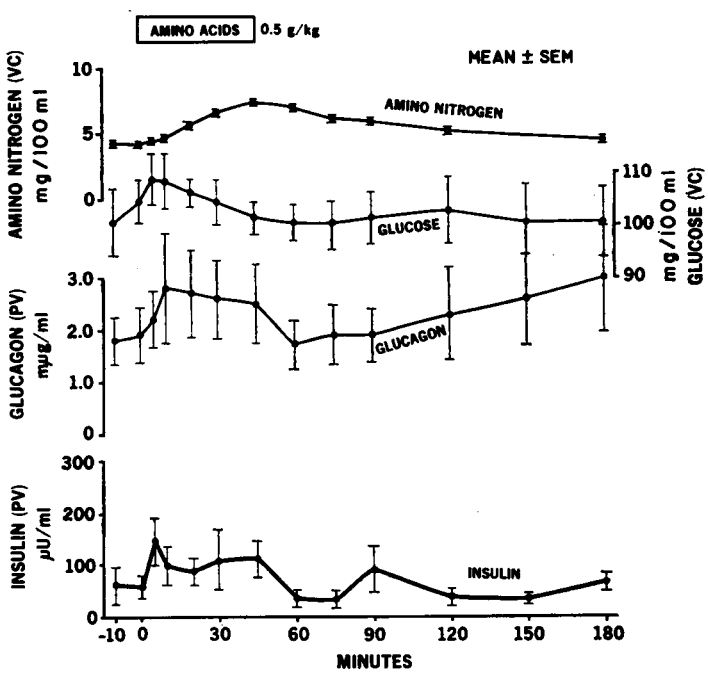

FIGURE 5 The effect of intraduodenal administration of 0.5 g/kg of amino acid mixture upon the mean pancreaticoduodenal vein concentrations of glucagon and insulin in a group of six conscious dogs. The mixture was adjusted to pH 4 and introduced via an intraduodenal tube which pointed cephalad and terminated in the upper portion of the duodenum.

TABLE IV

Effect of Pancreozymin Infusion, 8 U/min, upon

Dog No.	Measurement	Source	Control period	Minutes after amino acid infusion (A)					
				Amino acid infusion (100 mg/min)					
				5	10	20	30	45	60
			min						
2–55	Glucose (*mg/100 ml*)	VC	83	92	92	92	96	94	88
	Amino *N* (*mg/100 ml*)	VC	5.0	5.0	5.8	6.5	8.0	8.0	8.0
	Insulin (*μU/ml*)	PV	298	94	198	293	338	337	402
	Glucagon (*mμg/ml*)	PV	2.8	1.0	1.1	1.1	1.7	2.2	1.7
	Glucagon (*mμg/ml*)	VC	0.7	0.4	0.4	0.4	0.5	0.6	0.4
3–03	Glucose	VC	98	103	95	94	94	99	98
	Amino *N*	VC	3.4	6.8	7.6	9.8	10.2		12.8
	Insulin	PV	90	140	40	165	195	275	170
	Glucagon	PV	2.4	3.2	3.0	1.5	3.8	4.8	4.0
	Glucagon	VC	1.2	1.3	1.6	1.2	1.3	1.7	1.5
3–20	Glucose	VC	104	106	107	111	112	106	100
	Amino *N*	VC	3.8	6.2	6.5	7.8	8.5		8.5
	Insulin	PV	100	150	370	356	100	300	120
	Glucagon	PV	5.7	5.6	6.4	7.2	3.2	7.6	6.0
	Glucagon	VC	1.8	2.0	2.2	2.4	3.2	3.2	3.0
3–22	Glucose	VC	124	125	126	127	126	122	126
	Amino *N*	VC	4.3	5.2	5.8	6.1	6.1		6.9
	Insulin	PV	186	50	176	250	500	410	276
	Glucagon	PV	3.2	5.4	5.0	3.8	6.4	6.8	7.2
	Glucagon	VC	1.4	1.6	1.4	1.3	1.2	1.4	1.7
3–23	Glucose	VC	100	100	100	100	100	100	102
	Amino *N*	VC	4.0	5.8	6.4	6.5	7.0		8.5
	Insulin	PV	20	50	70	80	230	90	100
	Glucagon	PV	4.8	6.8	4.0	4.6	5.2	5.8	5.4
	Glucagon	VC	1.6	1.7	1.6	1.6	2.2	2.4	2.8
3–24	Glucose	VC	134	137	140	136	134	128	126
	Amino *N*	VC	5.4	7.8	9.3	9.8	10.5		11.0
	Insulin	PV	380	700	760	760	700	1000	860
	Glucagon	PV	2.1	2.9	2.6	2.5	2.0	3.0	2.6
	Glucagon	VC	1.1	1.2	1.2	1.0	1.0	1.0	1.1
Mean ± SEM									
	Amino *N*	VC	4.3	6.1	6.9	7.8	8.4		9.3
	± SEM		0.31	0.43	0.55	0.69	0.71		0.89
	Glucose	VC	107.1	110.5	110.0	110.0	110.3	108.2	106.7
	± SEM		7.6	6.9	7.8	7.4	6.8	5.6	6.4
	Insulin	PV	179.0	197.3	269.0	317.3	327.2	402.0	321.2
	± SEM		55.9	102.0	109.1	97.0	92.3	127.3	117.1
	Glucagon	PV	3.5	4.2	3.7	3.5	3.7	5.0	4.5
	± SEM		0.59	0.88	0.76	0.93	0.75	0.87	0.86
	Glucagon	VC	1.3	1.4	1.4	1.3	1.6	1.7	1.8
	± SEM		0.16	0.23	0.24	0.27	0.40	0.39	0.41

