Effects of Intraduodenal Administration of HCl and Glucose on Circulating Immunoreactive Secretin and Insulin Concentrations

GUENTHER BODEN, NOORJEHAN ESSA, OLIVER E. OWEN, and FREDERICK A. REICHLE with the technical assistance of WALTER SARAGA

From the Departments of Medicine and Surgery and the General Clinical Research Center, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140

ABSTRACT A new radioimmunoassay for secretin was used to investigate (*a*) serum secretin responses to intraduodenally infused HCl and glucose, (*b*) the metabolic half-life and the volume of distribution of exogenous secretin and (*c*) the effect of endogenously released secretin on insulin secretion in 25 anesthetized dogs. Portal and femoral venous blood samples were taken simultaneously before, during, and after intraduodenal infusion of HCl (21 meq/30 min) and glucose (131 ml/30 min). Control experiments were performed with intraduodenal infusion of saline.

Mean portal venous immunoreactive secretin concentration of six dogs rose from 313 μU/ml before to 1,060 μU/ml 10 min after initiation of the intestinal acidification ($P < 0.005$). Femoral venous immunoreactive secretin concentration rose from 220 μU/ml before to 567 μU/ml 15 min after intestinal acidification ($P < 0.01$). Secretin concentrations remained elevated during the remainder of the infusion.

In the same six dogs mean portal venous immunoreactive insulin concentration rose from 38 μU/ml before to 62 μU/ml at the end of the infusion ($P < 0.05$). Peripheral immunoreactive insulin, glucose, and free fatty acid concentrations, however, did not change significantly.

Pancreatic exocrine function was studied in four dogs. The rise in secretin concentration was followed promptly by a highly significant increase in exocrine pancreatic flow rate and bicarbonate secretion, indicating biological activity of the circulating immunoreactive secretin.

The effect of intraduodenal infusion of glucose on immunoreactive secretin concentration was studied in 12 dogs. Glucose in concentrations ranging from 2.5% to 10% had no detectable influence on portal or peripheral secretin concentration. Infusion of 50% glucose caused a slight decline in secretin concentration.

The metabolic clearance rate, half-life of disappearance, and volume of distribution of exogenous secretin was studied in three dogs by the constant infusion technic. The metabolic clearance rate was 730±34 ml/min, volume of distribution was 17.4±0.8% of body weight, and the half-life of disappearance was 2.8±0.1 min. It could be calculated that 1.38 U/kg-h^{-1} of endogenous secretin was released into the peripheral circulation during the steady state period of the HCl infusion experiments.

The data indicated that immunoreactive secretin was released rapidly after intestinal acidification, continued to be secreted throughout the duration of HCl infusion, and was promptly distributed in the extracellular compartment. Furthermore, they suggested that endogenously released secretin could stimulate insulin secretion. The HCl-mediated insulinogenic effect of immunoreactive secretin, however, was too weak to influence peripheral immunoreactive insulin, glucose, and free fatty acid concentrations.

The failure of intraduodenal glucose to stimulate secretin release suggests that secretin is not the insulin-stimulatory factor released from the gastrointestinal tract in response to glucose.

Received for publication on 27 February 1973 and in revised form 27 November 1973.

TABLE I

Effects of Intraduodenal Infusion of HCl and Physiologic Saline on

Dog no.	Source of sample	Control period		HCl (21 meq/30 min)									
		−10 min	0	2.5 min	5 min	10 min	15 min	20 min	25 min	30 min	40 min	50 min	60 min
								μU/ml					
4	PV	0*	0	480	1,160	1,200	1,160	1,120	1,040	800	480	320	0
	FV	0	0	0	440	520	440	600	440	480	480	0	0
5	PV	480	480	400	600	760	880	920	840	880	720	600	520
	FV	400	400	0	0	440	520	560	530	530	450	440	400
6	PV	400	400	480	960	1,280	1,120	1,280	1,280	1,240	1,000	600	520
	FV	0	0	0	0	440	640	520	600	600	400	0	0
7	PV	400	400	600	720	720	720	640	920	760	640	540	480
	FV	440	400	480	600	520	720	680	680	560	560	480	520
8	PV	600	600	800	880	1,600	1,200	1,120	1,720	1,840	1,120	760	640
	FV	640	520	560	600	600	680	720	800	800	760	680	680
9	PV	0	0	480	800	800	760	680	600	640	400	240	0
	FV	0	0	0	0	0	400	0	0	460	0	0	0
Mean		313	313	540	855	1,000	973	960	1,067	1,027	727	510	360
±SEM	PV	95	95	53	73	133	79	97	146	167	106	272	106
P Value‡				NS	<0.02	<0.005	<0.005	<0.005	<0.005	<0.005	<0.02	NS	NS
Mean		247	220	173	273	420	567	513	508	625	442	267	267
±SEM	FV	105	91	100	113	79	49	98	104	65	93	113	114
P Value‡				NS	NS	NS	<0.01	<0.025	<0.05	<0.01	NS	NS	NS

* Undetectable IRS concentrations are designated as 0.
‡ Significance of difference from IRS concentration at 0 min.
§ Significance of difference from IRS concentration at 120 min.

INTRODUCTION

Intestinal acidification is presently the only recognized stimulus for secretin secretion and this knowledge is based entirely on indirect evidence (1–4). Except for the unique experience of Young, Lazarus, Chisholm, and Atkinson (5), measurement of secretin in blood has not been reported.

