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The Effects of Secretin, Pancreozymin, and Gastrin on Insulin and Glucagon Secretion in Anesthetized Dogs *

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Summary. The effects upon islet hormone secretion of highly purified preparations of secretin and of pancreozymin–cholecystokinin and of a crude gastrin–containing extract of hog antrum have been studied in acutely operated dogs. All three preparations were shown to cause a striking increase in insulin concentration in the pancreaticoduodenal venous plasma after their rapid endoportal injection in anesthetized dogs. With each hormone preparation, the peak in insulin secretion occurred 1 minute after injection, and a rapid decline was observed immediately thereafter. Whereas secretin and gastrin failed to alter significantly the pancreaticoduodenal venous glucagon or arterial glucose concentration, pancreozymin caused a dramatic rise in pancreaticoduodenal venous glucagon concentration, which reached a peak 3 minutes after injection, and hyperglycemia was noted to occur soon thereafter. Endoportal infusion of secretin and pancreozymin for 20 minutes caused responses that were sustained but qualitatively identical to the responses noted after rapid injection of the hormones. The beta–cytotropic effect of secretin was abolished by the infusion of epinephrine.

These results could not be attributed to the small degree of contamination of the enteric hormone preparations with insulin or glucagon, and it would appear that secretin, pancreozymin, and probably gastrin have insulin–releasing activity and that pancreozymin has, in addition, glucagon–releasing activity.

The demonstration that these three hormones possess insulin-releasing activity suggests that there is in the gastrointestinal tract a chain of beta–cytotropic hormones from antrum to ileum that is capable of augmenting insulin secretion as required for disposal of substrate loads. It is suggested that the existence of this "entero-insular axis" prevents high substrate concentrations that would otherwise follow ingestion of large meals were the insular response entirely a function of arterial substrate concentration.

Introduction

The possibility that the secretory response of the islets of Langerhans to ingested food might be influenced by humoral factors of the gastrointestinal tract was apparently first considered in 1906, when Moore, Edie, and Abram (1) administered an extract of duodenum to several diabetics in the hope of augmenting insulin secretion. Although the results of this therapeutic trial were not conclusive, the concept of an entero-insular hormonal axis continued to receive the attention

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of investigators (2–7) until 1940, when a negative report by Loew, Gray, and Ivy (8) discredited the idea. In 1964 Dupré (9) revived interest in this question with a report that a crude secretin–containing extract of hog duodenal mucosa, prepared by the method of Crick, Harper, and Raper (10), accelerated the disappearance rate of intravenously administered glucose and increased insulin–like activity in man (11). Pfeiffer and associates (12) and McIntyre, Turner, and Holdsworth (13) noted in 1965 that the incubation of secretin with pieces of dog and rabbit pancreas enhanced the release of insulin. More recently, preliminary *in vivo* evidence of an insulin–releasing effect of secretin has been reported in dogs (14) and in humans (15).

The present study was designed to explore in dogs the effects of the enteric hormones secretin and pancreozymin–cholecystokinin and the antral hormone gastrin on insulin and glucagon secretion.

Methods

Mongrel dogs were anesthetized with Nembutal after an overnight fast, and a laparotomy was performed. An indwelling needle was placed in the pancreaticoduodenal vein in the direction of venous blood flow and its position stabilized. Obstruction to venous drainage was carefully avoided. A catheter was connected to the pancreaticoduodenal needle; catheters were also inserted in a femoral vein and a femoral artery. The patency of these vascular connections was maintained by local instillation of heparin. In addition a fourth catheter was inserted into a mesenteric vein, and normal saline was infused endoportally at a constant rate of 2 ml per minute throughout each experiment.

Simultaneous samples of blood were obtained from the pancreaticoduodenal and femoral veins and from the femoral artery at frequent intervals before and after the rapid injection of the enteric hormone and before, during, and after its infusion. Mean arterial blood pressure was monitored continuously, and the hematocrit was determined at frequent intervals. All dogs included in the study were considered to have tolerated well both the surgery and the removal of blood.

Plasma glucose concentration was determined by the Hoffman ferricyanide method (16) with the Technicon Autoanalyzer. Insulin was measured by the method of Yalow and Berson (17). Plasma glucagon concentration was measured by the following modification of the previously described radioimmunoassay (18): Rabbit anti-glucagon serum [diluted to 1:321 with 0.2 M glycine buffer (pH 8.9) containing 0.25% human albumin and 1:100 nonimmune rabbit serum] and 0.05 ml of either unknown undiluted plasma sample or a known crystalline beef–pork glucagon standard[1] were incubated at 4° C with 500 U of Trasylol[2] in 0.025 ml of normal saline for 2 days. After 48 hours, 30 $\mu\mu$g of glucagon-^{131}I in 0.05 ml glycine albumin buffer was added and the incubation continued for an additional 20 hours at 4° C. At the end of this time, 0.50 ml of either 0.25% human albumin or undiluted nonimmune rabbit plasma containing bromophenol blue was added, and 0.2 ml of this mixture was applied to the origin of a Whatman 3 MC paper strip. After 2 hours of chromatography in a barbital buffer, the plasma proteins migrated 18 cm from the origin, and the strips were heat-dried, bisected, and counted in a well–type gamma scintillation counter. Results were corrected for nonspecific migration by the method of Yalow and Berson (17).

This assay has a high degree of precision; a recent analysis of replicate determinations of both known and unknown samples at all concentrations of glucagon revealed a standard error of ± 1.2%. Recovery of crystalline glucagon is virtually quantitative. Although canine pancreatic glucagon appears to be immunologically similar to beef–pork glucagon (19), until canine standards are available the quantitative accuracy of assay results in absolute terms is uncertain. It seems probable, however, on physiologic grounds that the 0.4 to 2.0 mμg per ml range of normal fasting dogs is close to the true glucagon level in the fasting state (20). Although it may still be necessary to regard the glucagon assay as semiquantitative rather than quantitative, its precision and sensitivity make it possible to distinguish with 95% confidence differences in glucagon concentration 0.3 mμg per ml or more. It may, therefore, be regarded as fully capable of measuring small changes in plasma glucagon levels in the relative sense of immunologic equivalence to beef–pork glucagon.

The use of the proteinase inhibitor Trasylol has been shown to eliminate almost completely the problem of incubation damage by human plasma to glucagon-^{131}I (21). Although incubation damage poses more of a problem in the assay of human than canine plasma (21), Trasylol was added to the assay in this study because of the possibility that variation in release of pancreatic proteolytic enzymes into the pancreatic vein might result from manipulation of the pancreas before the experiment. It has been found that it is not necessary to collect blood specimens in Trasylol–containing tubes, however (21).

Highly purified secretin, estimated to contain from 4,330 to 17,500 U per mg, and pancreozymin–cholecystokinin, said to contain 6,000 U per mg (22), were provided[3] in vials containing 75 clinical U of secretin and 300 Crick U of pancreozymin. The contents of each vial were dissolved in 10 ml normal saline, giving a con-

[1] Kindly donated by Dr. W. R. Kirtley, Eli Lilly and Co., Indianapolis, Ind.

[2] Trasylol–bay A 128–kindly supplied by FBA Pharmaceuticals, Inc., New York, N. Y.

[3] Kindly provided by Professor Erik Jorpes and Dr. Viktor Mutt of Karolinska Institutet, Stockholm, Sweden.

