Suppressive Effect of Secretin upon Pancreatic Alpha Cell Function

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ABSTRACT Highly purified secretin, infused endoportally in five conscious mongrel dogs at a rate of 10 clinical units per min for 20 min, caused a prompt and statistically significant reduction in the pancreaticoduodenal vein level of pancreatic glucagon from a control average of 1130 pg/ml (SEM±312) to a nadir of 492 pg/ml (SEM±194) 15 min later ($P < 0.01$). During modest hyperglycemia of about 130 mg/100 ml, induced by glucose infusion, the infusion of secretin at the same rate elicited even more dramatic suppression of pancreaticoduodenal glucagon levels to virtually unmeasurable concentrations. At a lower rate of infusion (5 U priming injection followed by 1 U/min for 20 min) significant suppression of glucagon secretion during hyperglycemia was also observed. Stimulation of endogenous secretin release by the intraduodenal administration of 14 mEq of HCl in 10 dogs during intravenous glucose infusion was followed by a decline in pancreaticoduodenal vein glucagon from 130 pg/ml (SEM±34) to a nadir of 99 pg/ml (SEM±32) 5 min later ($P < 0.05$).

The infusion of secretin at a rate of 10 U/min in alloxan-diabetic dogs was associated with a significant decline in peripheral venous plasma glucagon, from a mean preinfusion level of 272 pg/ml (SEM±39) to a nadir of 128 pg/ml (SEM±22) ($P < 0.01$).

It was concluded that exogenous secretin in the doses employed in this study is a potent suppressor of glucagon secretion, particularly during hyperglycemia. HCl-stimulated endogenous secretin also suppresses glucagon secretion. The ability of secretin to augment the glucagon-suppressing effect of ingested glucose qualifies it uniquely for a physiologic role as a modifier of the islet cell response to ingested glucose. The fact that it lowers the hyperglucagonemia of alloxan-diabetic dogs suggests that its glucagon-suppressing activity may not be insulin dependent.

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INTRODUCTION

It has long been known that the route of glucose administration influences the magnitude of the effect of hyperglycemia on insulin secretion; ingested glucose elicits greater stimulation of insulin secretion than intravenously infused glucose at comparable levels of blood glucose (1–4). Reexamination of recently published studies from this laboratory (5, 6) favors a similar relationship between the route of glucose administration and its effect on glucagon secretion; ingested glucose (6) elicits greater suppression of plasma glucagon levels than intravenously administered (5) glucose. This suggests that the absorption of glucose through the gastrointestinal tract is associated with augmentation of the alpha cell suppressing effect as well as beta cell stimulating effect of hyperglycemia.

Ever since the discovery of secretin in 1903 by Bayliss and Starling, a relationship between this hormone and the islets of Langerhans has been suspected (7–11). In recent years studies by Dupré (12), McIntyre, Turner, and Holdsworth (13), Pfeiffer, Telib, Ammon, Melani, and Ditschuneit (14), Unger, Ketterer, Eisentraut, and Dupré (15, 16), and Kraegen, Chisholm, Young, and Lazarus (17), using highly purified secretin prepared by Mutt and Jorpes (18), indicate that secretin possesses the necessary biological qualifications to serve as an augmenter of the beta cell response to ingested glucose. The present study was designed to examine the possibility that secretin also serves as an augmenter of the decrease in alpha cell secretion which accompanies the ingestion of glucose.