PV, pancreaticoduodenal vein; VC, vena cava.

Minutes after amino acid and pancreozymin infusion (B)								
Amino acid infusion (100 mg/min)								
Pancreozymin infusion (8 U/min)								
1	3	6	10	15	20	30	45	60
88	88	90	98	85	73	64	56	60
9.0	9.0	9.0	9.5	9.5	9.5	9.5	9.5	9.5
546	1290	2133	2827	1240	942	446	50	84
1.7	2.5	3.9	4.1	4.0	4.4	3.0	2.7	2.4
0.4	0.6	0.9	1.0	1.0	1.2	1.1	0.9	1.5
97	95	96	97	101	102	104	96	96
12.8	14.0	13.4	13.2	13.8	13.6	13.4	11.2	10.3
165	1000	190	250	250	180	100	90	20
4.2	4.0	6.6	6.8	6.8	8.0	7.4	7.0	7.0
1.6	1.8	2.4	2.8	3.2	3.0	3.4	2.0	2.6
100	100	102	100	98	94	90	90	92
8.8	9.1	8.8	9.0	9.0	8.8	9.1	8.3	9.1
360	316	446	126	86	66	80	110	140
8.8	9.6	9.6	9.6	9.6	7.6	6.4	8.0	8.8
3.0	3.4	5.4	5.2	5.2	4.6	3.4	4.0	4.2
125	128	130	134	133	132	111	111	112
6.9	7.0	6.4	6.8		6.5	6.5	5.0	6.5
270	820	676	576	230	200	36	46	160
7.2	8.8	6.8	8.0	8.8	7.2	5.6	7.6	7.6
1.9	2.2	4.1	2.7	2.7	2.9	2.0	2.0	2.2
102	102	100	106	110	111	98	96	98
7.5	7.5	7.6	8.0	7.5	8.5	7.5	8.0	7.6
70	480	70	40	60	180	20	40	40
7.0	9.2	14.2	10.6	10.8	5.8	5.4	7.2	6.8
3.1	3.9	3.4	3.6	3.0	3.8	3.1	3.2	3.5
127	125	130	136	134	131	133	115	117
10.8	10.5	10.5	10.8	10.8	10.8	10.8	11.5	11.5
1000		1560	1500	900	1000	320	280	500
3.0		4.2	4.0	3.6	3.6	2.4	2.4	4.2
1.1	0.6	1.7	1.8	1.7	1.9	1.2	1.4	1.3
9.3	9.5	9.3	9.6	10.1	9.6	9.4	8.8	9.1
0.89	1.03	1.00	0.92	1.06	0.98	1.00	0.97	0.74
106.5	106.3	108.0	111.8	110.2	107.2	100.0	94.0	95.8
6.5	6.7	7.2	7.4	8.1	9.3	9.4	8.6	8.2
401.8	781.2	845.8	886.5	461.0	428.0	167.0	102.7	157.3
137.1	175.4	196.8	444.9	199.9	172.9	71.2	37.2	72.1
5.3	6.8	7.6	7.2	7.3	6.1	5.0	5.8	6.1
1.13	1.48	1.58	1.13	1.20	0.74	0.80	1.04	0.97
1.9	2.1	3.0	2.9	2.8	2.9	2.4	2.3	2.6
0.43	0.56	0.67	0.60	0.58	0.50	0.44	0.47	0.46