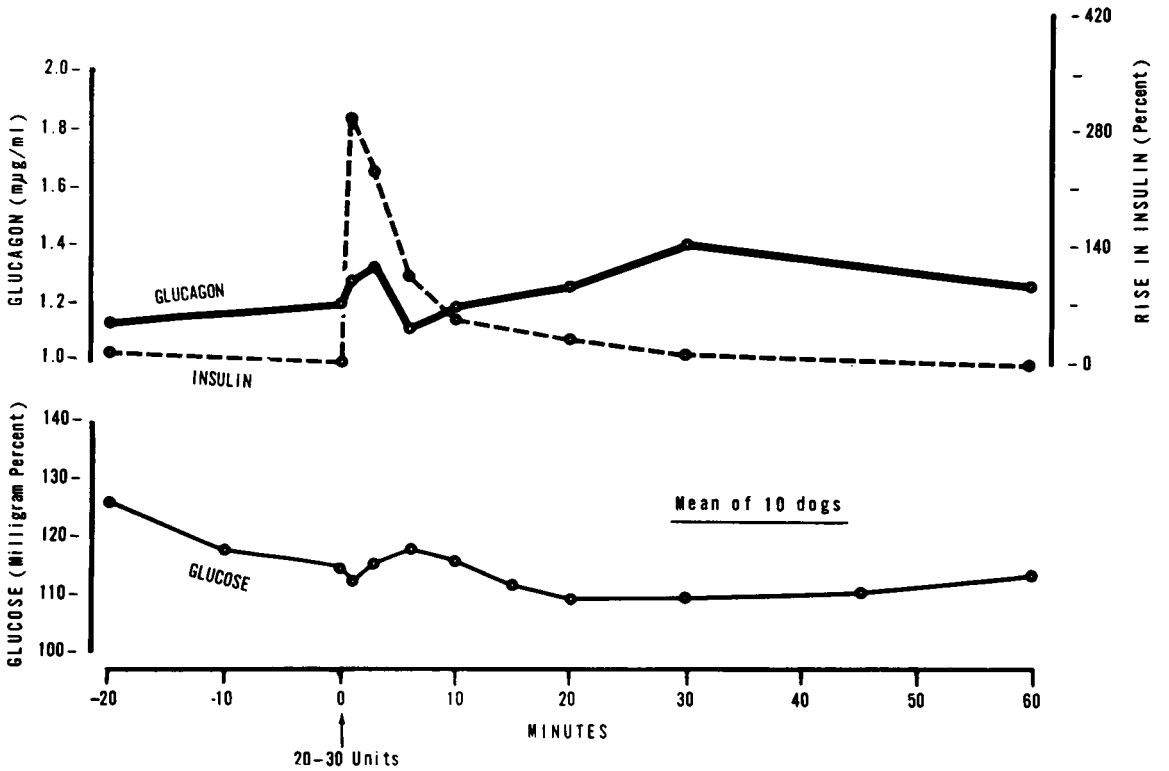

FIG. 1. EFFECT OF THE RAPID ENDOPORTAL INJECTION OF SECRETIN ON PANCREATICODUODENAL VENOUS PLASMA INSULIN AND GLUCAGON LEVELS AND ARTERIAL PLASMA GLUCOSE CONCENTRATION.

centration of 7.5 U per ml of secretin and 30 U per ml of pancreozymin. Secretin was administered through the mesenteric venous catheter by rapid injection of a dose of 1.5 U per kg or by constant infusion at a rate of 10 U per minute for 20 minutes; pancreozymin was administered by rapid injection in a dose of 100 U or by constant infusion at a rate of 30 U per minute for 20 minutes. Gastrin in the form of crude acetone powder,[4] starting material for the purification of gastrin by the method of Tauber and Madison (23), was administered by rapid injection in a dose of 135 to 203 mU; gastrin II,[5] prepared by the method of Gregory and Tracy (24), was administered in the same manner in a dose of 0.06 mg.

Every lot of each hormone was assayed for insulin and glucagon. All lots employed were free of insulin. Glucagon or glucagon-like immunoreactivity was from 0 to 3.7 mμg per U in secretin, from 0.02 to 0.06 mμg per U in pancreozymin, and 0.62 mμg per mU in gastrin.

Results

Secretin injection. Immediately after the rapid endoportal injection of 1.5 U per kg of secretin, a striking rise in pancreaticoduodenal venous insulin concentration was observed in each of 10 dogs (Figure 1). The mean insulin level for the group rose 294% from a preinjection value of 248 μU per ml (SD ± 270) to 1,175 μU per ml (SD ± 830) 1 minute after injection. The response was very brief, with a sharp decline occurring between 3 and 6 minutes after injection. Arterial insulin was measured in only one dog, 104, and reflected the changes in pancreatic venous insulin. There was no significant change in mean arterial glucose concentration for the group as a whole, although in some dogs a small rise was noted. However, such rises in plasma glucose were very small and followed the rise in insulin. Pancreatic venous glucagon concentration did not change significantly. The complete findings are recorded in Table I.

Pancreozymin injection. The rapid injection of 100 U of pancreozymin was also followed immediately by a sharp 484% rise in mean pancreaticoduodenal insulin concentration in each of

[4] Kindly donated by Dr. Stuart Tauber, Dallas, Texas.

[5] Kindly donated by Dr. Morton Grossman, Los Angeles, Calif.

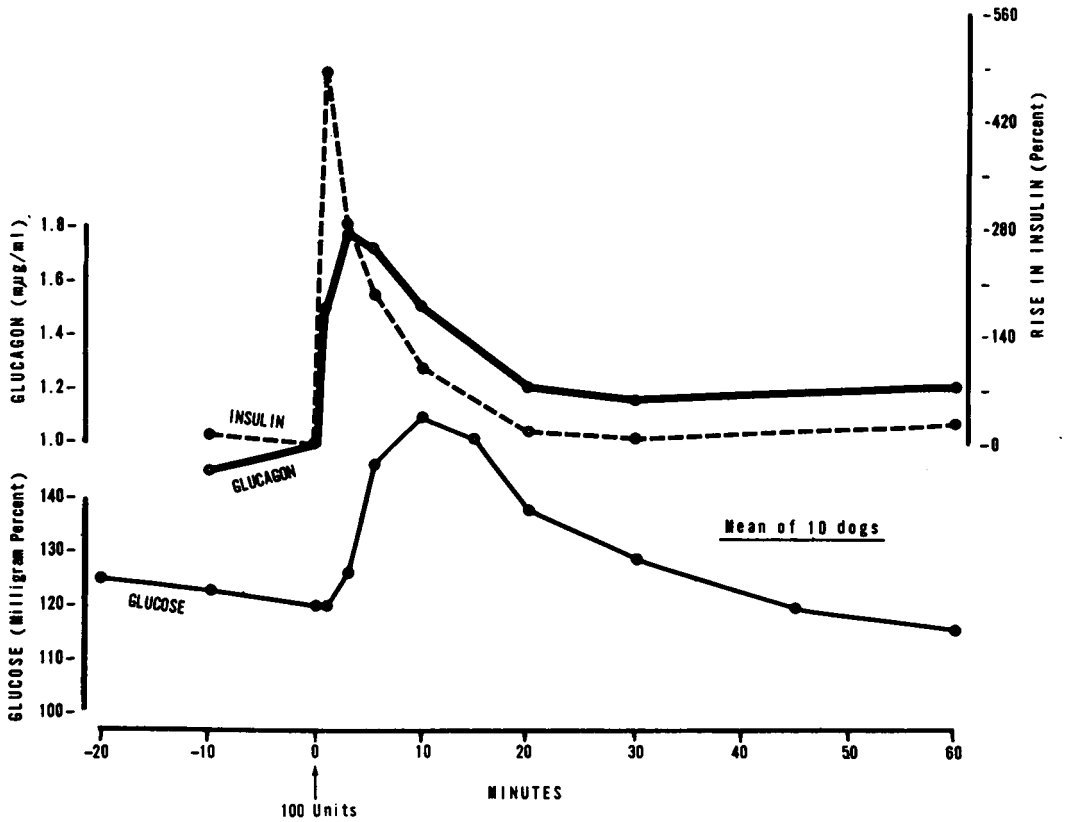

FIG. 2. EFFECT OF THE RAPID ENDOPORTAL INJECTION OF PANCREOZYMIN ON PANCREATICODUODENAL VENOUS PLASMA LEVELS OF INSULIN AND GLUCAGON AND ARTERIAL PLASMA GLUCOSE CONCENTRATION.

