Effects of Secretin, Pancreozymin, or Gastrin on the Response of the Endocrine Pancreas to Administration of Glucose or Arginine in Man

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ABSTRACT Intravenous administration of porcine secretin or pancreozymin or synthetic human gastrin II resulted in raised increments in serum immunoreactive insulin during intravenous infusion of glucose in normal man. Enhancement of serum immunoreactive insulin by each hormone was associated with accelerated disposal of glucose. In response to prolonged intravenous infusion of arginine with pancreozymin there was a maintained rise in immunoreactive insulin and glucagon-like immunoreactivity in the blood. These effects of pancreozymin and arginine were not reproduced with secretin and arginine, and may have been due to the stimulation of glucagon secretion together with insulin by pancreozymin.

Enteric infusion of hydrochloric acid, or stimulation of gastric acid secretion by betazole, presumed to cause release of endogenous secretin, led to enhancement of insulin secretion during intravenous infusion of glucose. Enteric infusion of arginine, presumed to cause release of endogenous pancreozymin, led to a rise in serum immunoreactive insulin not attributable to effects of circulating glucose and amino acids. It is concluded that secretin and pancreozymin released in response to physiological stimuli contribute to stimulation of the endocrine pancreas after ingestion of food.

INTRODUCTION

The rate of disposal of glucose taken orally by normal man is greater than that of glucose given by intravenous infusion (1, 2), and delivery of glucose into the stomach or the upper small intestine is associated with development of higher blood levels of immunoreactive insulin (IRI) than those attained when similar or even greater glycemia is produced by intravenous infusion of glucose (3, 4). It has also been shown in man that ingestion of glucose can provoke a rise in plasma glucagon-like immunoreactivity (GLI), whereas intravenous infusion of glucose leads to no increase in this activity (5, 6). The effect of alimentary glucose on glucose disposal may be impaired in liver disease but is not entirely dependent on portal perfusion of the liver (2). Potentiation of insulin release associated with intestinal absorption of glucose is not abolished by portacaval anastomosis (7). Moreover, infusion of glucose into the portal system in man does not evoke enhanced insulin secretion similar to that associated with absorption of glucose from the intestine (8). It therefore appears that some of the features distinguishing the response to glucose absorbed from the intestinal tract from that to glucose given parenterally are not related to portal hyperglycemia and depend on alimentary function. These findings have revived the question of the possible role of humoral agents derived from the intestine in the regulation of the endocrine function of the pancreas, a problem that was the subject of extensive but inconclusive investigation in the past (9, 10).

We have previously shown that administration of crude secretin improves tolerance to glucose given intravenously in normal man, and that this effect is associated with enhancement of the rise in the immunosuppressible insulin-like activity of the blood (2, 11). We

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have also demonstrated release of IRI into the splanchnic circulation in fasting man in response to intravenous injection of secretin (12). Similar effects of secretin, pancreozymin, or gastrin on insulin secretion and stimulation of glucagon secretion by pancreozymin in fasting anesthetized dogs were reported by Unger, Ketterer, Dupre, and Eisentraut (13). The effect of pancreozymin on insulin secretion in dogs was also described by Meade, Kneubuhler, Schulte, and Barboriak (14).

We report here studies of the effects of secretin pancreozymin or gastrin on the response to intravenous infusion of glucose or arginine in man. The possible physiological significance of effects of secretin or pancreozymin on insulin release was investigated in experiments in which stimuli believed to cause the secretion of these hormones were applied. The effect of infusion of hydrochloric acid (HCl) into the duodenum, a stimulus to the release of secretin (15), was compared with that of stimulation of gastric secretion of HCl with betazole during intravenous infusion of glucose. The response to infusion of amino acid into the duodenum, a stimulus to the release of pancreozymin (15), was compared with the response to intravenous infusion of amino acid. It has been shown that each of the hormones tested is capable of causing a rise in the blood IRI. Indirect evidence of similar effects of endogenous secretin and pancreozymin was obtained. The possible contribution of these effects to physiological responses to ingestion of nutrients is discussed.

METHODS

Nonobese male and female subjects aged 18–35 yr were studied in the morning after fasting overnight. Subjects adhered to their regular diet. All had normal blood glucose concentrations in the fasting state. Venous blood samples were drawn from a cannula in a superficial forearm vein. Glucose (10 g/100 ml in water) was infused at 7.6 ml/min for 40 min. Arginine hydrochloride (5 g/100 ml in water) was infused at 1.9 or 7.6 ml/min for 40 min. In some experiments 200 ml of arginine hydrochloride (5 g/100 ml in water) was infused intravenously at a steady rate for 2 min; control and test injections were administered on the same morning at an interval of not less than 60 min. When one of the hormones was administered it was dissolved in the infusate immediately before use. Porcine secretin, containing over 4000 U/mg, and porcine pancreozymin, containing approximately 6000 Crick Harper Raper units/mg, were prepared and assayed by Professor E. Jorpes and Dr. V. Mutt of Karolinska Institutet, Stockholm. Synthetic human gastrin II was obtained from the American Gastroenterological Association. Infusions into the duodenum were delivered through polyethylene tubes (PE/190) with multiple punctures in the distal 4 inches. A radio-opaque marker at the distal end of each tube was localized by fluoroscopy. When acid was administered, N/10 HCl was infused into the duodenum at 7.6 ml/min. When betazole[1] was administered, 50 mg was injected subcutaneously 3–5 min before starting the infusion. The order of control and test infusions was varied at random.

Blood glucose was determined by a glucose oxidase method in a manual procedure or with the same reagents on the Technicon AutoAnalyzer. Serum amino nitrogen was determined by the method of Lacy and Crofford on the Technicon AutoAnalyzer with standard solutions of arginine hydrochloride (16). Serum IRI was determined by a double antibody procedure (17), or by a modification in which dextran-coated charcoal was used to separate free and antibody-bound moieties of insulin. The dextran-coated charcoal was prepared according to the procedure of Herbert and colleagues (18) but was made up in the Veronal buffer used in the double antibody system. The results obtained with these two assays were indistinguishable. The accuracy of each assay was checked by the inclusion of samples from a reference pool of serum and by the recovery of human insulin from the same pool. These control estimates usually fell within 10% of the expected values, and assays in which this was not so were rejected. Plasma glucagon-like immunoreactivity was assayed by the method of Unger and his associates (13).