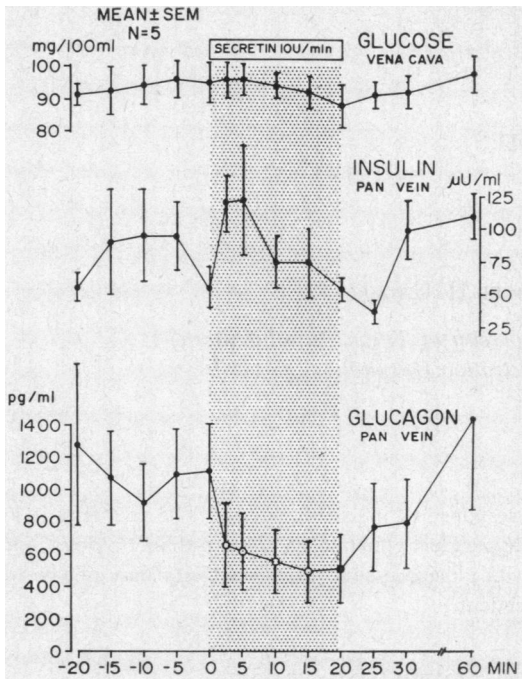

FIGURE 1 The effect of endoportal secretin infusion on pancreaticoduodenal vein glucagon and insulin in five dogs. Open circles signify statistically significant differences from the mean of the five base line values ($P < 0.02$).

METHODS

The experiments were conducted in male mongrel dogs surgically prepared as follows. Two days or more before each experiment a healthy dog, weighing between 15 and 25 kg, was anesthetized with nembutal and the abdomen was opened by midline incision. A small glass T-cannula, connected with teflon tubing, was inserted into the superior pancreaticoduodenal vein at a distance of about 3 cm from its junction with the portal vein. The tubing was fixed at the duodenum with a suture. A second teflon catheter was inserted through the left jugular vein with its opening reposing in the inferior vena cava between the heart and the hepatic vein. In some dogs a third catheter was passed through a mesenteric vein radicle into a major superior mesenteric vein. Finally, a plastic gastric tube was passed through a duodenostomy incision and sutured in place with its tip in the third portion of the duodenum. All tubes and catheters were exteriorized and heavily bandaged with tape. Postoperatively, the patency of the pancreatic vein catheter was maintained by a continuous infusion of 100 U of heparin in 20 ml of normal saline per hr. Each dog was given 600,000 U of penicillin G intramuscularly daily after surgery. Experiments were conducted 48 hr later.

Only dogs which appeared clinically well with a leucocyte count below 30,000 per mm^3 (normal 10,000–20,000), hematocrit above 35%, and a normal appetite were employed.

One group of four dogs was made diabetic by the intravenous administration of alloxan in saline solution in a dose of 75 mg/kg. In these only a jugular vein catheter was implanted 4 days later. Experiments were conducted on the following day. The alloxan diabetes was not treated. The dogs lost from 2 to 4 kg of weight despite a normal appetite in the 5 day interval between alloxanization and the experiment.

Dogs were studied after an overnight fast, in a fully conscious state. Blood specimens were obtained in syringes rinsed with 10% solution of EDTA and transferred to tubes containing 500 Kallikrein Inhibitor Units of Trasylor (FBA Pharmaceuticals, Inc., New York) per ml of blood. Plasma was separated immediately and stored at −15° to −20°C until the time of assay. Glucose concentration was measured by the ferricyanide method of Hoffman (19) using the Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.). Insulin was measured by the radioimmunoassay of Yalow and Berson (20), as modified by Herbert, Lau, Gottlieb, and Bleicher (21) and pancreatic glucagon was assayed by the previously described radioimmunoassay (22), modified as follows: the system included 7.5 pg of glucagon-^{125}I (Cambridge Nuclear Corp., Cambridge, Mass.), 1000 U of Trasylol, 0.5 ml of plasma sample, and antiserum 30K, which is highly specific for pancreatic glucagon in a final dilution of 1:100,000 making a final volume of 1.2 ml. This system can measure 8 pg/ml with better than 99% confidence.

RESULTS

Effect of secretin infusion. A group of five dogs received an endoportal infusion of purified secretin[1] at a rate of 10 clinical units per min for 20 min, administered via the mesenteric vein catheter. Within 2.5 min of the start of the infusion a statistically significant ($P < 0.01$) fall in pancreaticoduodenal vein glucagon was observed, the mean glucagon level declining from the zero value of 1130 (SEM±312) to 656 pg/ml and reaching a nadir of 492 pg/ml (SEM±194) at 15 min (Fig. 1). The statistical significance of these changes is based on comparison with the mean of all base line values. The apparent suppression of glucagon secretion continued until the infusion was terminated after 20 min, whereupon glucagon returned to the original level. This pattern was typical of all five dogs.