Injection of secretin has been shown to stimulate insulin secretion (6–8). The physiologic significance of this effect remained uncertain, since neither the physiologic dose nor the physiologic blood level of secretin were known. Moreover, widely different results have been obtained by several different laboratories when the effect of endogenous secretin (stimulated by intraduodenal or oral administration of HCl) on insulin secretion was studied (9–14).

Chisholm, Young, and Lazarus have recently reported dramatic increases in peripheral venous immunoreactive secretin (IRS) [1] concentrations in response to intraduodenal as well as oral glucose administration (14–15). These authors have postulated that the glucose-stimulated IRS release represented part of the gastrointestinal stimulus for the secretion of insulin. However, confirmation of these results is urgently needed.

We have developed a sensitive and specific radioimmunoassay for secretin using an antiserum produced in rabbits to synthetic secretin and ^{125}I-labeled synthetic secretin as tracer hormone (16). This assay has allowed us to (*a*) characterize in portal and peripheral venous serum changes in secretin concentrations after HCl infusion in anesthetized dogs; (*b*) determine the metabolic clearance rate (MCR) and volume of distribution (V) of serum secretin after continuous infusion of exogenous secretin; and (*c*) delineate the interrelationship between intraduodenal glucose administration and secretin release. In addition, identification of the endogenous IRS response to intestinal acidification and demonstration of the bioactivity of the released secretin have permitted us to reassess the effect of endogenous secretin release on insulin secretion.

METHODS

25 healthy mongrel dogs (15–23 kg), fasted overnight, were studied. In six dogs HCl was infused intraduodenally to study changes in IRS, immunoreactive insulin (IRI), glucose, and free fatty acid (FFA) concentrations. In four additional dogs pancreatic water and bicarbonate secretion in response to intestinal acidification was investigated. In 12 dogs the effect of intraduodenal administration of glucose on IRS concentration was studied and 3 dogs received

[1] *Abbreviations used in this paper:* FFA, free fatty acids; IRI, immunoreactive insulin; IRS, immunoreactive secretin; MCR, metabolic clearance rate; $t_{\frac{1}{2}}$, half-life of disappearance; V, volume of distribution.

Portal Venous (PV) and Femoral Venous (FV) Serum IRS Levels

Na Cl (131 ml/30 min)								HCl (21 meq/30 min)				
62.5 min	65 min	70 min	80 min	90 min	100 min	110 min	120 min	122.5 min	130 min	135 min	140 min	150 min
						μU/ml						
0	0	0	0	0	0	0	0	—	—	—	—	—
0	0	0	0	0	0	0	0	—	—	—	—	—
600	600	600	600	800	560	600	600	600	680	440	600	760
400	480	520	400	480	400	440	440	480	440	560	560	600
560	720	560	560	560	560	680	520	480	1,160	1,240	1,200	840
400	480	480	400	0	400	400	0	0	400	520	720	680
520	560	680	680	680	600	600	600	640	800	760	920	760
480	440	—	400	640	0	560	480	680	560	400	600	480
720	760	720	720	680	720	640	600	720	680	760	1,160	1,680
600	600	400	680	560	560	600	640	640	600	600	880	800
0	0	0	0	0	520	0	400	760	1,200	1,200	720	440
0	0	0	0	0	0	0	400	0	400	520	520	0
400	440	427	427	453	493	420	453	640	904	880	920	896
118	130	125	125	134	94	122	88	44	103	135	106	186
								NS	NS	NS	<0.05	NS
313	333	360	313	280	227	333	327	360	480	520	575	512
94	98	83	99	116	95	100	99	135	37	30	51	124
								NS	NS	NS	NS	NS

continuous i.v. infusion of exogenous secretin for determination of the MCR and V. The animals were anesthetized by i.v. injection of Nembutal (5.7 mg/kg) (Abbott Laboratories, North Chicago, Ill.), intubated, and connected to an artificial respirator. Laparatomy was performed and polyethylene catheters were inserted in the direction of venous blood flow into the right femoral vein and the portal vein, 1–2 in. distal from the portal area. The catheters were kept patent with a slow saline drip. A No. 32 French rubber tube was inserted through a gastrostomy opening into the stomach and passed through the pylorus, and its position was stabilized 1–2 in. distal from the duodenal bulb. A small polyvinyl catheter was inserted into the major pancreatic duct between the duodenal wall and the head of the pancreas. The accessory pancreatic duct was ligated together with the common bile duct.

The HCl infusion experiments included six test periods. After an initial control period (− 10 min until 0 time), 15 ml HCl (160 mM solution in distilled water) was rapidly infused intraduodenally. This was followed by a constant infusion (4 ml/min) for 30 min with a Harvard Pump (Harvard Apparatus Co., Inc., Millis, Mass.). This was followed by a 30-min rest. Saline was infused during the 60–90 min interval and again followed by a 30-min rest. From 120 to 150 min a second HCl (160 mM) infusion was performed, in the same fashion as the first.

Similarly, the glucose infusion experiments were started with an initial control period, followed by a 30-min intraduodenal infusion of NaCl, a 30-min rest period, a 30-min glucose infusion, a second rest period and a final 30-min HCl infusion.

Simultaneous blood samples were obtained from the portal and femoral veins at frequent intervals before, during, and after HCl, glucose, or saline infusions. The blood was collected in ice-cold test tubes, allowed to clot, and centrifuged at 4°C. Fibrin clots, when present, were removed and the serum recentrifuged. Serum was stored at − 15°C until assayed.

Pancreatic juice was collected at 15-min intervals into calibrated ice-cold conical glass tubes. Bicarbonate concentration was measured by adding 0.5 ml of pancreatic fluid to 1.0 ml of 0.1 N HCl, bringing the mixture briefly to boil, and back-titrating the residual HCl with 0.1 N NaOH to pH 7.0.