RESULTS

The tables show mean fasting values for total IRI, GLI, glucose, or alpha amino nitrogen with the mean changes in concentration from fasting values during or after infusions. When the same subjects received infusions of glucose or arginine with and without a further test procedure the tables show the means of paired differences in changes from fasting values. The quantities in terms of IRI microunits-minutes/ml and GLI millimicrogram-minutes/ml were obtained graphically from the areas under plots of increments in these activities during infusions and are referred to as integrated secretory responses (ISR). Rate constants for the fall in blood glucose concentration were derived graphically from plots of values for total blood glucose estimated in at least three specimens during the period of fall in blood glucose after completion of infusions at 40 min. The statistical significance of differences in changes in blood levels of IRI or GLI in control and test sets was estimated from the means and SEM of paired differences in integrated secretory responses, and from the means and SEM of paired differences between values at corresponding times during control and test infusions treated as sets (19). The validity of the latter procedure with respect to differences in concentrations attributable to the test agents was confirmed by analysis of variance.[2]

Effects of the infusion of secretin, pancreozymin, or gastrin in fasting subjects. Infusion of secretin (3.5 U/min for 20 min) caused a small rise in serum IRI in all four subjects which was greatest in the first specimen drawn at 5 min (17 $\pm$4.2 μU/ml) and was statistically significant only at this time ($P < 0.05$). Infusion

[1] Histalog.

[2] The tables of individual observations made in all experiments reported in this paper are available on request.

TABLE I

Effects of Enteric Hormones on Response to Intravenous Glucose

Part	Infusate	N		Fasting value*	Increments, time (min) 5	10	20	30	40	60	ISR* (0–40 min)	*K** value (after 40 min)	Mean of paired differences for IRI* (0–40 min)
A.	G	7	IRI	12 (4.0)	22	24	33	37	44	37	1129 (382)		
	G + sec	7	IRI	15 (3.0)	57	60	87	102	116	29	3181 (479)		
			pd IRI*‡		35 (5.1)	36 (9.1)	54 (11)	65 (10)	72 (10)	−8	2051 (385)		53 (5.0)
	G	7	BG	74 (3.5)	33	53	87	104	118	55		1.79 (0.13)	
	G + sec	7	BG	68 (3.7)	33	55	75	102	101	22		3.99 (0.66)	
			pd BG		0 (7.9)	2 (7.9)	−12 (9.8)	−2 (5.6)	−17 (4.9)	−33		2.20 (0.59)	
B.	G	8	IRI	13 (3.0)	14	16	24	32	38	18	964 (290)		
	G + PZ	8	IRI	16 (3.5)	56	69	98	112	98	36	3308 (740)		
			pdIRI		42 (6.6)	53 (12)	74 (21)	80 (24)	60 (15)1	18	2244 (665)		62 (7.8)
	G	8	BG	71 (3.6)	23	34	66	79	90	29		2.78 (0.35)	
	G + PZ	8	BG	67 (3.5)	30	46	67	76	73	−5.4		4.21 (0.68)	
			pd BG		7 (6.3)	12 (5.4)	1 (3.4)	−3 (11)	−17 (8.7)	−34		1.43 (0.48)	
C.	G	6	IRI	13 (2.8)	21	19	24	31	36	17	1047 (282)		
	G + GII	6	IRI	13 (3.8)	34	27	37	54	55	31	1496 (222)		
			pd IRI		13 (5.3)	8 (7.3)	13 (9.4)	23 (8.1)	19 (7.0)	14	449 (191)		16 (3.4)
	G	6	BG	69 (3.2)	26	46	73	89	99	35		2.54 (0.47)	
	G + GII	6	BG	65 (3.9)	34	54	80	97	101	26		3.32 (0.52)	
			pd BG		8 (3.4)	8 (1.3)	7 (.45)	8 (8.2)	2 (8.6)	−9		0.78 (0.30)	
	G	6	IRI	10 (3.2)	19	18	26	32	35	37	1021 (35)		
	G + HCl	6	IRI	14 (3.6)	25	20	46	48	51	25	1467 (309)		
			pd IRI*‡		6 (6.8)	2 (6.5)	20 (9.0)	16 (6.5)	16 (5.3)	−12	447 (208)		14 (3.2)
D.	G	6	BG	71 (3.7)	31	48	86	110	126	67		2.13 (0.44)	
	G + HCl	6	BG	61 (5.2)	33	51	81	93	107	39		2.52 (0.33)	
			pd BG		2 (4.0)	3 (1.8)	−5 (8.6)	−17 (12)	−19 (9.8)	−28		0.39 (0.22)	
E.	G	5	IRI	20 (4.2)	21	21	27	34	37	16	1087 (350)		
	G + BZ	5	IRI	25 (6.0)	29	32	46	43	48	23	1551 (285)		
			pd IRI		8 (4.8)	11 (4.8)	19 (3.8)	9 (5.0)	11 (4.6)	7	363 (123)		13 (4.6)
	G	5	BG	65 (4.4)	28	45	79	97	108	45		2.49 (0.47)	
	G + BZ	5	BG	62 (3.5)	34	50	79	102	115	45		2.83 (0.42)	
			pd BG		6 (2.4)	5 (3.2)	0 (4.0)	5 (6.5)	7 (9.4)	0		0.34 (0.23)	

Effects of i.v. secretin (Sec, part A), i.v. pancreozymin (PZ, part B), i.v. gastrin (GII, part C), intraduodenal HCl (part D), or subcutaneous betazole (BZ, part E) on serum IRI, on integrated secretory response for IRI (ISR), blood glucose (BG), and rate constant for glucose disappearance (*K*) in response to i.v. glucose (G, 30 g in 40 min). N = number of subjects. Units: IRI, μU/ml; ISR, μU −min/ml; BG, mg/100 ml.

* Value (±SEM).

‡ pd = paired differences.

of pancreozymin (30 U/min for 10 or 20 min) caused a rise in serum IRI in two of four subjects. Infusion of gastrin (25 μg in 5 min) caused a small rise in serum IRI in three of four subjects. These changes after administration of pancreozymin or gastrin were not statistically significant. Injection of betazole (50 mg subcutaneously) caused no detectable change in serum IRI in any of four subjects. In none of these studies in fasting subjects was there a detectable change in blood glucose concentration.