TABLE V

Effects of Intraduodenal Amino Acids (0.5 g/kg) on Glucose, Glucagon, and Insulin

Dog No.	Measurement*	Source	Control period −10	Control period 0	Minutes after start of ingestion: Intraduodenal infusion (11 mg/kg per min) 5	10	20	30	45	60	75	90	120	150	180
			min												
3–25	Amino *N*	VC	4.6	4.4	4.4	4.6	5.3	6.0	6.5	7.0	6.3	6.2	6.4		4.6
	Amino *N*	MV	4.6	4.8	5.8	6.0	8.0	11.8	8.8	6.8	7.0	6.8	6.3		5.3
	Glucose	VC	92	96	96	95	100	94	92	92	89	93	97	102	102
	Glucagon	PV	2.4	2.6	3.0	3.4	6.2	3.7	3.6	2.4	3.1	2.9	2.7	2.7	2.0
	Insulin	PV	20	30	316	20	106	20	20	10	0	10	20	70	60
3–30	Amino *N*	VC	4.4	4.4	4.6	5.3	7.3	7.7	12.5	6.9	7.1	6.3	5.6		5.0
	Amino *N*	MV	5.5	5.2	5.6	6.8	11.5	12.3	12.5	11.0	7.9	6.4	5.8		5.0
	Glucose	VC	106	110	116	112	110	106	108	105	100	93	82	81	82
	Glucagon	PV	3.4	3.2	3.0	5.2	3.2	4.0	5.2	3.8	3.6	3.6	6.4	6.4	8.0
	Insulin	PV	0	10	36	10	10	10	30	0	0	0	0	30	40
3–31	Amino *N*	VC	4.7	4.8	4.8	4.8	5.0	7.4	7.8	9.6	6.4	6.4	6.1		5.4
	Amino *N*	MV	5.8	5.8	5.5	5.2	5.8	8.8	11.5	10.0	6.8	7.6	6.4		5.6
	Glucose	VC	96	98	102	105	105	105	104	105	104	105	105	104	105
	Glucagon	PV	2.6	3.0	4.0	5.0	3.2	5.0	3.6	1.7	2.6	2.4	2.2	4.0	3.8
	Insulin	PV	166	90	46	206	26	336	226	40	110	190	40	20	
3–51	Amino *N*	VC	3.8	3.7	3.7	4.2	5.2	5.9	6.6	5.4	4.8	4.8	4.2		3.9
	Amino *N*	MV	3.9	5.0	5.0	5.9	6.5	6.5	7.0	6.0	5.4	5.6	4.6		
	Glucose	VC	116	118	112	110	110	109	109	110	118	122	125	128	128
	Glucagon	PV	0.7	0.7	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.7	0.7	0.6
	Insulin	PV	120	80	140	100	180	40	180	100	20	80	80	30	60
3–52	Amino *N*	VC	3.6	3.9	4.4	5.1	5.8	6.0	6.6	5.4	5.5	5.3	4.3	4.3	4.2
	Amino *N*	MV	4.9	4.6	5.3	7.5	9.0	10.5	7.8	6.5	6.6	6.3	5.0	4.4	4.4
	Glucose	VC	121	110	125	127	110	118	102	97	98	101	98	99	100
	Glucagon	PV	1.0	0.9	1.1	1.4	1.5	1.1	1.1	0.8	0.9	0.8	1.4	0.8	0.7
	Insulin	PV		50	170	150	90	220	130	40	30	240	40	20	20
3–54	Amino *N*	VC	4.4	4.8	5.1	5.4	5.9	6.7	7.3	7.4	6.6	6.2	4.4	4.5	4.8
	Glucose	VC	72	95	102	100	102	96	94	92	92	96	110	90	88
	Glucagon	PV	0.9	1.0	1.1	0.8	1.1	0.9	0.9	0.9	0.2	1.0	0.2	1.2	
	Insulin	PV	0	80	180	100	100	20	80	40	20	20	40	20	80
Mean ± SEM															
	Amino *N*	VC	4.3	4.3	4.5	4.9	5.8	6.6	7.4	7.0	6.1	5.9	5.2		4.7
	± SEM		0.2	0.2	0.2	0.2	0.3	0.3	0.5	0.6	0.4	0.3	0.4		0.2
	Amino *N*	MV	4.9	5.1	5.4	6.1	8.2	10.0	9.5	8.3	6.7	6.5	5.6		5.1
	± SEM		0.4	0.2	0.1	0.5	1.0	1.2	1.7	1.0	0.4	0.3	0.4		0.3
	Glucose	VC	100.5	104.5	108.8	108.1	106.1	104.6	101.5	100.1	100.1	101.6	102.8	100.6	100.8
	± SEM		7.3	3.9	4.6	4.9	2.5	4.0	2.9	3.5	4.5	4.8	6.0	6.7	6.6
	Glucagon	PV	2.0	1.9	2.2	2.8	2.7	2.6	2.5	1.7	1.9	1.9	2.3	2.6	3.0
	± SEM		0.46	0.51	0.55	1.04	0.83	0.76	0.76	0.49	0.58	0.5	0.9	0.92	1.4
	Insulin	PV	61	57	148	98	85	108	111	38	30	90	37	32	63
	± SEM		34.4	13.1	42.0	30.7	25.1	55.9	33.7	14.3	16.7	41.6	10.9	8.0	14.1