Insulin rose in all of the five dogs from a level of 97 μU/ml (SEM±23) at minus 5 min and 57 μU/ml (SEM±15) at zero time to 121 μU/ml (SEM±44) at 5 min and declined within 10 min. Glucose declined approximately 8 mg/100 ml. Neither of these changes were statistically significant when compared with the mean of all base line values.

Effect of secretin during glucose infusion. If secretin is released during intestinal glucose absorption, its influence on the islet cell response to hyperglycemia would be operative primarily during periods of alimentary hyperglycemia. For this reason, studies were designed to observe the effects of secretin in dogs made mildly hyperglycemic by an intravenous glucose infusion. Glucose was infused in a group of four dogs at a rate of 150 mg/min for 60 min so as to produce a relatively con-

[1] Kindly provided by Professors Erik Jorpes and Viktor Mutt, Karolinska Institutet, Stockholm, Sweden.

stant level of moderate hyperglycemia, which averaged 130 mg/100 ml in these experiments; secretin was infused via a peripheral vein at a rate of 10 U/min for 10 min beginning 30 min after the start of the glucose infusion. During the first 30 min of the glucose infusion, insulin rose to 323 μU/ml (SEM±151) and glucagon declined from 480 pg/ml (SEM±151) to 135 pg/ml (SEM± 28). Within 2.5 min of the start of the secretin infusion, glucagon declined further in all dogs to 85 pg/ml (SEM±11); at 5 min it fell to 12 pg/ml (SEM±9) ($P <$ 0.025) and at 10 min to 6 pg/ml (SEM±3) ($P < 0.02$), the lowest values ever observed in our laboratory (Fig. 2). When the secretin infusion was terminated after 10 min, but the glucose infusion continued for an additional 20 min, glucagon returned gradually to 112 pg/ml (SEM±29). Finally, after the glucose infusion was terminated, glucagon returned to the original concentration range. Although the infusion of secretin was not associated in these experiments with a rise in mean insulin above the levels reached during the glucose infusion alone, it did increase above the presecretin levels in three of the four experiments; furthermore, when the secretin infusion was terminated insulin fell sharply though not significantly below the original glucose-stimulated levels despite the continuing hyperglycemia. Mean glucose declined 13 mg/100 ml during the experiment. The fall in glucose was not statistically significant.

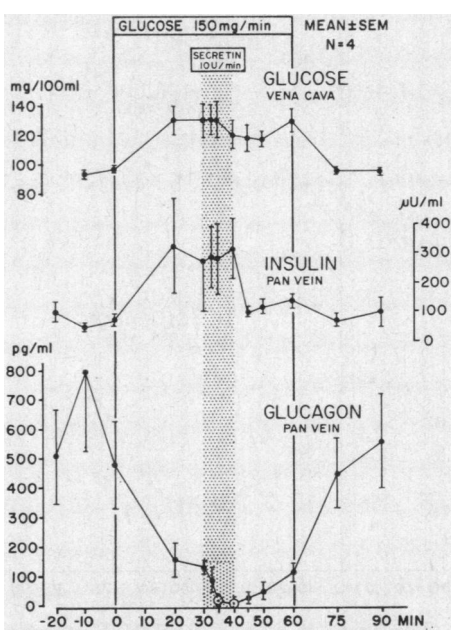

FIGURE 2 The effect of peripheral vein secretin infusion on pancreaticoduodenal vein glucagon and insulin in four dogs made hyperglycemic by glucose infusion. Open circles signify statistically significant differences from the 30 min level ($P < 0.025$).

TABLE I

The Effect of Glucose Infusion (5–7.5 mg/kg per min) without Secretin on Pancreatic Vein Glucagon and Insulin of Six Dogs

			Glucose infusion				
Minutes	−30	−15	0	30	40	50	60
Glucose mean (VC) ±SEM		98	97	122	123	121	120
		2	2	7	3	5	6
Insulin mean (PV) ±SEM	142	121	129	498	312	228	246
	38	40	40	138	102	37	135
Glucagon mean (PV) ±SEM	278	217	245	171	196	208	274
	60	59	53	49	44	46	107

VC, vena cava; PV, pancreatic vein.