Effect of secretin during the intravenous infusion of glucose. Infusion of secretin (1.9 or 3.5 U/min for 40 min) was associated with enhancement of the increment in serum IRI during intravenous infusion of glucose in all of seven subjects (Table I A). Fig. 1 *a* shows the mean increments in serum IRI attained in these experiments. The integrated secretory response for IRI was increased in all subjects and the mean of paired differences in these responses was statistically significant ($P < 0.02$). The mean of paired differences in increments in IRI during infusions was highly significant ($P < 0.001$). The glucose disposal rate was accelerated in all subjects and the mean of paired differences in the rate constants was statistically significant ($P < 0.02$). Infusion of secretin was associated with a mildly unpleasant epigastric sensation in approximately half the subjects.

Effect of pancreozymin during the intravenous infusion of glucose. Infusion of pancreozymin (300 Crick Harper Raper Units in 10–40 min) was associated with

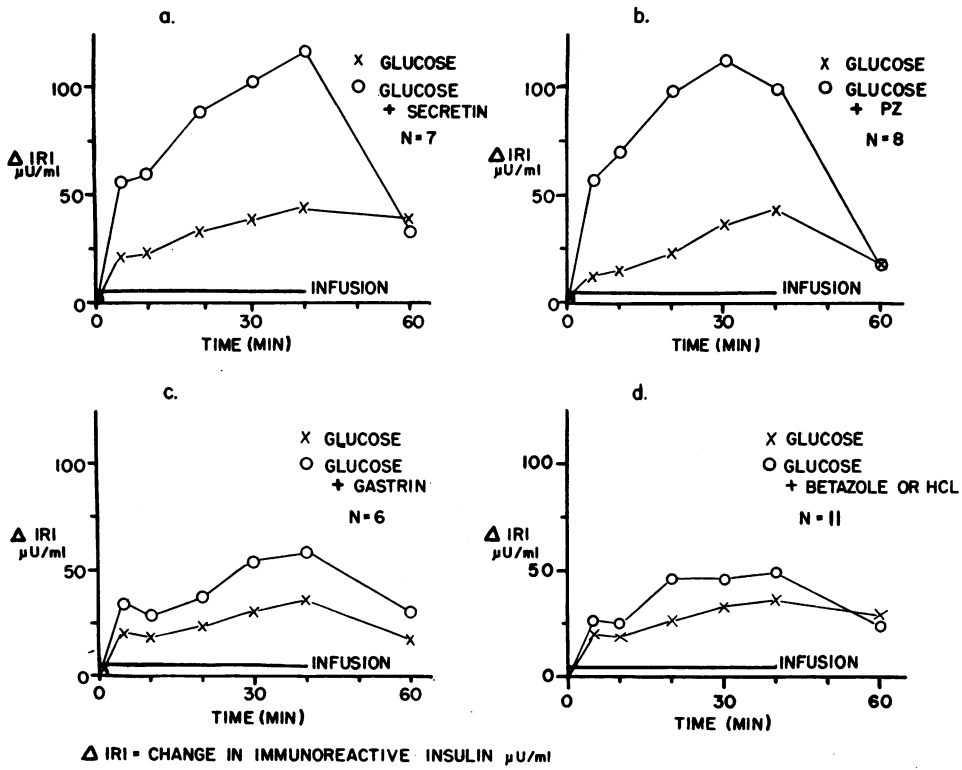

FIGURE 1 Effects of secretagogues on glucose-stimulated insulin release. Each subject received a control intravenous infusion of glucose, 30 g in 40 min, and on another occasion the same infusion with one of the following: (*a*) secretin, 150 or 300 U; (*b*) pancreozymin, 300 or 600 U; (*c*) gastrin, 25 μg or (*d*) betazole, 50 mg subcutaneously or intraduodenal HCl, 30 mEq. N = number of subjects.

enhancement of the rise in serum IRI in all subjects (Table I B). The mild epigastric discomfort that resulted from infusions at 15 U or more per min was negligible or absent during infusions at 7 U/minute. The increase in the rise in IRI fell off when the administration of pancreozymin was not continued throughout the infusion of glucose. Fig. 1 *b* shows the mean increments in serum IRI observed. The mean of paired differences in integrated secretory response for IRI in all eight subjects was statistically significant ($P < 0.02$). The mean of paired differences in increments in IRI was statistically significant ($P < 0.001$). The rate constant for glucose disappearance was substantially increased in the five subjects who received pancreozymin for 20 min or more and the mean of paired differences for all eight subjects was statistically significant ($P < 0.05$).

Effect of gastrin during the intravenous infusion of glucose. Infusion of gastrin in doses known to provoke maximal gastric secretion of acid was associated with enhancement of the increment in serum IRI in response to the rise in blood glucose in five out of six subjects (Table I C, Fig. 1 *c*). The mean increase in integrated secretory response for IRI was smaller than that produced by secretin or pancreozymin, and the mean of paired differences was not statistically significant. However, the mean of paired differences in increments in IRI during the infusions was statistically significant ($P < 0.001$). Glucose disappearance rate was accelerated in five out of six subjects and the mean of paired differences was statistically significant ($P < 0.05$). The effects of brief (5 min, four subjects) or sustained (20 min, two subjects) infusions of gastrin were not detectably different.