VC, vena cava; MV, mesenteric vein; PV, pancreaticoduodenal vein.

* Amino *N* (mg/100 ml); Glucose (mg/100 ml); Glucagon (mμg/ml); Insulin (μU/ml).

base line level of 2.8 mμg/ml to a mean of 1.6 mμg/ml, failed to rise even to its base line level during amino acid infusion, despite a rise in the mean amino nitrogen level to 7.0 mg/100 ml. These results, summarized in Table VII, reveal almost complete suppression by hyperglycemia of the normal hyperglucagonemic response to hyperaminoacidemia. It is perhaps significant that the mean plasma glucose concentration fell almost 35 mg/100 ml during the amino acid infusion.

In order to determine the effect of sudden termination of hyperglycemic suppression of aminogenic hyperglucagonemia upon glucagon secretion, four additional experiments of a similar nature were conducted. In these the glucose infusion was suddenly discontinued, while the amino acid infu-

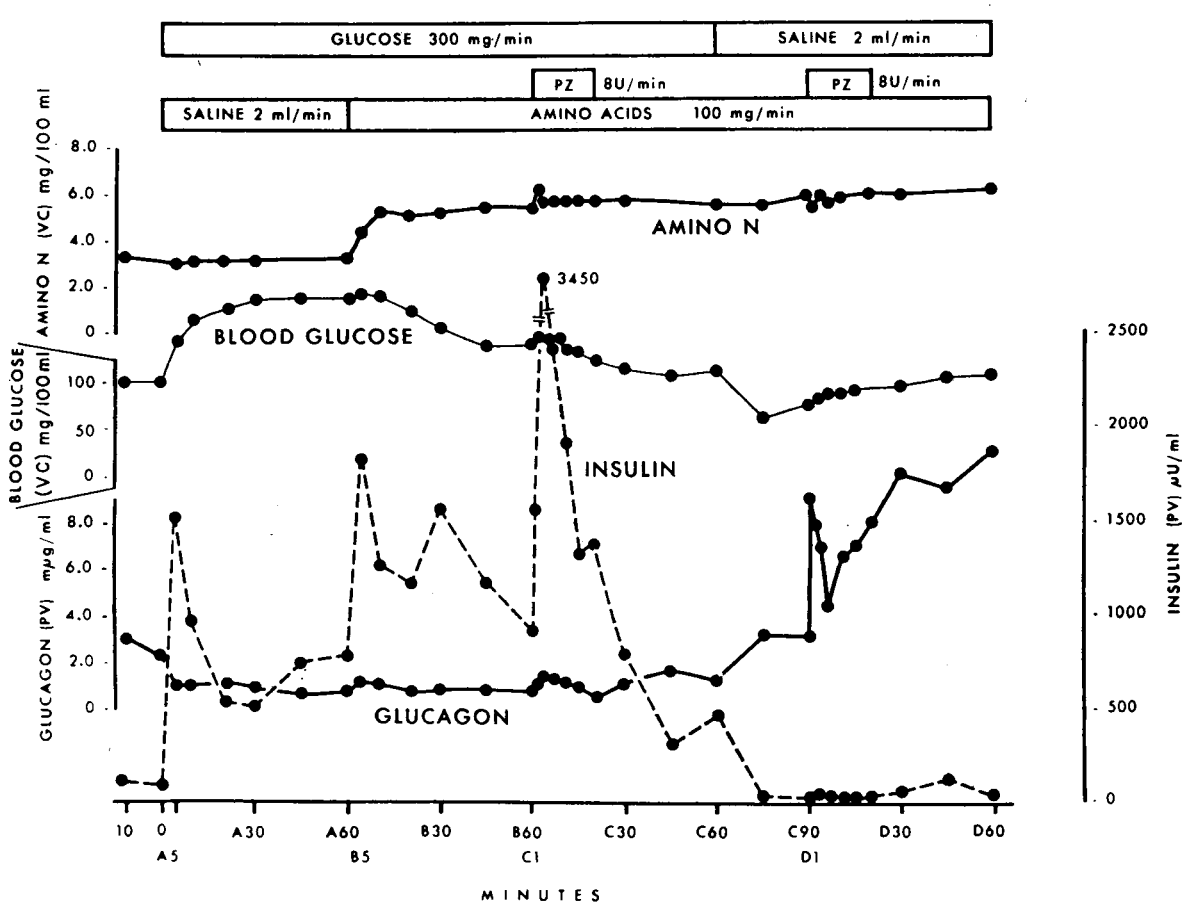

FIGURE 6 The effect of hyperglycemia upon the insulin and glucagon response to intravenously administered amino acid mixture and pancreozymin infusion (*PZ*).

sion was continued. Blood glucose fell from an average of 161 to 82 mg/100 ml, and under these conditions, mean glucagon concentration rose from 0.7 to 3.9 mμg/ml. Fig. 6 provides a representative example of this type of experiment; hyperglycemic suppression of glucagon was evident at first, and even the powerful augmenting action of pancreozymin infusion upon glucagon secretion was nullified during hyperglycemia of this magnitude. However, when the glucose infusion was terminated, with continued amino acid infusion, a remarkable rise in pancreatic glucagon secretion accompanied the decline in the blood glucose concentration, and at this point, the infusion of pancreozymin further augmented the striking hyperglucagonemia.