Secretin was measured by a radioimmunoassay that has recently been described (16). Highly purified porcine secretin mixed with cystein HCl (batch 17171, GIH Research Laboratory, Karolinska Institute, Stockholm, Sweden) was used for standards. Each ampule contained 75 clinical U but the mass of secretin was not given. Secretin activity was therefore expressed in units as stated on the label. Microgram amounts of the secretin-cystein HCl mixture were weighed out on a Cahn electrobalance (Cahn Div., Ventron Instruments Corp., Paramount, Calif.), dissolved in 1/10 N HCl, and diluted with buffer to contain 0.05, 0.1, 0.25, 0.5, 1.0, and 2.0 mU/ml. According to recent investigations from this laboratory, 1 mU of this material is immunologically equivalent to 250 pg of the purest synthetic secretin preparation (17). The sensitivity of the assay varied from 50 to 100 μU/sample (200–400 μU/ml of serum). Values below the calculated sensitivity were considered as zero. Coefficient of variation was 9.4% for intraassay and 17.1% for interassay reproducibility.

Serum insulin, glucose, and FFA were measured only in samples taken during the first HCl infusion and the following rest period. Insulin was determined by radioimmunoassay according to Soeldner and Slone (18). Serum

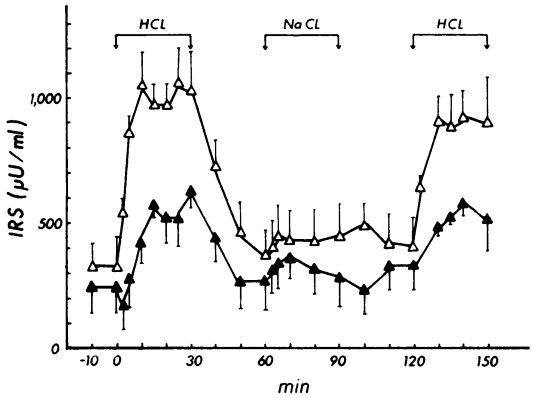

FIGURE 1 Effects of intraduodenal infusion of HCl (21 meq/30 min) and saline (131 ml/30 min) on portal venous (open triangles) and femoral venous (closed triangles) serum IRS concentrations. Shown are mean±SEM of six experiments (five experiments for the second HCl infusion).

FFA were determined by the microcolorimetric method of Duncombe (19), with the Dole extraction procedure (20). Serum glucose was measured with the method of Hill and Kessler (21).

The MCR of secretin was determined by the constant infusion technic. After a priming dose of 1 clinical U/kg, 8 clinical U/kg of GIH secretin were infused i.v. at a constant rate over a 60-min period with a Harvard pump. Blood samples were drawn before, during, and in rapid sequence after the infusion. Preinfusion (basal) IRS concentrations were subtracted and regression equations (log IRS concentrations vs. time) were determined for the post-infusion IRS concentrations by least square regression analysis. $t_{\frac{1}{2}}$ was determined from these equations. The following formulas were used to determine MCR, the fractional turnover rate (k) and V (22):

$$\text{MCR} = \text{infusion rate/final IRS concentration}, \quad (1)$$

$$k = \frac{0.693}{t_{\frac{1}{2}}}, \quad (2)$$

$$V = \frac{\text{MCR}}{k}. \quad (3)$$

Statistical analysis was performed with Student's t test for small paired samples, and least square regression analysis was performed as described by Snedecor and Cochran (23). Results are given as means±SEM.

RESULTS

Portal venous IRS after HCl and saline

First HCl infusion (*Table I, Fig. 1*). Preinfusion IRS levels were undetectable in two dogs. Mean preinfusion concentration was 470±41 μU/ml for the remaining four and 313±95 μU/ml for all six dogs (undetectable levels considered as zero). After HCl infusion there was a rapid IRS increase in all animals. A peak value of 1,060±133 μU/ml was seen at the 10-min interval. The increase over the preinfusion value was highly significant ($P < 0.005$). Thereafter IRS levels remained elevated throughout the HCl infusion. Within 30 min after discontinuation of the HCl infusion, IRS concentration declined from 1,027±167 μU/ml to 360±106 μU/ml ($P < 0.01$).

Saline infusion. Control IRS rose from 360±106 μU/ml to 440±130 μU/ml 5 min after the start of a saline infusion. This small increase was not statistically significant.

Second HCl infusion. A second infusion performed in five of the initial six dogs showed IRS responses similar to those seen during the first infusion. IRS concentration rose from 453±88 μU/ml before to 920 ±106 μU/ml 20 min after the start of the HCl infusion ($P < 0.05$). Peak IRS concentrations during both HCl infusions were similar in most dogs. An exception was dog 9, which had maximum IRS concentrations of 800 μU/ml and 1,200 μU/ml during the first and second infusion, respectively. The highest IRS concentrations observed were 1,840 μU/ml (infusion 1) and 1,680 μU/ml (infusion 2), both observed in dog 8.

Femoral venous IRS after HCl and saline

Peripheral IRS concentrations of sera taken before and 5 min after Nembutal injection did not differ significantly.