10 dogs. Mean insulin rose from 221 μU per ml (SD ± 113) before injection to a peak of 1,291 μU per ml (SD ± 734) 1 minute after injection (Figure 2). As with secretin, this rise was short-lived and declined rapidly between 1 and 10 minutes after injection. In contrast to the lack of a clear-cut effect of secretin upon glucagon concentration, however, the administration of pancreozymin was followed in 8 of the 10 dogs by a rapid rise in the pancreaticoduodenal venous glucagon concentration. The mean level rose from 1 mμg per ml (SD ± 0.25) before the injection to a 3-minute level of 1.78 mμg per ml (SD ± 0.91), after which it gradually declined; the mean of all 10 peak values, irrespective of time, was 2.15 mμg per ml (SD ± 0.73), a rise of 1.15 mμg per ml. Plasma glucose concentration rose slowly from 120 mg per 100 ml (SD ± 10.3) to a peak of 155 mg per 100 ml (SD ± 25.8) at 10 minutes. The time of the rise in glucose concentration was far too late to be causally related to the rise in insulin. The individual results of these experiments are recorded in Table II.

Gastrin injection. Rapid endoportal injection of a gastrin-containing extract of hog antrum in three dogs was followed by a rapid 385% rise in

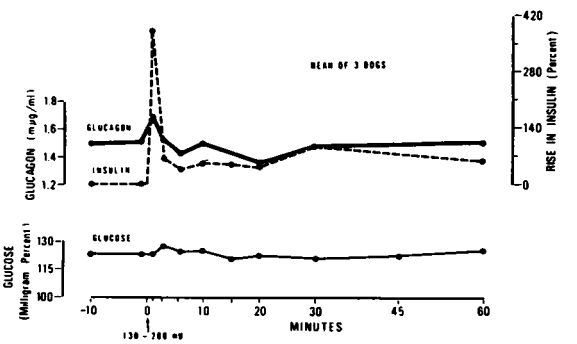

FIG. 3. EFFECT OF THE ENDOPORTAL INJECTION OF CRUDE GASTRIN UPON THE PANCREATICODUODENAL VENOUS PLASMA LEVELS OF INSULIN AND GLUCAGON AND THE ARTERIAL PLASMA GLUCOSE CONCENTRATION.

TABLE I

Effect of endoportal injection of 20 to 30 U of secretin on arterial glucose (AG), pancreatic venous insulin (PVI), and pancreatic venous glucagon (PVG)

Dog	Secretin dose		Minutes before injection			Minutes after injection								
			−20	−10	−0.5	1	3	6	10	15	20	30	45	60
	U													
96B	22.5	AG (*mg/100 ml*)	140	115	106	104	109	122	115	109	105	102	103	108
		PVI (*μU/ml*)	213		169	1,488	268	317	198		159	169		174
		PVG (*mμg/ml*)	0.9		0.92	1.06	1.36	0.84	0.72		0.8	1.2		1.16
103B	23.75	AG	110	120	126	119	130	122	122	118	119	121	125	130
		PVI	134		377	1,488	427	233	223		456	496		283
		PVG	1.16		1.74	1.4	1.16	1.44	1.42	1.96	1.92	2.4		1.72
59A	27.3	AG	123	122	120	118	120	119	112	108	106	104	109	120
		PVI	298	293	303	>2,480	1,488	645	397		347	303		472
		PVG	1.44	2.0	1.4	1.18	0.56	0.74	0.88	0.94	1.14	1.10	0.98	0.9
58C	29.4	AG	118	118	124	121	128	133	128	125	123	123	126	123
		PVI		422	461	>2,480	1,488	1,290	1,141		1,290	694		243
		PVG	1.76	2.0	1.78	2.6	1.52	1.34	1.68	1.6	1.56	1.7	1.76	1.88
95B	30	AG	100	84	82	80	84	86	84	81	78	81	80	84
		PVI	139		268	1,240	327	198	144		193	169		268
		PVG	0.92		1.04	0.7	0.92	0.86	0.82		1.16	1.24		1.16
78A	30	AG	145	134	127	134	135	139	143	135	131	125	131	148
		PVI	253		595	645	2,728	1,885	1,290		942	412		436
		PVG	1.2		1.2	1.48	3.0	1.9	1.98	1.92	1.8	2.0		
104B	30	AG	130	120	109	102	104	107	115	108	107	104	98	96
		PVI	1,190		595	744	>2,480	1,240	942		546	794		496
		AI* (*μU/ml*)			74	79	161	164	136		114			
		PVG	0.94		0.98	1.04	1.58	1.44	1.26		1.08	1.32		1.14
109B	30	AG	146	122	108	106	106	114	104		100	98	95	88
		PVI	84		45	129	104	461	139		35	79		40
		PVG	0.82		0.7	1.02	1.11	0.9	1.2		1.06	1.12		1.12
112A	30	AG	129	130	125	128	124	124	125	122	124	125	125	122
		PVI		109	74	942	119	89	89		94	124		188
		PVG		1.22	1.0	1.0	0.9	0.9	1.14		1.04	1.12		1.12
111A	30	AG	116	115	118	119	121	116	113	106	104	114	122	121
		PVI		69	94	109	322	134	35		15	50		60
		PVG		1.2	1.28	1.34	1.16	0.88	0.88		0.94	0.9		1.26
Mean		AG	126	118	115	113	116	118	116	122	110	110	111	114
SD (±)			15.2	13.4	13.9	15.5	15.4	14.5	15.6	15.3	13.2	12.5	16.9	19.9
Mean		PVI	330		248	1,175	975	649	460		407	329		262
SD (±)			400		270	830	1,000	614	474		410	262		154
Mean		PVG	1.14		1.20	1.28	1.33	1.12	1.20		1.25	1.14		1.27
SD (±)			0.33		0.37	0.21	0.66	0.39	0.39		0.38	0.46		0.33
Mean maximal rise and SD														
		PVI	1,313 ± 713			p < 0.01								
		PVG	0.56 ± 0.49			p > 0.1								

* Arterial insulin concentration.

the mean pancreaticoduodenal venous insulin concentration from a preinjection value of 193 μU per ml (SD ± 123.3) to a peak of 937 μU per ml (SD ± 661.4) 1 minute after injection (Figure 3). Again a rapid decline occurred, with a return to the base-line value within 6 minutes. A 0.19 mμg per ml rise in mean pancreaticoduodenal venous glucagon concentration was noted at 1 minute after injection in parallel with a 4 mg per 100 ml rise in glucose concentration. The administration of 0.06 mg of pure porcine gastrin II elicited the same type of insulin response; the pancreaticoduodenal insulin level rose more than tenfold, from 169 to 1,835 μU per ml 3 minutes after the injection.

Glucagon content of injected hormone solutions

TABLE II

Effect of endoportal injection of pancreozymin–cholecystokinin on AG, PVI, and PVG