Effect of intestinal infusion or endogenous secretion of hydrochloric acid during the intravenous infusion of glucose. 10 or 30 mEq of N/10 HCl was infused into the duodenum during intravenous infusion of glucose for 40 min in six subjects. The maximum rise in serum IRI was increased when acid was infused in five of the six subjects (Table I D). The mean of paired differences in integrated secretory response for IRI was not statistically significant. However, the mean of paired differences in increments in IRI was statistically significant ($P < 0.01$). The rate constant for glucose disappearance was increased in five of the six subjects when acid was infused, but the mean of paired differences was not

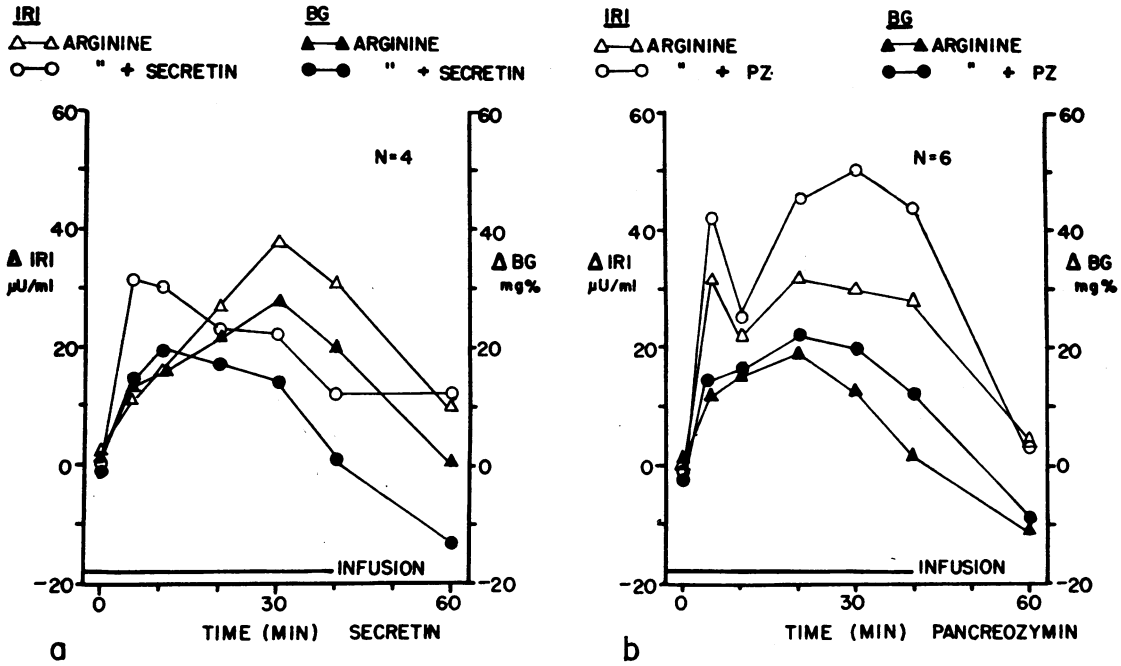

FIGURE 2 Effects of secretagogues on arginine-stimulated insulin release (prolonged stimulation). Each subject received a control intravenous infusion of arginine, 15 g in 40 min, and on another occasion the same infusion with either secretin, 150 U, or pancreozymin, 600 U.

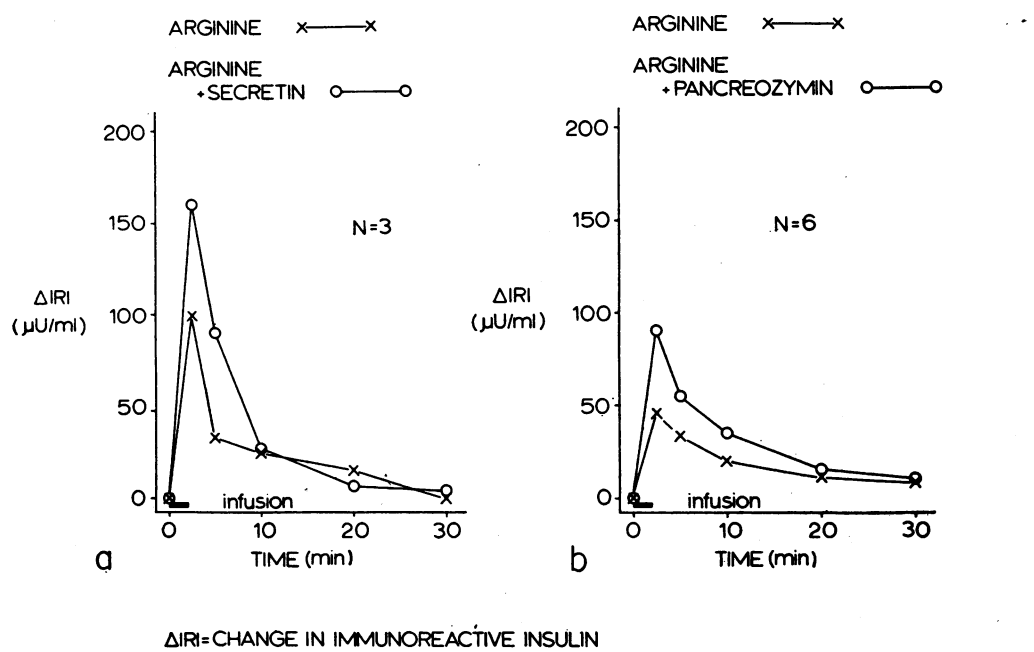

FIGURE 3 Effects of secretagogues on arginine-stimulated insulin release (brief stimulation). Each subject received a control intravenous infusion of arginine, 10 g in 2 min, and on another occasion the same infusion with either secretin, 150 U, or pancreozymin, 300 U.

TABLE II

Effects of Enteric Hormones on

Part	Infusate	N		Fasting value*	Increments time (min) 2.5	5	10
A	A 15‡	4	IRI	15 (2.1)	—	11	16
	A 15 + Sec	4	IRI	15 (1.3)	—	32	32
			pd IRI*‡			21 (3.8)	16 (4.0)
	A 15	4	BG	62 (1.9)	—	13	16
	A 15 + Sec	4	BG	59 (2.2)	—	14	19
			pd BG			1.5 (4.5)	3.0 (2.1)
B	A 15	6	IRI	13 (0.9)	—	32	21
	A 15 + PZ	6	IRI	10 (4.4)	—	42	25
			pd IRI			10 (5.1)	3.3 (5.0)
	A 15	6	BG	75 (4.9)	—	12	15
	A 15 + PZ	6	BG	71 (3.1)	—	14	16
			pd BG			1.2 (3.6)	0.3 (5.2)
C	A 10‡	3	IRI	20	99	33	24
	A 10 + Sec	3	IRI	21	153	90	28
			pd IRI		54 (15)	57 (14)	3.7 (0.9)
	A 10	3	BG	67	5	11	14
	A 10 + Sec	3	BG	66	9	11	18
			pd BG		3.7 (2.5)	0.3 (1.1)	5.0 (2.0)
D	A 10	6	IRI	21 (5.7)	32	45	21
	A 10 + PZ	6	IRI	21 (4.9)	91	53	35
			pd IRI		59 (27)	7.5 (5.3)	15 (3.3)
	A 10	6	BG	65 (2.4)	3	7	9
	A 10 + PZ	6	BG	64 (0.9)	2	9	13
			pd BG		−1.5 (1.0)	2.5 (1.1)	2.7 (2.3)

Effect of i.v. secretin (Sec, parts A, C) or i.v. pancreozymin (PZ, parts B, D) on serum IRI, integrated secretory response for IRI (ISR) and blood glucose (BG) in response to i.v. arginine (15 g in 40 min, parts A, B, or 10 g in 2 min, parts C, D). N = number of subjects. Units: IRI, μU/ml; ISR, μU−min/ml; BG, mg/100 ml.