First HCl infusion. Femoral venous IRS concentration could not be measured in three dogs. Preinfusion IRS concentration was 440±33 μU in the remaining three, and was 220±91 μU/ml in all six dogs. After HCl infusion femoral IRS rose in all six dogs. A peak of 567±49 μU/ml ($P < 0.01$) was reached at the 15-min interval, 5 min after the portal venous IRS peak was observed. IRS concentrations remained

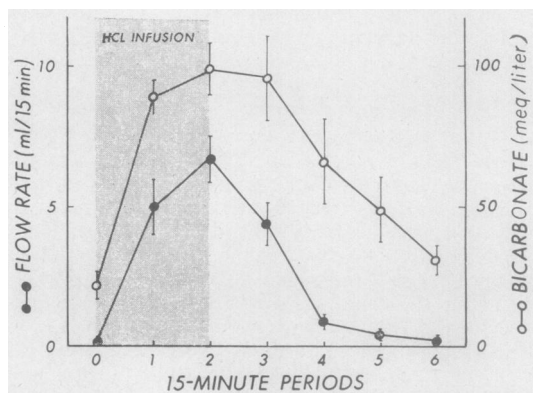

FIGURE 2 Effect of intraduodenal infusion of HCl (21 meq/30 min) on pancreatic flow rate (closed circles) and bicarbonate concentration (open circles). Shown are mean ±SEM of four experiments.

TABLE II

MCR, $t_{\frac{1}{2}}$, and V in Dogs Studied with Constant Infusion of Unlabeled Secretin

Dog	Weight	IRS (final)	Infusion rate	$t_{\frac{1}{2}}$	MCR	V	V
	kg	*mU/ml*	*mU/min*	*min*	*ml/min*	*ml*	*% of body wt*
SM-1	17.7	3.27	2,360	2.6	722	2,749	15.5
SM-2	15.9	3.20	2,120	3.1	663	2,964	18.6
D-10	17.7	2.93	2,360	2.8	805	3,209	18.1
Mean	17.1	3.13	2,280	2.8	730	2,974	17.4
SEM	0.5	0.09	65	0.1	34	109	0.8

elevated throughout the infusion period and returned to baseline 30 min after discontinuation of the HCl infusion.

Saline infusion. No statistically significant changes in IRS concentrations occurred during saline infusions.

Second HCl infusion. IRS rose in all dogs after a second HCl infusion. The IRS concentration increased from 327±99 μU/ml before to 575±51 μU/ml 20 min after the start of the infusion. The rise was observed in all five dogs; however, it failed to reach statistical significance ($t = 2.269$, $P > 0.05$).

Water and bicarbonate secretion after HCl (Fig. 2)

Pancreatic excretory responses to intraduodenally infused HCl (21 meq/30 min) were determined in four anesthetized dogs. The rate of pancreatic secretion rose from 0.15±0.01 ml/15 min before to a maximum of 6.70±0.90 ml/15 min ($P < 0.001$) during the second half of the HCl infusion. Thereafter, the rate of secretion declined to preinfusion levels within 1 h. Similarly, bicarbonate rose from a preinfusion concentration of 21.7±5.2 meq/liter to a maximum of 99.2±10.6 meq/liter during the last 15-min infusion period and then declined to 31.3±5.7 meq/liter 1 h after infusion.

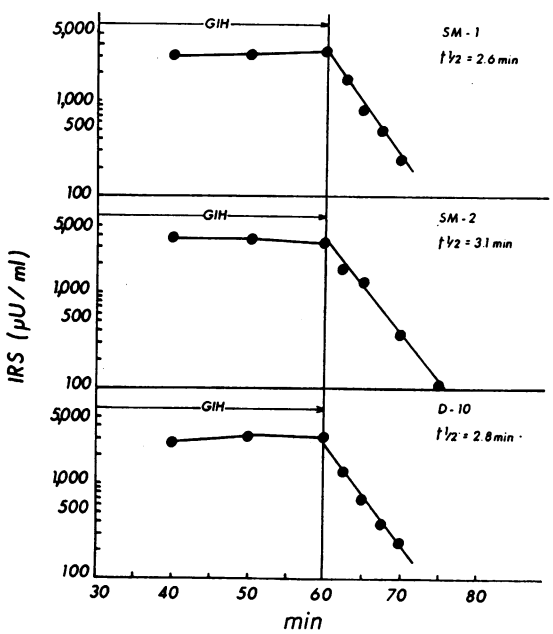

FIGURE 3 IRS concentrations during and after infusion at a constant rate of exogenous secretin. Shown are the results of three individual experiments.

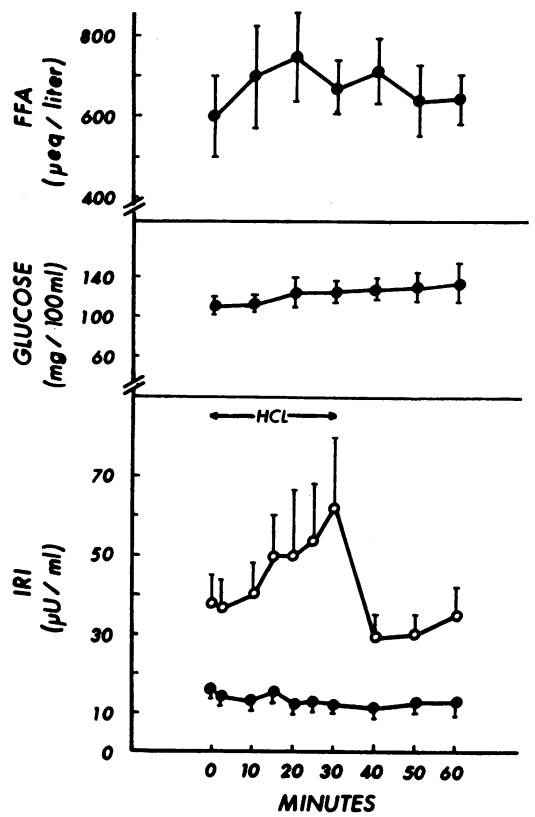

FIGURE 4 Effects of intraduodenal HCl infusion (21 meq/30 min) on portal venous serum IRI (open circles) and on femoral venous serum insulin, glucose, and FFA concentrations (closed circles). Shown are mean±SEM of six experiments.