DISCUSSION

The results indicate that in dogs hyperaminoacidemia is a potent stimulus of glucagon as well as of insulin. Assan and coworkers were the first to demonstrate an apparent rise in glucagon in peripheral venous blood of human subjects during the infusion of arginine at a rate of 25 g for 30 min (8). The present study confirms their findings and demonstrates, first, that glucagon is released during a physiologic elevation of amino nitrogen, whether intravenously or intraduodenally administered, and that glucagon release takes place in concert with aminogenic insulin release. Second, it demonstrates that aminogenic hyperglucagonemia is derived from pancreatic glucagon rather than from gut glucagon-like immunoreactivity, whereas after intraduodenal administration of glucose the gut is the source of the rise in circulating glucagon-like immunoreactivity (15).

Pancreozymin, a gut hormone known to be stimulated most effectively by the ingestion of protein and of amino acids, has been shown to aug-

TABLE VI A

Comparison of "Insulinogenic Indexes" and "Glucagonogenic Indexes"‡ after Intraduodenal (i.d.) and Intravenous (i.v.) Amino Acid Administration (1 g/kg)*

Dog No.	Insulinogenic indexes		Glucagonogenic indexes	
	i.d.	i.v.	i.d.	i.v.
2–07	3.8	22.4	250.0	224.9
2–11	30.5	63.5	19.2	36.0
2–12	74.9	16.9	237.6	54.8
2–15	98.7	44.4	164.0	14.2
2–32	142.9	20.0	161.0	43.0
2–33	276.7	58.8	333.3	8.6
2–35	67.8	22.6	318.2	109.5
2–36	0	21.0	103.7	72.9
2–69	43.6	41.8	245.2	162.9
2–70	35.0	22.1	33.3	16.7
2–71	26.0	36.9	0	129.8
2–81	71.6	84.5	73.6	412.6
2–82	76.4	65.1	320.0	88.6
2–84	199.4	55.8	207.1	128.7
2–85	14.5	19.0	213.3	195.9
2–86	45.1	59.1	259.2	62.9
Mean	75.4	40.9	183.7	110.1
± SEM	18.7	5.3	27.3	25.9
P value		<0.1		<0.1