* Value (±SEM).

‡ pd = paired differences.

statistically significant. However, omission of one subject whose glucose disposal rate was exceptionally rapid in the control experiment raised the significance of the increase in rate constant in the remaining five subjects ($P < 0.05$).

Betazole-stimulated gastric secretion was associated with similar effects (Table I E). The rise in serum IRI in response to intravenous infusion of glucose was enhanced in five out of five subjects. The mean of paired differences in integrated secretory response for IRI was not statistically significant, but the mean of paired differences in IRI during infusions was statistically significant ($P < 0.01$). Glucose disappearance rate was accelerated in three of the subjects, but the mean of paired differences was not statistically significant.

Effect of secretin during the intravenous infusion of arginine. Infusion of secretin (3.5 U/min) throughout infusions of arginine (15 g in 40 min) in four subjects was associated with an increased rise in serum IRI at 5 and 10 minutes in all four, but this enhancement was not maintained through the full period of the infusion (Table II A). As seen in Fig. 2 *a*, an early increment in the mean rise in serum IRI on the occasions when secretin was given was followed by a period in which the level fell below that observed in the control experiments. As a result, the means of paired differences in integrated insulin response or in increments in IRI during infusions were not statistically significant. The infusion of arginine was associated with a rise in blood glucose concentration in all subjects. The increment in blood glucose

Increments time (min)			ISR* (0–40 min)	Mean of paired differences for IRI*	Mean of paired differences for BG
20	30	40			
27	38	31	994 (276)	0–40 min	
23	22	12	851 (290)		
−4.0 (4.2)	−16 (4.6)	−19 (7.8)	−143 (92)	−2.45 (18)	
22	28	20			
17	12	1.2			
−4.5 (5.8)	−16 (6.5)	−19 (11)			−6.65 (2.91)
32	30	28	1041 (463)	0–40 min	
46	50	44	1562 (354)		
14 (4.4)	20 (5.4)	16 (6.4)	521 (118)	12 (2.54)	
19	13	2.0			
22	20	14			
2.7 (5.7)	6.7 (3.1)	12 (10)			4.53 (2.92)
15			382 (96)	0–10 min	
28			725 (177)		
13 (9.0)			343 (87)	38 (10)	
7					
5					
1.7 (2.8)					2.66 (1.56)
13			291 (63)	0–10 min	
15			503 (111)		
1.9 (6.5)			212 (94)	27 (10)	
5					
4					
−0.9 (2.3)					1.2 (4.4)

concentration was reduced after 10 min in all subjects on the occasion when secretin was administered, and the mean of paired differences was statistically significant ($P < 0.05$).

In further experiments 10 g arginine was infused rapidly at a steady rate for 2 min with or without addition of 150 U of secretin (Table II C, Fig. 3 *a*). The increment in serum IRI associated with infusion of arginine at this rate was enhanced as a result of addition of secretin in all subjects, and the mean of paired differences in IRI after infusion was statistically significant ($P < 0.01$). The mean of paired differences in integrated secretory responses for IRI was not statistically significant, but the mean proportionate increase (+193 ±12%) was significant ($P < 0.05$). There was no consistent difference between increments in blood glucose concentration on the two occasions in the experiments with rapid infusion of arginine.

Effect of pancreozymin during the intravenous infusion of arginine. Infusion of pancreozymin (7 or 15 U/min) was associated with enhancement of the increment in serum IRI in response to the intravenous infusion of 15 g arginine over 40 min in all of six subjects (Table II B). Fig. 2 *b* shows the mean increments in IRI attained. Although the paired differences were small at 10 min, a difference was maintained in each subject throughout the infusion and the mean of paired differences in integrated secretory responses for IRI was statistically significant ($P < 0.05$). The mean of paired differences in increments in IRI during infusions was

TABLE III

Effects of Arginine and Pancreozymin on Plasma Glucagon-Like Immunoreactivity

Infusate	N		Fasting value*	Increments time (min) 2.5	5	10	20	30	40	ISR* (0–40 min)	Mean of paired differences for GLI*
A 15	3	GLI	0.10 (0.10)	—	0.0	0.0	0.0	0.0	0.0		0–40 min
A 15 + PZ	3	GLI	0.07 (0.06)	—	0.03	0.17	0.10	0.27	0.23		1.71 (0.34)
				—	(0.03)	(0.09)	(0.06)	(0.09)	(0.04)	6.15 (0.28)	
A 10	4	GLI	0.75 (0.19)	0.02	0.15	0.05	—	—	—	0.69 (0.26)	0–10 min
A 10 + PZ	4	GLI	0.50 (0.12)	0.22	0.27	0.35	—	—	—	2.40 (0.41)	
		pd GLI‡		0.20 (0.04)	0.12 (0.03)	0.30 (0.04)				1.71 (0.54)	2.08 (0.52)

Effect of i.v. pancreozymin (PZ) on plasma GLI and integrated secretory response for GLI (ISR) in response to i.v. arginine 15 g in 40 min (A15) or 10 g in 2 min (A10). N = number of subjects. Units: GLI, mμg/ml; ISR, mμg−min/ml.

* Value (±SEM).

‡ pd = paired differences.

also statistically significant ($P < 0.001$). Infusion of arginine was associated with a rise in blood glucose concentration in all subjects. In contrast to the effect of secretin in corresponding experiments with arginine, administration of pancreozymin led to a small rise in the mean increase in blood glucose concentration throughout the infusion, but this was not statistically significant.