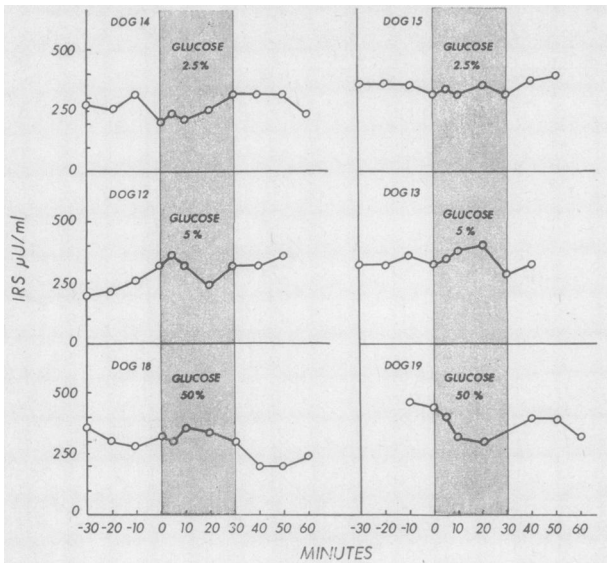

FIGURE 5 Effects of intraduodenal infusion of glucose (in concentrations ranging from 2.5% to 50%) on portal venous IRS concentrations. Shown are results of six individual experiments.

Half-life of disappearance ($t_{\frac{1}{2}}$) of IRS (Table II, Fig. 3)

After the end of the HCl infusion, portal and femoral venous IRS concentrations declined sharply in all dogs. The apparent $t_{\frac{1}{2}}$ of disappearance of endogenous IRS from the portal circulation was 11.9±1.3 min with a range from 6.3 to 15 min. However, this was probably an overestimation of the real $t_{\frac{1}{2}}$, since there was no evidence that secretin release ceased abruptly after cessation of HCl infusion. To determine the true metabolic $t_{\frac{1}{2}}$, GIH secretin was continuously infused into a femoral vein in three dogs. Steady-state conditions were reached after approximately 30–40 min (Fig. 3). The disappearance of IRS was measured after discontinuation of the infusion. As can be seen from Fig. 3, IRS concentration declined linearly with a mean $t_{\frac{1}{2}}$ of 2.8±0.1 min. The calculated MCR was 730±34 ml/min. (Table II). V was 2,974±65 ml or 17.4±0.8% of body wt.

IRI, glucose, and FFA after HCl (Fig. 4)

Portal venous IRI rose significantly from a preinfusion concentration of 38±8 μU/ml to a peak concentration of 62±18 μU/ml 30 min after the start of the HCl infusion ($P < 0.05$). This rise was caused by insulin increments of 94%, 90%, and 60% at 25, 20, and 15 min in dogs 4, 5, and 7, respectively. After discontinuation of the HCl infusion, IRI decreased to below preinfusion concentration within 10 min. Femoral serum IRI concentration was 16±2 μU/ml. It did not change significantly during HCl infusion. Preinfusion glucose and FFA concentrations were 111±5 mg/100 ml and 597±100 μeq/liter, respectively. There were no significant changes in the concentrations of these substrates during or after HCl infusion.

IRS after glucose

Fig. 5 shows the effect of intraduodenal infusion of 2.5%, 5%, and 50% glucose in water (131 ml/30 min) on portal venous IRS in two dogs each. As can be seen, there was no consistent effect of any of the glucose concentrations on circulating IRS. The only exception, perhaps, was dog 19, where IRS concentration declined from 440 μU/ml to 300 μU/ml during infusion with 50% glucose. The decrease of 140 μU/ml considerably exceeded possible interassay variation (16). Fig. 6 shows a comparison of the effects on portal and femoral venous serum IRS concentrations of intraduodenal infusion of physiologic saline, 10% glucose, and HCl (21 meq/30 min). Again, saline and 10% glucose had no effect, whereas HCl elicited the same prompt IRS rise seen in the previous experiments (Fig. 1).

DISCUSSION

In this study a sustained increase in IRS concentration in response to intestinal acidification was demonstrated by direct measurement of the hormone in serum. The acid load utilized to achieve this effect (21 meq/30 min) in anesthetized dogs was greater than that used by Preshaw, Cooke, and Grossman (4) to provoke maximal pancreatic secretory response in alert dogs (8–12 meq/30 min). However, it was within the range of gastric acid output observed by Rune and Henricksen in dogs after a protein meal (24) and was therefore considered to be physiologic.

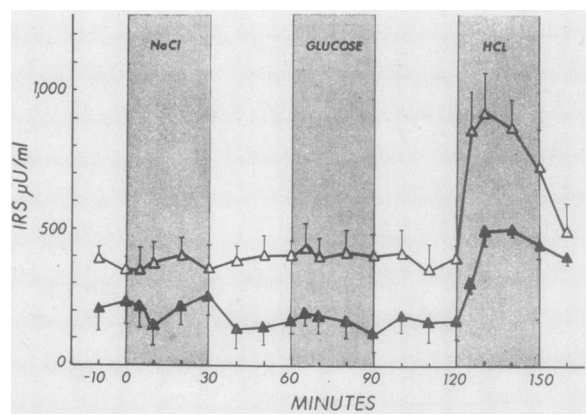

FIGURE 6 Effects of intraduodenal infusions of saline, 10% glucose in water and HCl (21 meq/30 min) on portal venous (open triangles) and femoral venous (closed triangles) serum IRS concentrations. Shown are mean±SEM of six experiments.