Dog	Dose		Minutes before injection			Minutes after injection								
			−20	−10	−0.5	1	3	6	10	15	20	30	45	60
	U													
95	100	AG (*mg/100 ml*)	100	98	94	90	104	114	132	120	111	100	84	82
		PVI (*μU/ml*)		263	258	1,438	744	794	595		243	139		268
		PVG (*mμg/ml*)		0.68	0.8	1.48	2.0	1.28	1.0		0.92	0.92		1.04
96	100	AG	121	124	121	118	120	161	177	188	169	140	115	106
		PVI		124	238	>2,600	843	595	397		238	213		169
		PVG		0.84	1.02	1.82	2.4	1.68	1.4		1.04	0.90		0.92
99	100	AG	118	121	122	122	122	131	120	119	109	110	118	122
		PVI		119	114	1,091	451	188	119		74	109		139
		PVG		0.68	0.74	1.17	0.83	0.8	0.8	1.0	0.88	0.94		0.96
103	100	AG	129	122	124	123	135	138	132	120	113	110	120	126
		PVI		283	298	1,488	893	744	188		79	134		377
		PVG		0.72	0.76	0.88	0.9	0.94	0.96	1.2	1.0	1.16		1.74
104A	100	AG	131	124	129	129	137	167	180	169	152	145	134	127
		PVI		293	382	1.091	942	744	476		427	253		595
		PVG		0.88	0.96	1.98	1.54	1.56	1.28	1.26	1.16	1.2		1.2
108A	100	AG	133	131		129	131	136	144	158	139	130	120	109
		PVI		446	342	794	>2,480	1,240	942		546	794		496
		PVG		1.0	0.9	0.98	1.62	2.2	1.32		1.0	0.94		0.98
109A	100	AG	132	132	119	117	136	178	200	196	155	146	122	108
		PVI		30	60	>2,480	233	794	432		109	84		45
		PVG		0.7	0.88	1.96	1.26	1.66	1.08		0.94	0.82		0.7
110B	100	AG	142	134	128	127	125	153	171	165	160	145	134	134
		PVI		382	268	694	1,290	744	844		694	456		337
		PVG			1.54	1.94	4.0	4.0	2.6		2.2	1.9		1.94
111	100	AG	114	122	121	122	129	145	144	136	130	124	123	122
		PVI	50		60	436	322	223	144		30	60		263
		PVG	0.9		1.26	1.43	1.58	1.48	1.48		1.12	1.16		1.34
112B	100	AG	125	125	122	125	124	138	147	143	140	134	126	126
		PVI	124		188	794	223	362	188		174	114		134
		PVG	1.12		1.12	1.38	1.66	1.56	3.2		1.8	1.76		1.4
Mean		AG	125	123	120	120	126	146	155	151	138	129	120	116
SD		±	11.9	10.0	10.3	11.4	9.9	18.9	25.8	28.3	21.6	16.5	14.0	15.2
Mean		PVI		243	221	1,291	842	643	433		261	245		282
SD		±		141	113	734	674	316	303		223	239		173
Mean		PVG		0.79	1.00	1.50	1.78	1.72	1.51		1.21	1.17		1.22
SD		±		0.12	0.25	0.41	0.91	0.89	0.77		0.44	0.37		0.39
Mean maximal rise and SD														
		PVI	1,298 ± 744			$p < 0.01$								
		PVG	1.15 ± 0.73			$p < 0.01$								

and its effect on the results. Glucagon has been shown to have potent insulin-releasing activity in man (25–27) and in dogs (20), even in small doses of less than 100 mμg; since glucagon-like immunoreactivity is present in the gut (28, 19, 29), its presence as a contaminant in preparation of gut hormones would not be unexpected and might play a role in the rise in insulin excretion observed. For this reason, in each experiment a sample of the hormone solution was removed from the syringe before its injection and its glucagon concentration measured. Secretin solutions contained from 0 to 3.7 mμg per U, pancreozymin from 0.02 to 0.06 mμg per U, and gastrin 0.62 mμg per U. These quantities are known to be insufficient to cause a rise in insulin of the magnitude observed (20), although potentiation by glucagon of the effect of another hormone is a possibility. The large rise in glucagon concentration observed after the injection of pancreozymin cannot be explained by its glucagon content; however, the small rise in pancreatic venous glucagon ob-

TABLE III

Effects of endoportal injection of crude gastrin on AG, PVI, and PVG, and of purified porcine gastrin II on AG and PVG

Dog	Gastrin dose		Minutes before injection		Minutes after injection								
			−10	0–1	1	3	6	10	15	20	30	45	60
	mU												
		A. crude gastrin											
125	203	AG (*mg/100 ml*)	113	115	115	120	119	120	112	112	111	112	114
		PVI (*μU/ml*)	471	169	1,640	486	392	312		327	416		288
		PVG (*mμg/ml*)	1.48	1.34	1.40	1.30	1.32	1.30		1.26	1.26		1.44
126	135	AG	129	123	125	129	123	126	127	130	132	123	135
		PVI	442	327	844	342	332	482		432	644		546
		PVG	1.50	1.44	1.84	1.54	1.46	1.58		1.44	1.48		1.46
127	180	AG	127	132	130	133	129	129	122	123	120	132	125
		PVI	74	84	327	109	74	94		79	89		94
		PVG	1.48	1.72	1.84	1.72	1.48	1.60		1.36	1.68		1.58
Mean		AG	123	123	123	127	124	125	120	122	121	122	125
		PVI	329	193	937	312	266	296		279	383		309
		PVG	1.49	1.50	1.69	1.52	1.42	1.49		1.35	1.47		1.49
		B. purified porcine gastrin II											
	mg												
183	0.06	AG	127	132	130	135	142	131	123	117	115		
		PVI	129	169	1,835	169	357	154	45	60	90		

served after gastrin injection may well be the consequence of its glucagon contaminant.

It seemed barely possible that the rise in pancreaticoduodenal venous glucagon concentration observed after pancreozymin administration was a consequence of reflux of the glucagon contaminant from the portal vein into the indwelling pancreatic venous needle. For this reason, six pancreozymin experiments were performed with a catheter inserted into the pancreatic vein in a retrograde direction and tied in place; thus, the entire pancreaticoduodenal vein effluent was collected, making reflux from the portal vein impossible. In these experiments, the rise in mean glucagon concentration after the injection of pancreozymin was substantially greater than in the other experiments, with a peak at 3 minutes of 3.19 mμg per ml as compared with 1.78 mμg per ml (Table IV).

These results not only exclude reflux of glucagon from the portal vein as a cause of the rise in glucagon concentration, but they point to the pancreas rather than to the gut as the principal source of the pancreozymin–induced rise in glucagon concentration.

Effects of inactive secretin and other polypeptide hormones. Inasmuch as each of the four hormones examined thus far, secretin, pancreozymin, gastrin, and glucagon, have been shown to elicit a very prompt and short–lived rise in pancreaticoduodenal venous insulin concentration when injected endoportally in anesthetized dogs, it seemed possible that this might be a nonspecific response to the rapid injection of a polypeptide. For this reason, the effects on insulin secretion of several other polypeptide hormones not of gastrointestinal origin and of a preparation of secretin that had been shown to have lost its secretagogue activity (22) were studied.

The administration via a peripheral vein of large doses of three other polypeptide hormones, ovine growth hormone, vasopressin, and ACTH, failed to elicit the same pattern of insulin response as observed after injection of the gastrointestinal hormones. After 20 U of vasopressin, insulin secretion seemed to decline at first and then rise in parallel with the blood glucose level (Figure 4). One mg ACTH seemed to induce a small early rise in insulin concentration lasting 3 minutes and an apparent decline in glucagon concentration (Figure 4). In a 2-mg dose, ovine growth hormone had no immediate effect on insulin concentration, but a late rise in plasma glucose and insulin concentration was noted (Figure 4). Al-

TABLE IV

Effect of endoportal injection of cholecystokinin–pancreozymin on AG or venous glucose (VG), PVI, and PVG during retrograde catheterization of the pancreatic vein

Dog	Dose		Minutes before injection −3	−2	−1	Minutes after injection 1	3	6	10
	U								
PZ3	100	VG (*mg/100 ml*)			91	86	92	88	95
		PVI (*μU/ml*)	104	69	94	372	367	402	134
		PVG (*mμg/ml*)	1.3	1.3	1.3	2.2	2.2	2.8	2.2
PZ4	100	VG			90	92	88	103	109
		PVI	283	193	159	1,538	1,637	1,141	1,290
		PVG	1.44	1.48	1.58	3.4	4.4	3.4	3.2
PZ5	100	VG			103	105	102	98	102
		PVI	45	35	50	139	432	308	109
		PVG	1.02	1.02	1.0	1.28	1.46	1.46	1.2
PZ9	100	AG (*mg/100 ml*)			186	189	200	224	232
		PVI	114	169	139	>2,480	546	92	149
		PVG		1.28	1.18	2.8	4.2	4.2	3.8
PZ10	100	AG			147	146	149	174	183
		PVI	233	184	159	>2,480	2,133	794	546
		PVG		1.3	1.49	2.6	4.6	3.0	1.92
PZ11	100	AG			133	142	174	225	273
		PVI	30	30	30	94	74	55	32
		PVG		1.06	1.12	1.78	2.3	3.0	2.2
Mean		VG			95	94	94	96	102
SD					±7.2	9.7	7.2	7.6	7.0
		AG			155	159	174	208	229
					±27.5	26.1	25.5	29.2	45.1
		PVI	135	113	105	>1,184	865	465	377
			±107	77	56	1,134	821	424	483
		PVG		1.24	1.28	2.34	3.19	2.98	2.42
				±0.17	0.22	0.76	1.36	0.90	0.93