In further experiments with six subjects 10 g of arginine was infused intravenously at a steady rate during a 2 min period with and without the addition of 300 U of pancreozymin (Table II D, Fig. 3 *b*). The increment in serum IRI associated with the rapid infusion of arginine was enhanced by the addition of pancreozymin in all subjects. The mean of paired differences in IRI in the first two samples after injection was significant

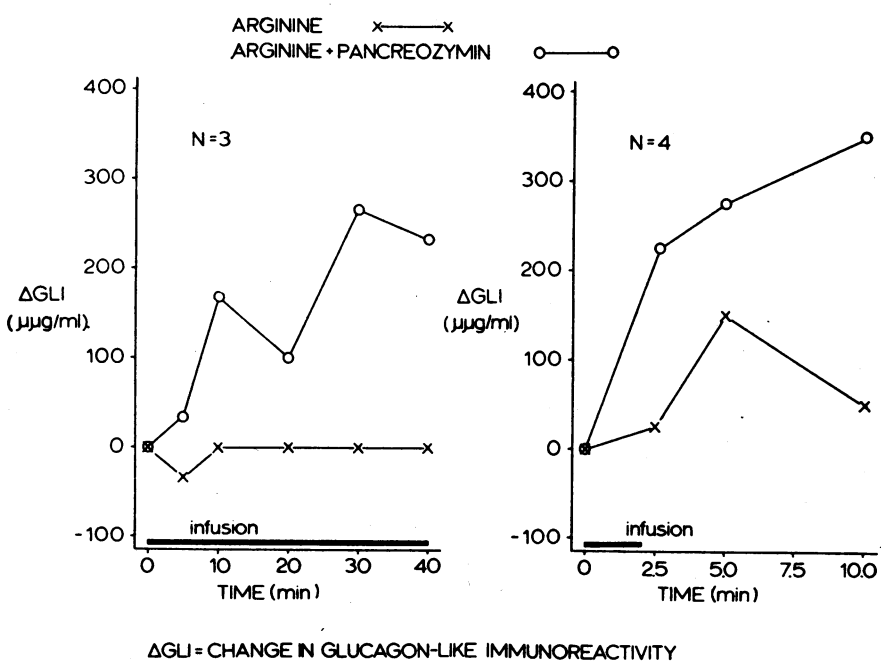

FIGURE 4 Effects of pancreozymin on plasma glucagon-like immunoreactivity in response to prolonged or brief stimulation with arginine. Each subject received a control intravenous infusion of arginine, 15 g in 40 min, or 10 g in 2 min, and on another occasion the same infusion with pancreozymin, 600 U in 40 min or 300 U in 2 min.

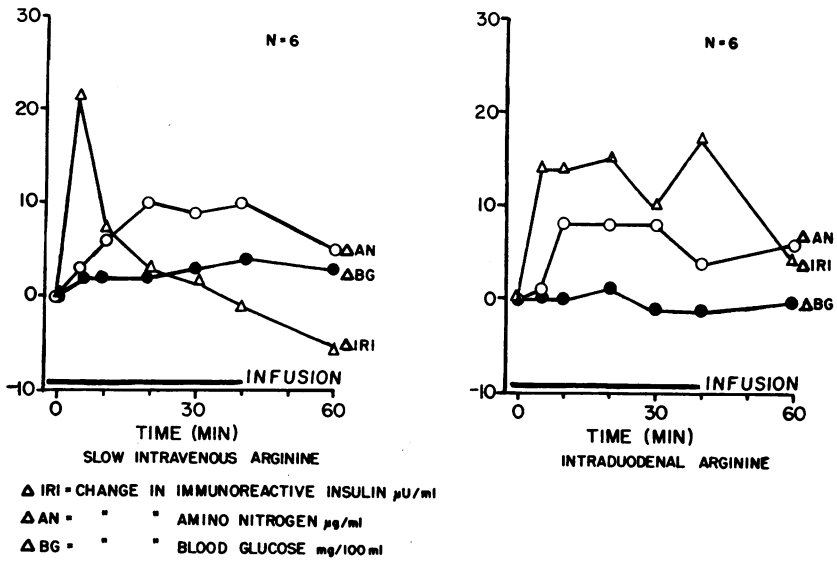

FIGURE 5 Effects of intravenous or enteric infusions of arginine on alpha-amino-nitrogen, glucose, and immunoreactive insulin in peripheral venous blood. Subjects received intravenous infusions of arginine, 3.8 g in min, or intraduodenal infusions of arginine, 15 g in 40 min.

($P < 0.01$). The mean of paired differences in integrated secretory response was significant ($P < 0.05$), as was the mean proportionate effect on integrated secretory response (220 ±54%, $P < 0.02$). In the experiments with rapid infusions of arginine there was no detectable effect of pancreozymin on the change in blood glucose concentration.

Effects on plasma glucagon-like immunoreactivity. Glucagon-like immunoreactivity was assayed in specimens from at least two experiments in each group of studies in which glucose or arginine was infused intravenously with and without enteric hormones. A consistent effect on plasma GLI was observed only when arginine was administered together with pancreozymin (Table III, Fig. 4).

When arginine was administered intravenously alone in a dose of 15 g in 40 min, there was no detectable change in plasma GLI, but when pancreozymin was given together with the same dose of arginine a rise in GLI occurred and exceeded the minimum change considered significant in the assay in all of three subjects. The mean integrated secretory response for GLI and the mean change in plasma GLI were statistically significant ($P < 0.02$ and $P < 0.05$, respectively). When arginine was administered intravenously alone in a dose of 10 g given in 2 min plasma GLI was transiently elevated in three out of six subjects but the minimum change considered significant in the assay was exceeded in only two of these (13). However, when this dose of arginine was accompanied by pancreozymin, plasma GLI was elevated in all of four subjects in excess of the minimum change considered significant, and the mean of paired differences in integrated change in GLI and the mean of paired differences in change in GLI were statistically significant ($P < 0.01$ in both cases).