The increase in pancreatic flow rate and bicarbonate concentration, which closely followed the rise in peripheral IRS concentrations, demonstrated the biological effectiveness of the measured immunoreactive secretin. The mean peak flow rate, 6.7 ml/15 min (range 4.2–8.5) was approximately one-half of the flow rate observed by Preshaw et al. in alert dogs with chronic pancreatic fistulas (4). It has long been known, however, that anesthesia depresses pancreatic exocrine secretion for reasons that are not entirely clear.

Preinfusion IRS levels were too low to be detected in portal venous serum in two and in femoral venous serum in three of the six dogs. Mean IRS concentrations in the remaining animals were 470 μU/ml in portal and 440 μU/ml in femoral venous serum. IRS concentration was about 10–30% higher in portal than femoral venous serum during periods of nonstimulation, i.e., the preinfusion and saline infusion periods. This suggests that under the conditions of these experiments there was continuous basal secretion of secretin into the portal circulation. The observed base-line pancreatic exocrine secretion supports this conclusion.

Intestinal acidification resulted in a rapid increase in IRS concentrations in both portal and femoral venous blood of all animals. The portal venous IRS increase was evident at the first blood sampling, collected 2.5 min after the start of the HCl infusion. It became statistically significant at 5 min and peaked at 10 min. The femoral venous IRS response was slightly delayed. IRS remained elevated and rather constant throughout the acid infusion and declined promptly when the infusion of HCl was discontinued.

Wang and Grossman investigated the effect of intraduodenal NaCl instillation on pancreatic excretory volume in dogs and saw no significant changes (25). Our observation of an insignificant portal IRS rise is in agreement with their findings. The possibility that the absence of IRS changes during saline infusion might have been due to unresponsiveness of the intestinal mucosa could be excluded by the results of the second HCl infusion. This HCl stimulation resulted in IRS increases quantitatively comparable to the increments seen 120 min earlier, during the first HCl infusion.

In the past, attempts have been made to determine the disappearance rate for secretin by indirect means. Lehnert, Stahlheber, and Forell (26) determined the change in pancreatic exocrine secretion after secretin injection in dogs. They observed a 50% decline in secretory activity in 3.2 min. Lagerlof, Ek, and Nyberg (27), however, using similar indirect methodology, arrived at a $t_{\frac{1}{2}}$ of 18 min in human subjects. In this study the disappearance rate of secretin was determined by direct measurement of IRS by the continuous infusion technic. Mean $t_{\frac{1}{2}}$ of disappearance from peripheral venous serum was found to be 2.8 min. This value is similar to recently reported disappearance rates of the gastrin heptadecapeptide (28–29). The calculated V averaged 17.4% of the body weight. This suggests that secretin is distributed promptly throughout the extracellular compartment (30).

In this study, the release of endogenous secretin into the peripheral circulation during the second half of the HCl infusion (when IRS concentrations had reached a steady state) was estimated to be 1.38 U/kg-h^{-1}. (The mean values for the MCR of 730 ml/min and the femoral venous IRS concentrations of 0.55 mU/ml were used for this calculation.) Meyer, Way, and Grossman found that acidification of the first 45 cm of the upper intestinal tract with HCl (16 meq/liter/1 h) resulted in bicarbonate responses approximately equivalent to the responses obtained with 1.0 U/kg-h^{-1} of exogenous secretin (31). The results of our study are in good agreement with the estimate of Meyer et al., particularly when considering the larger HCl load used in our study, which probably resulted in acidification of a longer part of the intestine and consequently a greater secretin release (31). Furthermore, it would appear that the similarity of the results observed in alert (Meyer et al.) and anesthetized dogs (our study) makes it unlikely that the depressed pancreatic exocrine response in anesthetized animals is caused by inhibition of secretin release from the intestinal mucosa.

Mean measurable femoral venous IRS concentration was 440 μU/ml in the three unstimulated dogs. This concentration is considerably lower than the mean level of 1,870 μU/ml found in fasting human subjects (16). The reason for this difference is presently unknown but several possibilities have to be considered. First, it might simply reflect a species difference. Second, the higher IRS levels in overnight-fasted humans might not represent true basal IRS conditions. Third, it is important to realize that porcine secretin was used as tracer and reference standard in the measurements of both human and dog secretin levels. The possibility that human secretin has greater immunoreactivity than dog secretin in a porcine system cannot be excluded. For instance, it is possible that human serum contains several species of secretin with different molecular sizes and different immunological reactivity with certain antisera, as has been demonstrated recently for parathyroid hormone (32). Lastly, it has been found that greater than usual tracer-degrading activity was responsible for spuriously high IRS concentrations in some human sera (33).

The ability of secretin to stimulate insulin secretion

under physiological conditions has not been proved. In fact, secretin doses frequently used in the past to stimulate insulin (1–4 U/kg) were found to result in unphysiologically high serum IRS concentrations.[2] On the other hand, several laboratories have obtained contradictory results when endogenous IRS release was stimulated via oral or intraduodenal administration of HCl. Chisholm et al., for instance, found significant increases in peripheral venous insulin after intraduodenal infusion of 5–20 meq of HCl/30 min in seven normal human subjects (15). Kaess, Schlierf, and Mikulicz-Radecki (9) infused HCl (20 meq for 10 min) intraduodenally into five patients with portocaval shunts. They found a small but significant insulin rise from 41 to 57 μU/ml, 22 min after the start of the HCl infusion. Other laboratories, however, failed to detect peripheral insulin changes after HCl instillation (11–13). In this study IRS release was physiologically stimulated through intestinal acidification with HCl. In addition to previous studies, however, the increase of IRS release was verified by direct measurement and its biological activity by demonstration of increased pancreatic exocrine secretion of water and bicarbonate. With this approach, a significant increase in insulin concentration was found in the portal venous circulation, which followed closely the rise of IRS in the peripheral venous circulation. This finding supports the concept that physiologically induced release of endogenous secretin is able to stimulate insulin secretion.