Mean maximal rise
PVI: 1,149 ± 1,037 $p < 0.01$
PVG: 2.13 ± 1.04 $p < 0.01$

TABLE V

Effect of endoportal injection of inactive secretin (1.7 μg) on AG and PVI

Dog		Minutes before injection	Minutes after injection 1	3	6	10	15	20	30	45	60
35	AG (*mg/100 ml*)	138	126	128	122	129	127	135	130	134	138
	PVI (*μU/ml*)	362	392	252	193	169	159	243	262	348	282

though only one experiment with each of these hormones was performed, it would appear that they do not resemble the gastrointestinal hormones with respect to their effect upon insulin release.

The endoportal injection of 1.7 μg of the inactive secretin preparation failed to elicit the customary response in insulin secretion (Table V).

Epinephrine suppressibility of secretin-induced insulin release. Porte, Graber, Kuzuya, and Williams (26) have demonstrated that the stimulatory effect of intravenously administered glucose and of glucagon upon insulin secretion is inhibited by epinephrine. It seemed of interest, therefore, to evaluate the effect of epinephrine infusion on the stimulation of insulin secretion induced by secretin. During the infusion of epinephrine by peripheral

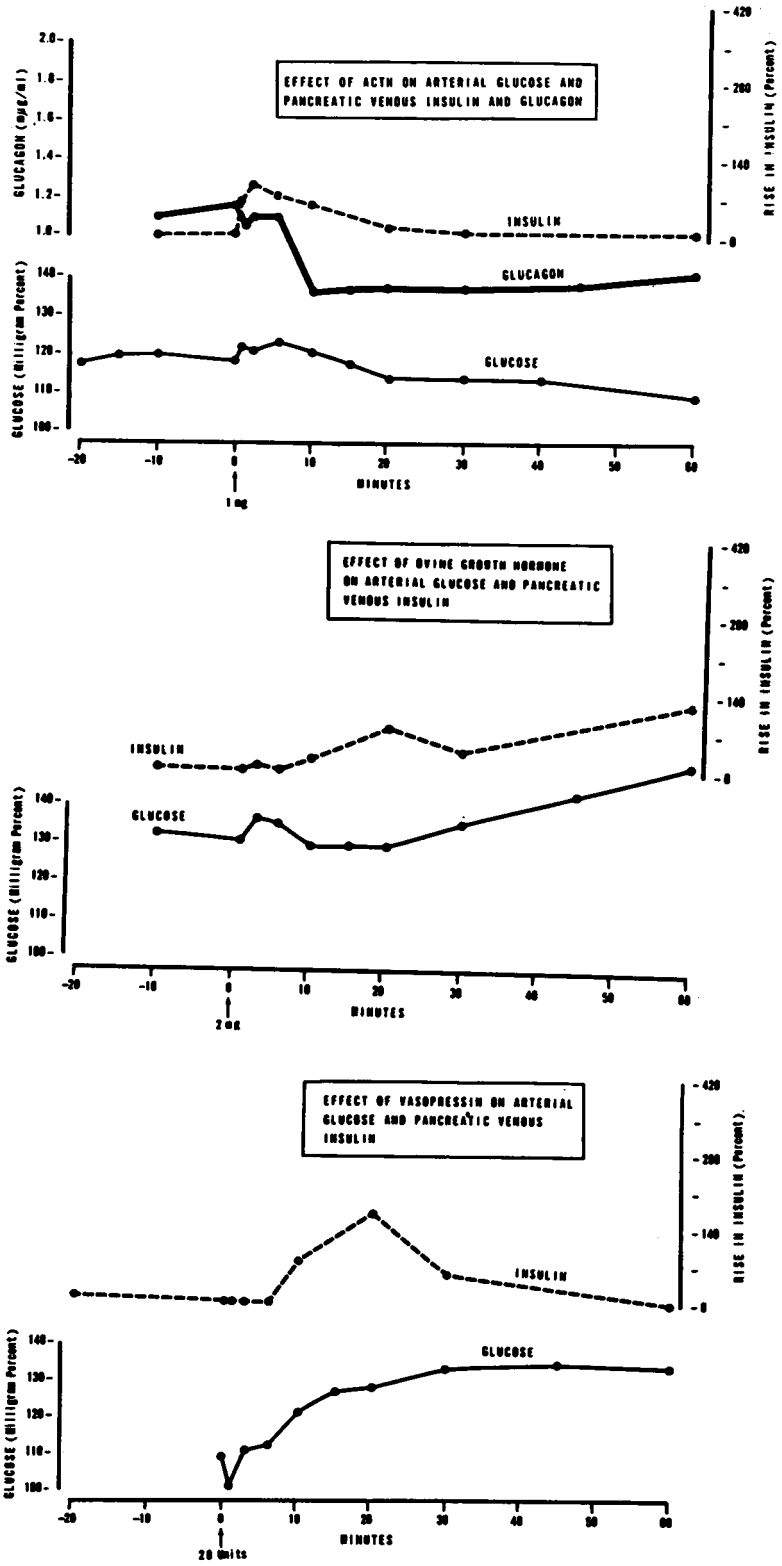

FIG. 4. EFFECT OF PERIPHERAL INTRAVENOUS ADMINISTRATION OF THREE POLYPEPTIDE HORMONES NOT FOUND IN GASTROINTESTINAL TISSUES UPON THE PANCREATICODUODENAL VENOUS PLASMA LEVEL OF INSULIN AND ARTERIAL PLASMA GLUCOSE LEVEL.

TABLE VI

Effect of epinephrine infusion on AG and PVI response to endoportal secretin and glucagon

Dog	Epinephrine		−10	−0.5	Secretin	1	3	6	10	15	20	30	Glucagon	1	3	6	10	15	20	30
	μg/min				*U*								*mμg*							
113	2	AG (*mg/100 ml*)	252	255	30	252	258	246	243	252	255	258	680	252	285	279	294	282	276	270
		PVI (*μU/ml*)	99	64		179	60	45	35		64	99		139	129	144	109		164	223
114	2	AG	231	243	30	246	246	252	255	255	258	255	700	258	270	270	258	252	258	246
		PVI	15	42		69	94	74	89		99	94		89	55	40	60		84	94
115	2	AG	236	232	30	220	226	226	224	222	216	228	720	238	250	256	254	250	250	244
		PVI	253	332		223	114	149	228		263	293		303	308	312	317		308	184
Mean		AG	240	243		239	243	241	241	243	243	247		249	268	268	269	261	261	253
		PVI	122	146		157	89	89	117		142	162		177	164	165	162		185	167

vein at a rate of 2 μg per minute, 30 U of secretin was injected rapidly into the portal vein; 30 minutes later, 700 mμg of glucagon was administered. As shown in Figure 5, no rise in mean pancreaticoduodenal insulin level was observed after either injection. The individual results of three such experiments are recorded in Table VI.