Effect of secretin or pancreozymin on serum amino acid concentration during the intravenous infusions of arginine. Serum amino nitrogen was estimated in the following 12 pairs of experiments: two subjects who received slow (40 min) infusions of arginine with and without the addition of pancreozymin; in two other subjects who received slow infusions of arginine with and without addition of secretin; in five subjects who received rapid (2 min) infusions of arginine with and without pancreozymin; and in three subjects who received similar infusions with and without secretin. On all seven occasions when pancreozymin was given together with arginine, the increment in serum amino nitrogen concentration was smaller than in the control experiment. The mean of paired differences in amino nitrogen at corresponding times in experiments with arginine and arginine plus pancreozymin was statistically significant ($P < 0.02$) and the mean maximum increment was reduced by more than 40% during rapid or prolonged infusions. In contrast, no consistent effect of secretin on the change in blood amino nitrogen concentration resulting from brief or prolonged infusions of arginine was detected.

Effect of infusion of arginine into the small intestine (*Fig. 5*). The infusion of arginine (5 g/100 ml in wa-

TABLE IV

Effects of Intraduodenal or Intravenous Infusions of Arginine

Part	Infusate	N		Fasting value*	Increments time (min) 5	10	20	30	40	ISR* (0–40 min)
A	A 15 (ID)	6	IRI	13 (3.3)	15.0	15.0	16.0	11.0	0.7	489 (94)
		6	BG	60 (1.9)	0.0	0.0	1.0	−1.0	−1.3	
		6	AN	51 (11)	1.3	1.3	6.5	8.0	4.5	
B	A 15 (IV)	6	IRI	10 (1.2)	39.0	26.0	31.0	31.0	29.0	1166 (310)
		6	BG	53 (6.0)	8.1	13.0	17.0	17.0	15.0	
		6	AN	49 (4.1)	11.0	17.0	25.0	31.0	37.0	
C	A 3.9 (IV)	6	IRI	12 (1.2)	21.0	6.5	3.1	1.5	0.0	194 (49)
		6	BG	60 (4.0)	1.1	2.3	1.9	3.1	4.0	
		6	AN	55 (2.1)	3.0	6.5	11.0	10.0	11.0	

Effect of intraduodenal arginine 15 g in 40 min (A15 ID) i.v. arginine 15 g in 40 min (A15 i.v.) or i.v. arginine 3.0 g in 40 min (A3.9 i.v.) on serum IRI, integrated secretory response for IRI (ISR) blood glucose (BG) and serum alpha aminonitrogen (AN). N = number of subjects. Units: IRI, μU/ml; ISR, μU−min/ml; BG mg/100 ml; AN, μg/ml.
* Value (±SEM).

ter) at 7.6 ml/min for 40 min into the duodenum was associated with increments in peripheral serum IRI in all subjects (Table IV A). This was accompanied by a small increment in mean serum amino nitrogen and by no detectable change in blood glucose concentration. Intravenous infusion of arginine at the same rate was associated with a rise in blood glucose concentration, with a greater mean rise in blood amino nitrogen concentration, and with a greater mean increase in serum IRI (Table IV B). In further experiments the effect of intravenous infusion of arginine at a rate leading to increments in serum amino nitrogen concentration resembling those observed during intraduodenal infusion of 15 g arginine was studied (Table IV C). Intravenous infusion of 3.9 g arginine in 40 min resulted in no significant change in blood glucose concentration, and in a mean rise in serum amino nitrogen concentration slightly more rapid in onset and slightly greater than that resulting from the duodenal infusions. However, in contrast with the sustained effect of duodenal infusions on serum IRI, the rise in IRI that occurred at the beginning of these intravenous infusions was not maintained, and the mean integrated secretory response for insulin was significantly smaller ($P < 0.01$).

DISCUSSION

Administration of secretin, pancreozymin, or gastrin has been shown to stimulate release of insulin into the splanchnic circulation in animals (13) and similar effects of secretin (12) or pancreozymin [3] have been observed in man. Stimulation of release of insulin from animal pancreas in vitro by secretin or pancreozymin has also been reported (20, 21). The increments in serum IRI observed after administration of these agents in the present experiments are therefore attributed to changes in the rate of secretion of this hormone. Evidence that effects of the intestinal hormones used in the present experiments are not the result of nonspecific actions on the pancreas or of contamination of the preparations with insulin or glucagon was obtained by Unger and his colleagues (13).

Infusion of secretin together with glucose showed that this hormone led to enhancement of the rise in systemic IRI. This was associated with acceleration of glucose disposal after the infusions. More insulin was mobilized by secretin during hyperglycemia than in the fasting state. The effect of secretin in these experiments can therefore be regarded as a potentiation of the secretion of IRI in response to hyperglycemia.

The effects of pancreozymin on serum IRI and blood glucose resembled those of secretin. In view of consistent findings in the present studies, in which four batches of hormone were used, and in view of similar findings with pancreozymin in dogs (13, 14), we believe that reliance cannot be placed on earlier negative reports of experiments which failed to reproduce these effects (22). The enhancement of increments in IRI in response to intravenous infusion of glucose suggests that this action of pancreozymin, like that of secretin, may be regarded as a potentiation of the response to hyperglycemia.

The effect of synthetic human gastrin on the rise in serum IRI during the intravenous infusion of glucose was similar to that of secretin or pancreozymin, although its magnitude was smaller. The effect of gastrin on pan-

[3] Dupre, J. Unpublished observations.

creaticoduodenal vein IRI in anesthetized dogs recorded by Unger and his associates (13) was likewise smaller than that of secretin and pancreozymin, but occurred within seconds of the injection and was probably produced by a direct action on the pancreas. Maintenance of a high rate of administration of gastrin throughout glucose infusions in two subjects in the present study was not associated with a greater effect on the serum IRI than that observed in subjects who received briefer infusions of gastrin. Clearly the possibility that circulating gastrin may have a direct effect on insulin release in man is not excluded by these results. However, intestinal infusions of HCl or gastric stimulation with betazole led to effects similar to those of intravenous infusion of gastrin, and analysis of the data obtained in these three sets of experiments revealed no significant differences between them. Treatment of pooled data from the 11 studies with HCl or betazole showed that differences in the blood levels of IRI (Fig. 1 *d*), in the integrated secretory responses for insulin ($+498 \pm 98$ μU-minutes/ml), and in the rate constants for glucose disappearance ($+0.41\%$/min ± 0.17) were statistically significant ($P < 0.005$, < 0.01, and < 0.05 respectively). It is suggested that these effects of HCl or betazole and also those of gastrin depended on a common mechanism related to the presence of acid in the intestine, and were probably mediated by endogenous secretin.