Quantitatively, however, the betacytotrophic effect of physiologic amounts of secretin appears to be rather weak. Doubling of the femoral venous IRS concentration resulted in a less than twofold augmentation of portal venous IRI concentration. In contrast, doubling of the glucose concentration has been shown to increase portal venous IRI concentration tenfold (34). The weakness of the insulin response to endogenous secretin is further underlined by the fact that it was demonstrable in only three of the six dogs and that the changes in portal venous insulin levels were not reflected in peripheral venous insulin changes. Moreover, peripheral FFA levels, a sensitive indicator of peripheral insulin action, did not change. Nevertheless, hepatic metabolism has been shown to be exquisitely sensitive to minute amounts of insulin (35). Therefore, it is possible that the weak insulinogenic effect of endogenous IRS may serve to prime the liver in preparation for postprandial fuel metabolism. However, other factors, such as cholecystokinin, gut glucagon, or perhaps the newly discovered gastric inhibitory peptide (36), which were not measured, also could conceivably have contributed to the HCl-mediated insulin increase.

[2] Boden, G. Unpublished observations.

Chisholm et al. have recently reported dramatic increases in peripheral venous IRS concentrations after oral as well as intraduodenal administration of glucose (14–15). In this study no consistent changes in portal or peripheral IRS concentrations were observed after intraduodenal infusion of hypo-, iso-, or hypertonic glucose solutions. These findings are supported by the lack of exocrine pancreatic response to intraduodenal glucose in our experiments (data not shown) and in the experience of others (4). Moreover, oral glucose administration (2 g/kg) to three adult human subjects did not change IRS concentrations significantly in portal or peripheral venous serum (37).

Reichle, Sovak, Soulen, and Rosemond found portal venous blood flow to increase by approximately 50% in conscious human subjects in response to oral administration of hypertonic glucose (38). This suggests the possibility that the decline in IRS concentration seen in the dogs receiving 50% glucose intraduodenally may have been caused by a rise in portal blood flow rather than a decrease in secretin release. By the same token, it cannot completely be ruled out that in the experiments where 10% glucose was infused, a small increase in secretin release might have been obscured by a concomitant rise in portal blood flow. At any rate, the lack of changes in peripheral venous IRS concentration excludes the possibility of major alterations in secretin release. Therefore, it appears unlikely that secretin represents the as yet unidentified insulin stimulatory factor released from the gastrointestinal tract in response to oral glucose. Kraegen, Chisholm, Young, and Lazarus have recently proposed that secretin may act by potentiating the glycemic effect on insulin release (39). However, the *conditio sine qua non* for this concept also would be a glucose-stimulated IRS release. This could not be demonstrated in this study.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Laurence Lundy for help in preparing the manuscript, to Dr. Subhash Gulati for assistance in the dog experiments, and to Mrs. Rachelle Masloff for secretarial help.

This work was supported in part from U. S. Public Health Service, Research Grants No. 1 RO1 AM 16348-01 MET and 5 MO1 RR 349, N.I.H., General Clinical Research Centers Branch.

REFERENCES

1. Bayliss, W. M., and E. H. Starling. 1902. The mechanism of pancreatic secretion. *J. Physiol.* (*Lond.*). **28**: 325.
2. Mellanby, J. 1925. The mechanism of pancreatic digestion: the function of secretin. *J. Physiol.* (*Lond.*). **60**: 85.
3. Wormsley, K. G. 1970. Response to duodenal acidification in man. III. Comparison with the effects of