Effects of endoportal infusion of secretin. Although the sudden burst of insulin release that follows the injection of the enteric hormones is dramatic, it is extremely short-lived. Whereas the rise in insulin concentration observed in dogs after the endoportal injection of 1 μg of glucagon lasted for 6 minutes or more (20), 6 minutes after the injection of secretin, pancreozymin, or gastrin, the insulin concentration in the pancreaticoduodenal vein was nearing its preinjection level. Even if one overestimates pancreatic blood flow

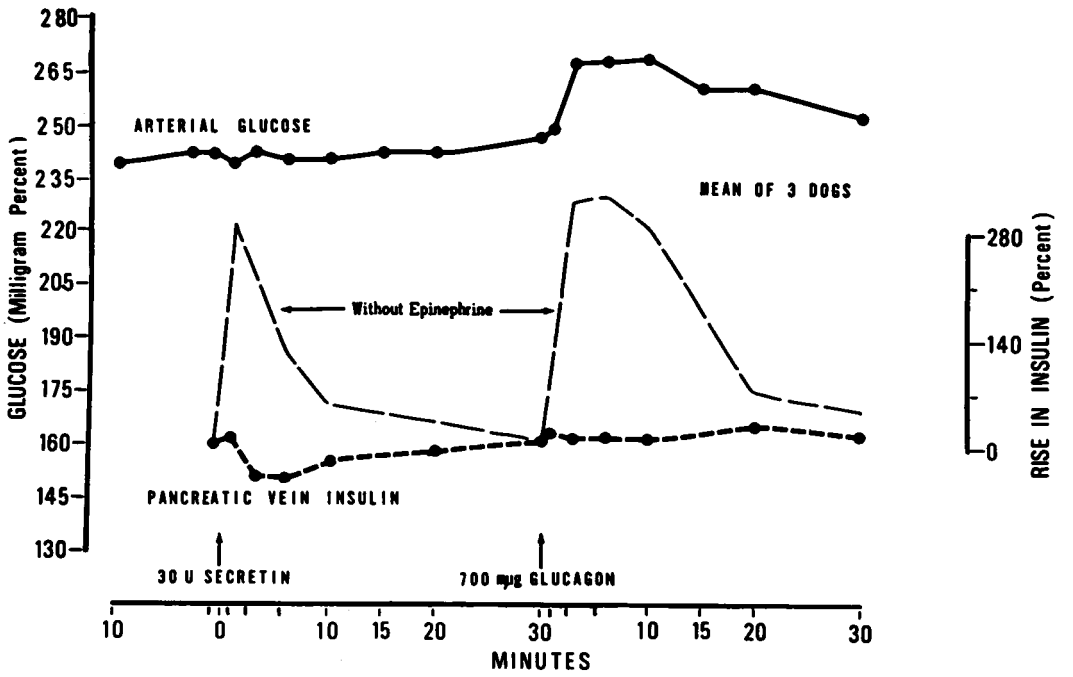

FIG. 5. EFFECTS OF THE RAPID ENDOPORTAL INJECTION OF SECRETIN AND GLUCAGON UPON PANCREATICODUODENAL VENOUS PLASMA LEVELS OF INSULIN DURING EPINEPHRINE INFUSION AT A RATE OF 2 μG PER MINUTE.

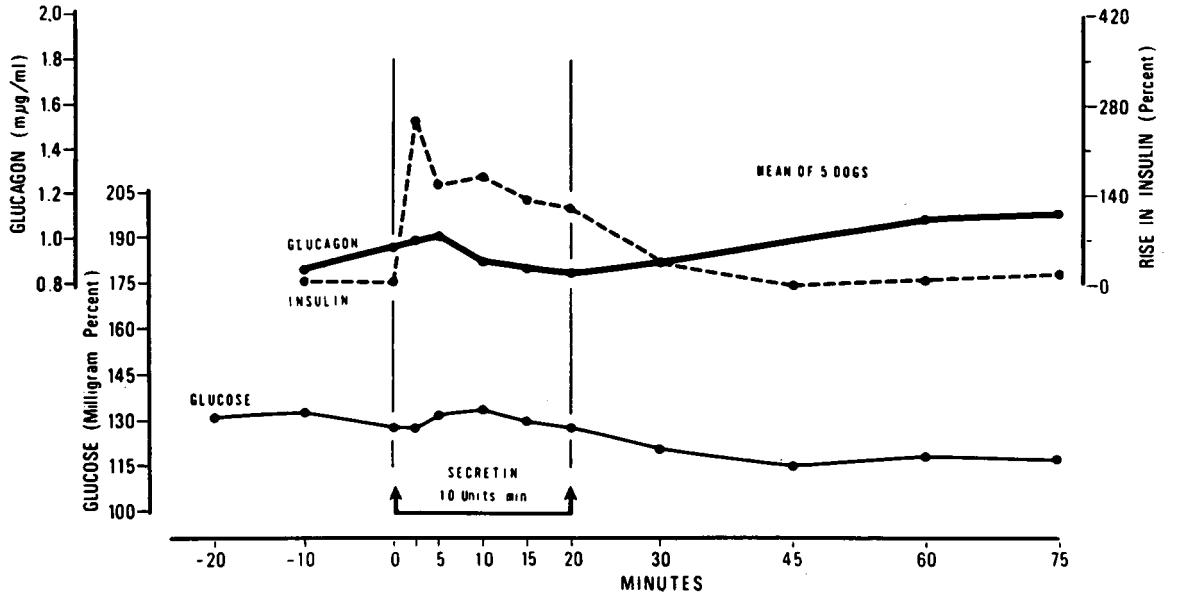

FIG. 6. EFFECT OF ENDOPORTAL INFUSION OF SECRETIN UPON PANCREATICODUODENAL VENOUS PLASMA LEVELS OF INSULIN AND GLUCAGON AND ARTERIAL PLASMA GLUCOSE CONCENTRATION.

to be 20 ml per minute, the total increment in insulin output resulting from the endoportal injection of these three hormones in these experiments is not more than a few milliunits. Its physiologic importance can, therefore, be questioned.

To determine if sustained endoportal infusion of secretin would elicit a sustained increase in insulin secretion, we infused secretin at a rate of 10 U per minute in a group of five dogs. The mean insulin level (Figure 6) rose initially from 173 μU per ml (SD ± 91.7) to a peak at 2.5 minutes of 604 μU per ml (SD ± 406.8), an increase of 249%, and declined to 435 μU per ml (SD ± 417.3) 5 minutes after the start of the infusion. There appeared to be a slight downward trend in the mean pancreaticoduodenal insulin concentration throughout the remainder of the infusion, and its rate of decline accelerated only slightly when the infusion ended. The mean level of pancreaticoduodenal venous glucagon did not change significantly during the infusion, nor did the small rise in mean arterial glucose concentration appear to be significant. The individual results are recorded in Table VII.

If we assume the same overestimated value of 20 ml per minute for pancreatic blood flow, it can be calculated that insulin secretion was augmented by approximately 105 mU during the 20-minute infusion of secretin, i.e., by 5.24 mU per minute.

Effects of endoportal infusion of pancreozymin. The effects upon insulin and glucagon secretion of pancreozymin infused endoportally at a rate of 30 U per minute for 20 minutes were examined in a group of four dogs. The mean pancreaticoduodenal concentration of insulin rose rapidly from a preinjection level of 127 μU per ml (SD ± 17.4) to a peak of 1,191 μU per ml (SD ± 319.3) at 10 minutes, a rise of more than 600% (Figure 7); during the last 10 minutes of the infusion, there was a decline to a mean level of 968 μU per ml, which was still more than six times the preinjection level. When the infusion was terminated, the insulin level fell to 372 μU per ml (SD ± 164.8) within the first 10 minutes and reached the base-line value within 25 minutes after the end of the infusion. If we estimate a pancreatic blood flow of 20 ml per minute, it can be calculated that a total of 346 mU of insulin was added during the 20-minute infusion of pancreozymin, or about 17.3 mU per minute. The changes in pancreatic venous insulin were reflected by appropriate, though smaller, alterations in arterial insulin concentration.