* Area of increment in insulin (PV)/area of increment in amino nitrogen (VC) during 20 min

$$\left(\mu U \times \min \Big/ \frac{mg \times min}{100\ ml}\right)$$

‡ Area of increment in glucagon (PV)/area of increment in amino nitrogen (VC) during 20 min

$$\left(m\mu g \times \min \Big/ \frac{mg \times min}{100\ ml}\right).$$

TABLE VI B

Comparison of "Insulinogenic Indexes" and "Glucagonogenic Indexes" after Intraduodenal (i.d.) and Intravenous (i.v.) Amino Acid Administration (0.5 g/kg)

Dog No.	Insulinogenic indexes		Glucagonogenic indexes	
	i.d.	i.v.	i.d.	i.v.
3–25	301.7	32.8	4333	315
3–30	5.8	70.2	674	569
3–31	870.0	63.9	7636	123
3–51	84.4	38.8	111	72
3–52	79.5	123.4	397	28
3–54	65.2	81.1	87	53
Mean	103.9	68.4	2648	193.3
± SEM	41.4	13.3	1090	86.1
P value		<0.5		<0.25

See Table VI A for explanation of words in quotes.

ment aminogenic insulin and glucagon secretion during hyperaminoacidemia. This fact prompted an unsuccessful effort to obtain evidence of augmentation by endogenous pancreozymin by comparing the effects of intraduodenal and intravenous amino acid administration upon the response of the islet hormones; but these experiments failed to demonstrate a significantly greater response of the hormones to intraduodenal administration. This does not, however, necessarily exclude the existence of an enterohumoral system for augmentation of the insulin and glucagon response to ingested amino acids, particularly since the islet hormone response seemed to anticipate hyperaminoacidemia in the intraduodenal experiments. It may be that the use of amino acids with weaker insulinogenic and glucagonogenic activities than those in the Floyd mixture would make more visible any existing effect of endogenous pancreozymin upon islet hormone output, and further studies seem indicated. It should also be pointed out that in dogs augmentation of insulin secretion during glucose absorption has been far more difficult to demonstrate than in man (15), and Dupré et al. have recently obtained data suggesting a greater bihormonal response to the enteric administration of arginine.[8]

Of considerable physiologic interest is the demonstration that hyperglycemia in excess of 160 mg/100 ml completely prevented both the aminogenic hyperglucagonemia and its augmentation by pancreozymin. However, when the glucose infusion was terminated and blood glucose fell towards normal, glucagon secretion rose to unprecedentedly high levels during the continuing hyperaminoacidemia, and this rise was further augmented by pancreozymin. The ability of hyperglycemia, which does not prevent aminogenic hypersomatotropinemia (26), to suppress completely the most potent known stimulus to glucagon secretion, combined infusion of amino acids and pancreozymin, suggests that the function of aminogenic glucagon secretion may be to prevent hypoglycemia (27) rather than to lower amino acid levels, and that glucagon secretion is unnecessary when exogenous

[8] Dupré, J., J. D. Curtis, R. H. Unger, R. W. Waddell, and J. C. Beck. 1968. Effects of secretin, pancreozymin, or gastrin on insulin and glucagon secretion in response to administration of glucose or arginine in man. Submitted for publication.