The dose of exogenous HCl given during glucose infusions did not exceed the recent estimates of gastric acid secretion in response to ingestion of food or pharmacological stimulation in normal man (23). In fasting normal subjects stimulation of a rise in serum IRI during infusion of HCl into the duodenum was observed by Young and his colleagues (24). The dose of acid was similar to that used in the present experiments, and the insulin response was preceded by a rise in the blood secretin as measured by radioimmunoassay. However Boyns, Jarrett, and Keen observed no effect of infusion of 5 mEq citric acid into the duodenum while glucose was infused intravenously in three normal subjects (25). The absence of an effect on insulin release in these experiments may have been due to the lower intensity of the stimulus to the release of secretin. The failure of larger doses of acid to affect insulin release in the achlorhydric subjects of Mahler and Weisberg (26) raises the question whether the alimentary function involved in this response is normal in such patients, but it is also possible that the action of endogenous secretin is less readily demonstrated while the blood glucose concentration falls from a high peak after the rapid intravenous infusions which were used in those experiments.

Although the results of the present study lead to the conclusion that endogenous secretin is capable of increasing the insulin response to hyperglycemia, there is doubt whether secretin is released from the intestine in all situations in which an alimentary stimulus to insulin secretion is detectable (27). It has been suggested that potentiation of insulin secretion during the response to delivery of glucose into the small intestine is attributable to the secretion of an enteric material which is detected in the immunoassay for glucagon (28). However, studies with an immunoassay system capable of detecting secretin have shown a rise in circulating secretin-like immunoreactivity in man after infusion of glucose into the duodenum (24). This question will not be resolved until the natures of these immunological activities are defined. It is also apparent that the increment of approximately 50% in integrated secretory response for IRI here attributed to endogenous secretin falls well short of the 300% enhancement attributed to the net physiological stimulus in the study of Perley and Kipnis (29). It is not possible to assess the significance of this discrepancy without taking account of possible interactions between humoral and nervous influences on the pancreas. The potential importance of an interaction between secretin and the parasympathetic nervous system is suggested by reports of inhibition of the response of the exocrine pancreas to exogenous secretin by anticholinergic drugs (30), by reports of stimulation of insulin release by cholinergic agents in vitro (31), and by reports of inhibition of the insulin response to alimentary glucose but not to intravenous glucose by atropine in man (32).

With regard to the effect of insulin on glucose disposal, it is notable that even when integrated insulin secretion was increased to 300% or more by the action of exogenous secretagogues, the glucose tolerance curve was little affected during the 40 min period of intravenous infusion, although the rate of fall of blood glucose after the infusion was greatly increased. In contrast, the relatively greater tolerance to glucose infused into the duodenum is apparent from the outset (3), a difference which almost certainly does not depend on a limited rate of absorption of glucose or on intestinal metabolism of glucose. In the present experiments the secretagogue or the substrate or both were delivered into peripheral veins, and the physiological combination of relative portal hyperglycemia and enhanced insulin secretion was not obtained. It seems unlikely that this feature of these studies accounts for the failure of enteric hormone–stimulated insulin secretion to modify the glucose tolerance curve during glucose infusions, and it may therefore be inferred that the alimentary effect on glucose disposal probably does not depend solely on stimulation of insulin secretion.

The present study also shows that exogenous secretin or pancreozymin enhances the insulin response to rapid intravenous infusions of arginine (10 g in 2 min). The

responses were not attributable to effects of either hormone on blood levels of amino acid or glucose. However, in experiments with prolonged infusions of arginine (15 g in 40 min) maintained enhancement of the insulin response was produced by pancreozymin and not by secretin. Stimulation of insulin secretion by secretin may have been limited by reduction of the rise in blood glucose, which did not occur with pancreozymin. The administration of pancreozymin in dogs is capable of causing a rise in blood glucose in the fasting animal, and this has been attributed to stimulation of release of glucagon by this hormone (13). It has also been shown by Ohneda and his colleagues that stimulation of glucagon secretion by intravenous infusion of amino acids in dogs is potentiated by pancreozymin (33). It therefore appears possible that the same mechanism was responsible for maintenance of the blood glucose concentration in the face of sustained stimulation of insulin secretion by pancreozymin during arginine infusions. With respect to this question, evaluation of the results of the assays of plasma GLI in the present experiments cannot be attempted without making assumptions regarding the source of this activity. Fasting plasma GLI varied widely, but when the change in GLI from the values observed immediately before starting the infusions was examined, a consistent increment was observed when pancreozymin was administered together with arginine. The mean increment in plasma GLI if attributable to glucagon would be associated with changes in portal glucagon which would be expected to exert physiological effects (34). Thus while the present observations on plasma GLI are subject to difficulties in interpretation due to the detection of cross-reacting material in this assay, and to the fact that its sensitivity is close to the range of physiological changes in glucagon in peripheral blood, it is suggested that the results obtained are consistent with the conclusion that glucagon secretion is stimulated by amino acids and by pancreozymin in man as it is in the dog.

The infusion of arginine into the duodenum, which is presumed to cause release of pancreozymin, led to a rise in the blood IRI which was not attributable to direct effects of glucose or arginine on the pancreas. These experiments therefore furnish evidence of the operation of an alimentary stimulus to the endocrine pancreas in this response. The effects of exogenous pancreozymin here reported show that this hormone is qualified to mediate such a stimulus, and the rise in plasma GLI which would be expected in man after ingestion of arginine has been observed by Assan, Rosselin, and Dolais (35). The possible importance of humoral effects on the disposal of amino acids absorbed from the intestine cannot be inferred from the results of these studies of blood amino nitrogen, since the direct consequences of portal hyperaminoacidemia and the rates of intestinal absorption and metabolism of the amino acid are not known.

It is concluded that stimuli to the release of endogenous secretin or pancreozymin are capable of producing enhancement of the pancreatic endocrine response to circulating nutrients. The rates of disposal and patterns of distribution of glucose and amino acids derived from ingested nutrients may be dependent on the effects of these hormones, and possibly those of other enteric agents. The relative importance of these secretions, their interactions with other stimuli, and their significance in the pathophysiology of diseases of metabolism or of the gastrointestinal system remain to be determined.

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