The mean glucagon level in pancreaticoduodenal

TABLE VII

Effect of endoportal infusion of secretin (10 U/minute) on AG, PVI, and PVG

Dog	Dose		Minutes before infusion			Infusion					Minutes after infusion						
			−20	−10	−0.5	2½	5	10	15	20	30	45	60	75	90	105	120
	U																
116	200	AG (*mg/100 ml*)	122	127	123	122	121	116	115	112	103	106	113	113	110		
		PVI (*μU/ml*)		50	40	104	129	94	60	40	104	184	164	174	114		
		PVG (*mμg/ml*)		0.7	0.78	0.82	0.96	0.56		0.48	0.88		0.90		1.04		
129	200	AG	137	137	132	133	133	129	126	125	118	118	133	133	136		
		PVI		104	164	794	382	164	193	114	84	134	253	303	317		
		PVG		0.82	1.04	0.88	0.90	0.96		0.82	0.78		0.98		0.96		
130	200	AG	124	124	122	120	120	123	130	139	141	136	148	143	136		
		PVI		84	114	238	99	35	55	60	50	84	179	213	179		
		PVG		0.94	1.00	0.92	1.0	1.0		1.0	0.96		1.02		1.1		
149	200	AG	133	132	134	137	155	169	156	143	130	110	100	98	102	103	104
		PVI		253	248	1,240	1,240	1,488	1,290	1,290	794	367	179	179	149	129	144
		PVG		0.7	0.8	1.14	1.26	1.04	1.04	0.94	0.88	1.02	1.1		1.2	1.1	1.4
150	200	AG	144	138	130	126	130	132	126	121	112	106	103	105	108	110	108
		PVI		258	298	645	327	546	397	402	124	129	179	174	144	184	144
		PVG		1.12	1.18	1.2	1.0	0.96	0.96	1.0	1.02	1.08	1.44		1.32	1.56	1.28
Mean		AG	132	132	128	128	132	134	131	128	121	115	119	118	118		
SD		±	8.1	5.5	4.8	5.8	12.6	18.4	13.6	11.5	13.4	11.3	18.4	17.0	14.6		
		PVI		150	173	604	435	465	399	381	231	180	191	209	181		
		±		88.8	91.7	406.8	417.3	541.8	462.5	472.8	282.6	98.2	30.4	47.7	70.2		
		PVG		0.86	0.96	0.99	1.02	0.90		0.85	0.90		1.09		1.12		
		±		0.14	0.15	0.16	0.15	0.19		0.19	0.12		0.38		0.16		

TABLE VIII

Effect of endoportal infusion of pancreozymin–cholecystokinin (30 U/minute) on AG, PVI, PVG, AI, and arterial glucagon (Ag)

Dog	Dose		Minutes before infusion			Infusion					Minutes after infusion							
			−20	−10	−0.5	1	2½	5	10	15	20	30	45	60	75	90	105	130
	U																	
131	600	AG (*mg/100 ml*)	125	126	124		126	124	126	126	123	112	110	96	103	100		
		PVI (*μU/ml*)		149	154		694	1,042	1,389	893	546	169	144	99	144	139		
		AI (*μU/ml*)		37	45		181	119	206		161	79		57				
		PVG (*mμg/ml*)		2.6	2.2		3.2	4.0	3.8		3.4	2.6		2.4		3.2		
		Ag (*mμg/ml*)		0.8	0.8		0.8	0.8	1.0		1.0			0.8				
132	600	AG	118	122	113		119	142	209	235	238	239	215	159	130	111	93	
		PVI		74	109		644	596	644	694	596	253	144	184	119	84		
		AI		47	50		141	246	335		322	184		50				
		PVG		0.84	0.92		1.32	1.42	2.2		2.0	1.44		0.94		0.82		
		Ag		0.8	0.6		0.8	1.0	1.4		1.2	1.0		0.8				
142	600	AG	124	124	121	124	122	136	178	202	208	189	144	114	103	103	100	103
		PVI		179	139	124	892	1,140	1,440	1,400	1,440	546	283	129	114	129	124	99
		AI		40	37		87	273	546		54	243		47				
		PVG		0.62	0.6	0.54	1.04	1.5	1.56	1.8	1.78	0.96	0.84	0.78	0.74	0.86	0.96	0.78
		Ag		0.6	0.6		0.8	1.0	1.4		1.6	1.0		0.8				
143	600	AG	137	138	135	139	140	159	215	246	263	236	164	108	91	95	103	110
		PVI		104	104	243	794	1,042	1,290	1,339	1,290	521	208	89	79	94	89	89
		PVG		0.52	0.72	0.66	1.02	1.7	1.66	1.72	1.72	0.84	0.66	0.64	0.62	0.62	0.6	0.62
Mean		AG	126	128	123		127	140	182	202	208	194	158	119	107	102	99	
SD		±	6.9	6.2	11.1		8.8	14.2	35.5	47.9	49.3	51.3	39.1	25.1	12.4	9.2		
		PVI		127	127		756	955	1,191	1,092	968	372	195	125	114	112		
		±		38.8	17.4		95.3	211.1	319.3	306.5	400.8	164.8	56.4	37.8	22.4	23.0		
		PVG		1.15	1.11		1.64	2.16	2.31		2.23	1.46		1.19		1.38		
		±		0.84	0.64		0.91	1.05	0.88		0.67	0.69		0.71		1.05		

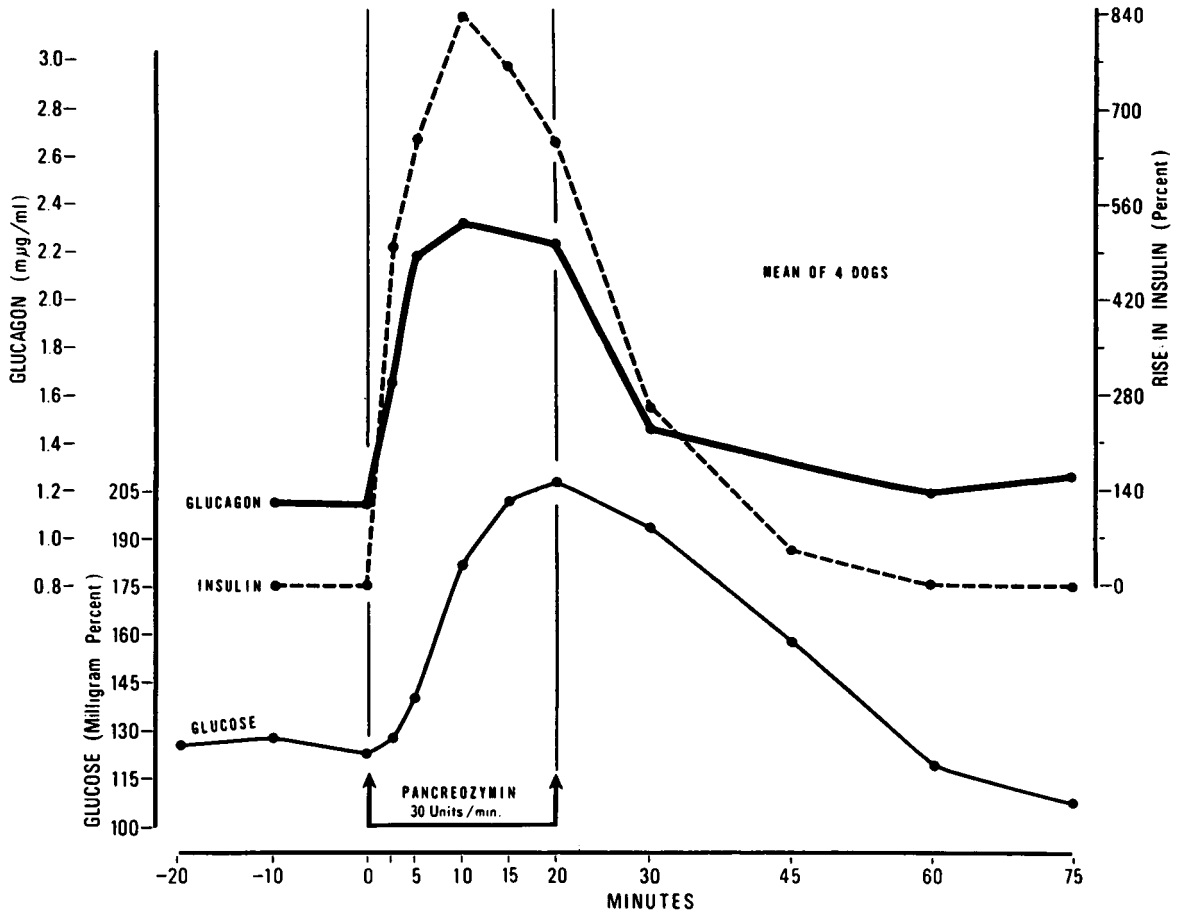

FIG. 7. EFFECT OF ENDOPORTAL INFUSION OF PANCREOZYMIN UPON PANCREATICODUODENAL VENOUS PLASMA LEVELS OF INSULIN AND GLUCAGON AND ARTERIAL PLASMA LEVELS OF GLUCOSE.