TABLE VII

Effect of Hyperglycemia on Insulin and Glucagon Response to Amino Acids

Dog No.	Measurement	Source	Minutes before amino acid infusion (A)					Minutes after start of amino acids infusion (B)					
			Control period		Glucose infusion (200 mg/min)			Amino acids infusion (100 mg/min)					
			−10	0	−30	−15	0	5	10	20	30	45	60
			min										
3–95- I	Glucose (*mg/100 ml*)	VC	97	97	198	195	188	196	191	194	182	165	158
	Amino *N* (*mg/100 ml*)	VC	5.2	5.3			4.4	5.4	6.0	6.4	6.8	6.6	7.1
	Insulin (*μU/ml*)	PV	156	220	690	580	660	900	1100	1560	1320	1280	1550
	Glucagon (*mμg/ml*)	PV	4.8	5.4	2.0	1.8	1.6	1.9	1.8	1.7	1.8	2.0	2.2
3–98-III	Glucose	VC	102	102	145	149	142	162	154	139	133	125	117
	Amino *N*	VC	5.0	4.7			3.6	3.6	4.9	5.6	5.8	6.0	6.3
	Insulin	PV	0	10	160	146	98	186	120	120	700	376	180
	Glucagon	PV	0.8	0.8	0.8	0.8	1.0	1.0	0.9	0.9	0.9	0.7	0.6
4–02- II	Glucose	VC	94	96	169	168	168	172	170	160	140	128	129
	Amino *N*	VC	5.1	5.2			4.6	5.4	6.2	6.8	6.8	6.6	6.6
	Insulin	PV	20	16	386	306	460	2080	1700	1400	850	146	2330
	Glucagon	PV	2.7	2.7	2.1	2.0	1.9	2.2	2.5	2.4	2.2	1.9	2.6
4–05- II	Glucose	VC	124	123	152	165	158	160	160	154	147	137	132
	Amino *N*	VC	4.5	4.2			3.7	4.6	5.9	6.1	6.1	6.1	6.6
	Insulin	PV	178	268	566	476	836	1050	1080	900	840	1130	1430
	Glucagon	PV	3.8	2.4	1.8	2.0	2.0	1.8	1.8	1.8	1.7	1.5	1.5
4–06- II	Glucose	VC	128	128	197	186	196	200	184	165	148	142	142
	Amino *N*	VC	5.5	5.9			5.0	6.8	6.9	7.4	7.8	7.8	8.0
	Insulin	PV	56	80	760	480	670	1230	1030	1480	1580	1180	1050
	Glucagon	PV	2.5	2.8	1.9	1.6	1.8	1.7	1.4	1.4	1.6	1.9	1.7
Mean ± SEM													
	Glucose	VC	109.0	109.2	172.2	171.8	170.0	178.0	171.8	162.4	150.0	139.4	135.6
	± SEM		7.1	6.8	11.0	9.7	9.9	8.4	7.0	9.0	8.4	7.1	6.9
	Amino *N*	VC	5.1	5.1			4.3	5.2	6.0	6.5	6.7	6.6	6.9
	± SEM		0.2	0.3			0.3	0.5	0.3	0.3	0.3	0.3	0.3
	Insulin	PV	82.0	116.8	512.4	398.8	544.8	1089	1006	1092	1058	821.2	1228
	± SEM		36.0	53.1	108.6	77.1	126.6	304.5	205.3	268.9	149.7	233.5	346.0
	Glucagon	PV	2.9	2.8	1.7	1.6	1.7	1.7	1.7	1.6	1.6	1.6	1.7
	± SEM		0.67	0.74	0.24	0.22	0.18	1.98	0.26	0.25	0.21	0.24	0.34

VC, vena cava; PV, pancreaticoduodenal vein.

glucose is avaliable, as with a mixed meal. Aminogenic hyperglucagonemia would then be particularly important during ingestion of large carbohydrate-free protein meals, as is the case in carnivorous animals. Along the same line, one might speculate that, if a glucagon deficiency state exists, it would be manifested by aminogenic hypogylcemia. It is perhaps noteworthy that, when aminogenic hyperglucagonemia was prevented by hyperglycemia, a 35 mg/100 ml fall in mean plasma glucose concentration occurred, whereas, when hyperaminoacidemia was accompanied by hyperglucagonemia, only a trivial change in glucose occurred. Other studies still in progress suggest that this fall in glucose concentration is, at least in part, related to the absence of aminogenic hyperglucagonemia (27).

ACKNOWLEDGMENTS

The authors express their thanks to Miss Virginia Harris, Mrs. Ava M. Jones, Mrs. Shirley Harvey, Mrs. Margaret Bickham, and Mrs. Genevieve Thompson for technical assistance and to Mrs. Ila Fite and Mrs. Mary M. Minor for secretarial work. In addition, the authors wish to thank Dr. Paul A. Srere for his valued advice.

This study was supported by U. S. Public Health Grant No. AM-02700-09, The Upjohn Co., Kalamazoo, Mich., Ames Co., Inc., Elkhart, Ind., Syntex Laboratories, Inc., Palo Alto, Calif., and Chas. Pfizer & Co., Inc., New York.

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