secretin and pancreozymin. *Scand. J. Gastroenterol.* **5**: 353.
4. Preshaw, R. M., A. R. Cooke, and M. I. Grossman. 1966. Quantitative aspects of response of canine pancreas to duodenal acidification. *Am. J. Physiol.* **210**: 629.
5. Young, J. D., L. Lazarus, D. J. Chisholm, and F. F. V. Atkinson. 1968. Radioimmunoassay of secretin in human serum. *J. Nucl. Med.* **9**: 641.
6. Unger, R. H., H. Ketterer, A. Eisentraut, and J. Dupré. 1966. Effect of secretin on insulin secretion. *Lancet.* **2**: 24.
7. Unger, R. H., H. Ketterer, J. Dupré, and A. M. Eisentraut. 1967. The effects of secretin, pancreozymin, and gastrin on insulin and glucagon secretion in anesthetized dogs. *J. Clin. Invest.* **46**: 630.
8. Pfeiffer, E. F., M. Telib, J. Ammon, F. Melani, and H. Ditschuneit. 1965. Direkte Stimulierung der Insulin-Sekretin in vitro durch Sekretin. *Dtsch. Med. Wochenschr.* **90**: 1663.
9. Kaess, H., G. Schlierf, and J. G. von Mikulicz-Radecki. 1970. Effect of intraduodenal instillation of hydrochloric acid on plasma insulin levels of patients with portocaval shunts. *Metab. (Clin. Exp.).* **19**: 214.
10. Mahler, R. J., and H. Weisberg. 1968. Failure of endogenous stimulation of secretin and pancreozymin release to influence serum insulin. *Lancet.* **1**: 448.
11. Kaess, H., and G. Schlierf. 1969. Veränderungen des Blutzuckers und der Plasmainsulinkonzentration nach Stimulierung der endogenen Sekretinfreisetzung. *Diabetologia.* **5**: 228.
12. Boyns, D. R., R. J. Jarrett, and H. Keen. 1966. Intestinal hormones and plasma insulin. *Lancet.* **1**: 409.
13. Boyns, D. R., R. J. Jarrett, and H. Keen. 1967. Intestinal hormones and plasma insulin: an insulinotropic action of secretin. *Br. Med. J.* **2**: 676.
14. Chisholm, D. J., J. D. Young, and L. Lazarus. 1969. The gastrointestinal stimulus to insulin release. I. Secretin. *J. Clin. Invest.* **48**: 1453.
15. Chisholm, D. J., E. W. Kraegen, J. D. Young, and L. Lazarus. 1971. Comparison of secretin response to oral intraduodenal or intravenous glucose administration. *Horm. Metab. Res.* **3**: 180.
16. Boden, G., and W. Y. Chey. 1973. Preparation and specificity of antiserum to synthetic secretin and its use in a radioimmunoassay (RIA). *Endocrinology.* **92**: 1617.
17. Boden, G., V. Dinoso, and O. E. Owen. 1973. Immunological comparison of natural and synthetic secretins. *Horm. Metab. Res.* **5**: 237.
18. Soeldner, J. S., and D. Slone. 1965. Critical variables in the radioimmunoassay of serum using the double antibody technique. *Diabetes.* **14**: 771.
19. Duncombe, W. G. 1963. The colorimetric micro-determination of long-chain fatty acids. *Biochim J.* **88**: 7.
20. Dole, V. P. 1956. A relation between nonesterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35**: 150.
21. Hill, J. B., and G. Kessler. 1961. An automated determination of glucose utilizing a glucose oxidase-peroxidase system. *J. Lab. Clin. Med.* **57**: 970.
22. Baird, D. T., R. Horton, C. Longcope, and J. F. Tait. 1969. Steroid dynamics under steady-state conditions. *Recent Prog. Horm. Res.* **25**: 611.
23. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 6th edition. 147.
24. Rune, S. J., and F. W. Henriksen. 1967. Secretory rate of gastric acid and pancreatic bicarbonate in the dog after feeding. *Gastroenterology.* **52**: 930.
25. Wang, C. C., and M. I. Grossman. 1951. Physiological determination of release of secretin and pancreozymin from intestine of dogs with transplanted pancreas. *Am. J. Physiol.* **164**: 527.
26. Lehnert, P., H. Stahlheber, and M. M. Forell. 1969. Bestimmung der Halbwertszeit von Sekretin. *Klin. Wochenschr.* **47**: 1200.
27. Lagerlof, H., S. Y. Ek, and A. Nyberg. 1962. The duodenal secretion in man as a function of secretin dose and secretin inactivation. *Gastroenterology.* **43**: 174.
28. Walsh, J. H., H. T. Debas, and M. I. Grossman. 1973. Pure natural human big gastrin: biological activity and half-life in dog. *Gastroenterology.* **64**: 873 (abstr.).
29. Clendinnen, B. G., D. D. Reeder, E. N. Brandt, Jr., and J. C. Thompson. 1973. Effect of nephrectomy on the half-life of exogenous gastrin in dogs. *Gastroenterology.* **64**: 711 (abstr.).
30. Edelman, I. S., and J. Leibman. 1959. Anatomy of body water and electrolytes. *Am. J. Med.* **27**: 256.
31. Meyer, J. H., L. W. Way, and M. I. Grossman. 1970. Pancreatic response to acidification of various lengths of proximal intestine in the dog. *Am. J. Physiol.* **219**: 971.
32. Silverman, R., and R. S. Yalow. 1973. Heterogeneity of parathyroid hormone. Clinical and physiologic implications. *J. Clin. Invest.* **52**: 1958.
33. Boden, G. 1974. The secretin radioimmunoassay. *In* Methods of Hormone Radioimmunoassay. E. M. Jaffe, and H. Berman, editors. Academic Press, Inc., New York. In press.
34. Blackard, W. G., and N. C. Nelson. 1970. Portal and peripheral vein immunoreactive insulin concentrations before and after glucose infusion. *Diabetes.* **19**: 302.
35. Felig, P., and J. Wahren. 1971. Influence of endogenous insulin secretion on splanchnic glucose and amino acid metabolism in man. *J. Clin. Invest.* **50**: 1702.
36. Brown, J. C. 1974. Proceedings of the 8th International Diabetes Federation. Brussels, July 1973. Excerpta Medica Foundation, Publishers, Amsterdam, The Netherlands. In press.
37. Boden, G., W. Y. Chey, and V. P. Dinoso. 1972. A sensitive and specific radioimmunoassay (RIA) for secretin. *Excerpta Med. Int. Congr. Ser.* **256**: 70.
38. Reichle, F. A., M. Sovak, R. L. Soulen, and G. P. Rosemond. 1972. Portal vein blood flow determination in the unanesthetized human by umbilicoportal cannulation. *J. Surg. Res.* **12**: 146.
39. Kraegen, E. W., D. J. Chisholm, J. D. Young, and L. Lazarus. 1970. The gastrointestinal stimulus to insulin release. II. A duel action of secretin. *J. Clin. Invest.* **49**: 524.