venous plasma also rose promptly after the start of the infusion from a preinjection level of 1.11 mμg per ml (SD ± 0.64) to 2.16 mμg per ml (SD ± 1.05) at 5 minutes and remained at or above this level until the termination of the infusion, at which point it declined rapidly to a level of 1.46 mμg per ml 10 minutes after termination. If we assume a pancreatic blood flow of 20 ml per minute, glucagon secretion was augmented by approximately 408 mμg or 20.4 mμg per minute during the infusion. The changes in pancreatic venous glucagon were reflected by small changes in arterial glucagon levels.

The mean arterial plasma glucose level rose from a preinjection level of 123 mg per 100 ml (SD ± 11.1) to 140 mg per 100 ml (SD ± 14.2) during the first 5 minutes of the infusion. During the second 10 minutes, however, it ascended more rapidly to a level of 182 mg per 100 ml (SD ± 35.5) at 10 minutes and reached a peak level of 208 mg per 100 ml (SD ± 49.3) at the end of the infusion. Upon termination of the infusion, the mean plasma glucose concentration declined at a gradual rate of 2.2 mg per minute and required more than 30 minutes to reach the preinjection level. The individual results of all experiments are recorded in Table VIII.

Discussion

These results provide evidence of a relationship between the hormones of the gastrointestinal tract and those of the islets of Langerhans. They thus support the 60-year-old concept that secretin is biologically capable of augmenting insulin secretion. These studies also reveal, however, that

TABLE IX

Effects of gastrointestinal hormones and pancreatic glucagon

Hormone	Arterial glucose	Insulin secretion	Glucagon secretion
Glucagon, 1 μg	Prompt 50 mg/100 ml rise; peak at 6 minutes.	Prompt 250% rise; peak at 6 minutes	
Secretin, 20–30 U (1–2 μg)	No effect	Vertical 290% rise; peak at 1 minute.	None
Gastrin, 0.13–0.2 U (30–34 mg)	No effect	Vertical 390% rise; peak at 1 minute.	None, or very slight.
Pancreozymin–cholecystokinin, 100 U (17 μg)	Late rise; peak at 10 minutes.	Vertical 480% rise; peak at 1 minute.	Prompt 80% rise; peak at 3 minutes.

secretin is but one of several beta–cytotropic hormones present in the gastrointestinal tract. The observations of Meade (30) and the results of the present study reveal a brisk release of insulin after the rapid injection and the continuous infusion of the highly purified pancreozymin preparation of Jorpes and Mutt. In addition, both crude gastrin–containing antral extract and purified porcine gastrin II exhibited insulin–releasing activity similar in pattern to that of secretin and pancreozymin. Although the doses employed were large, particularly in the case of pancreozymin, Meade (30) has noted a similar response in portal venous insulin concentration after the rapid administration of 6 to 7.5 U of pancreozymin by peripheral vein. Glucagon has recently been shown to have potent insulin–releasing activity by Samols, Tyler, Megyesi, and Marks (19) and by Crockford, Porte, Wood, and Williams (27) in man, and by Ketterer, Eisentraut, and Unger (20) in dogs. In the latter study, the pattern of the response of pancreaticoduodenal insulin concentration and of arterial plasma glucose concentration to the rapid endoportal injection of 1 μg of glucagon was distinctly different from the response to the three hormones studied here; after glucagon injection, the mean insulin and glucose levels rose concomitantly to a plateau peak at 3 to 6 minutes after injection and remained above the preinjection level for almost 20 minutes. The patterns of response for each of the four beta–cytotropic hormones are compared in Table IX.

The ubiquity of "glucagon–like" biologic activity (31, 32) and immunoreactivity (19, 28, 29) raises the possibility of its presence as a contaminant in other hormone preparations of the gut. Furthermore, there appears to be a structural similarity between glucagon, composed of 29 amino acids (33), and secretin, composed of 27 (34), that has led to the suggestion of an overlap in their biologic properties (35). The crude gastrin preparation employed here contained from 17 to 38 mμg of glucagon–like immunoreactivity per milligram, but since glucagon–free gastrin II caused a similar response, it seems unlikely that glucagon was an important factor in the observed response. Pancreozymin contained only 0.02 to 0.06 mμg per U of glucagon–like immunoreactivity, not enough to have played a significant role in the genesis of the insulin response. Finally, glucagon–like immunoreactivity of the secretin preparation ranged from 0 to 3.9 mμg, not nearly enough to stimulate insulin secretion to the degree observed. (Despite a possible structural similarity of the glucagon and secretin molecules, the absence of hyperglycemia after the endoportal injection of large doses of secretin and the immunologic displacement by 20 μg of secretin of the equivalent of only 0.0009 μg of glucagon reveal major biologic and immunologic differences.)

It would appear that the pattern of insulin release induced by gastrin, secretin, and pancreozymin is a characteristic of these gastrointestinal hormones and is not shared by any of the non-gastrointestinal polypeptides studied. The insulinogenic effect of ACTH, previously reported *in vitro* by Sussman, Vaughn, and Timmer (36), differed both in timing and magnitude.)

In these experiments, pancreozymin had a direct glucagon–releasing effect and is consequently the first hormone thus far shown to have this property. Although the pancreozymin prepara-

tion did contain traces of glucagon, the quantities were far too low to account for a rise in pancreatic venous glucagon of the magnitude and duration observed. The relative timing of the arterial hyperglycemia and the hyperglucagonemia suggests that the former could be a consequence of the release of endogenous glucagon rather than of a glycogenolytic effect of the pancreozymin itself. The occurrence of hyperglycemia in the retrograde catheterization experiments does not weigh against this possibility, since venous channels other than the pancreaticoduodenal vein drain the endocrine pancreas and arterial glucagon levels rose in those experiments.

The metabolic importance of the quantities of insulin released in response to the enteric hormones is not apparent from these data, since, surprisingly, glucose did not decline significantly and free fatty acid levels were not measured. However, Meade has observed a fall in FFA concentration after the administration by peripheral vein of much smaller doses of pancreozymin to dogs (30).

Proof of the physiologic significance of the enteric hormones in the control of islet hormone secretion will require demonstration that similar responses in islet hormone secretion are provoked by maneuvers that cause the secretion of endogenous enteric hormones. Thus far, reported attempts to enhance insulin secretion by intraduodenal instillation of acid, a stimulus for secretin secretion have been unsuccessful (37). On the other hand, the intraduodenal instillation of protein hydrolyzates has been shown to cause a striking rise in both insulin and glucagon secretion (38). Pancreozymin, which is secreted in response to amino acid ingestion (39) and which duplicates qualitatively the effects of amino acids upon islet hormone secretion, could be that hormone.

The fact that four hormones of the gastrointestinal tract, gastrin, secretin, pancreozymin–cholecystokinin, and "glucagon" of intestinal origin (40), all have insulin–releasing activity suggests a chain of beta-cytotropic hormones extending from the antrum to the ileum. Early augmentation of insulin secretion by these hormones to a degree appropriate to the quantity and type of nutrients ingested before the attainment of peak blood levels of substrates would permit prompt substrate disposal without excessive hyperglycemia or hyperaminoacidemia, which would occur after large meals if insulin release were entirely dependent upon arterial substrate concentration.

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