The effect of glucose alone, infused for 60 min in six dogs at a similar rate for control purposes, is shown in Table I. In contrast to the remarkable reduction in mean glucagon from 135 pg/ml after 30 min of glucose alone to 6 pg/ml observed during the 10 min secretin infusion, in the control experiments in which secretin was omitted glucagon did not decline below the 30 min level of 171 pg/ml.

Effect of a lower dose of secretin during hyperglycemia. The previous amount of infused secretin must produce plasma secretin levels well above the physiologic range. To determine if a small quantity of the hormone would also augment hyperglycemia suppression of glucagon, the dose of secretin employed in humans by Kraegen et al. (17), a rapidly administered priming dose of 5 U followed by 1 U/min infusion for 20 min, was begun 60 min after the start of the glucose infusion. The results are recorded in Table II.

Glucagon, which averaged 344 pg/ml (SEM±147) after 60 min of glucose infusion and 241 (±44) 10 min earlier, declined to 178 pg/ml (SEM±70) 2 min after the start of secretin administration and reached a nadir of 140 pg/ml (SEM±54) at 20 min. These changes were observed in every dog. These values are significantly below the average of the three base line glucagon values preceding the start of the secretin (40, 50, and 60 min after the start of the glucose infusion) ($P < 0.005$ and < 0.02, respectively). The effect on insulin was equivocal; insulin rose in two of the four dogs and declined in two during the infusion of secretin and the changes in mean insulin were not significant. Mean plasma glucose declined from 120 mg/100 ml (SEM±10) to a nadir of 112 mg/100 ml (SEM±5) after 10 min of the secretin infusion but this was not a statistically significant change.

Effects of intraduodenal hydrochloric acid administration during glucose infusion. To evaluate further the possible physiologic significance of the preceding observations experiments were designed to determine if

TABLE II

The Effect of Peripheral Vein Infusion of Physiological Doses of Secretin on Glucose, Insulin, and Glucagon during a Glucose Infusion in Four Dogs

				Glucose				5U ↓ Secretin (1 U/min) infusion (150 mg/min)									
Minutes	−30	−15	0	30	40	50	60	62	65	70	75	80	90	105	120	135	150
Glucose mean (VC) ±SEM		98	96	118	119	120	120		114	112	114	115	118	115	120	97	97
		4	2	10	3	8	10		8	5	7	6	5	6	7	8	4
Insulin mean (PV) ±SEM	169	112	100	576	362	255	455	297	193	209	217	191	571	179	388	107	117
	54	47	43	170	154	65	186	65	53	51	78	29	264	27	149	49	33
Glucagon mean (PV) ±SEM	300	229	246	160	216	241	344	178*	171‡	232§	171‖	140‖	151§	205§	149§	357	473
	64	74	24	38	39	44	147	70	82	140	86	54	23	44	39	86	163

VC, vena cava; PV, pancreatic vein.
* P value vs. mean of 40, 50, 60 min values < 0.005.
‡ < 0.01.
§ NS.
‖ < 0.02.

endogenous secretin release would elicit similar evidence of suppression of pancreatic glucagon. In order to stimulate release of endogenous secretin, hydrochloric acid was administered intraduodenally to a group of 10 dogs during the intravenous infusion of glucose at a rate of 5 mg/kg per min so as to produce a constant level of moderate hyperglycemia similar to that produced by the ingestion of glucose. After the glucose infusion had been in progress for 30 min, 14 mEq of HCl were instilled into the duodenum over a 10 min period. A decline in mean pancreaticoduodenal vein glucagon from a control level of 130 pg/ml (SEM±34) before the HCl administration to a nadir of 99 pg/ml (SEM±32) 5 min later was observed (Fig. 3). The latter value is barely significantly less than the final base line value immediately before the administration of HCl ($P < 0.05$), when analyzed by a t test based on paired comparisons of the two points, although not by a test of the significance of the difference between two means, and reflected a decline in 8 of the 10 dogs. This is in contrast to the control experiments of Table I in which glucose was infused at the same or a slightly greater rate but without intraduodenal HCl; in these no statistically significant change in the glucagon level from the 30 min point was observed.

Insulin rose from a 30 min value of 141 (SEM±31) to a level of 177 (±31) μU/min at 35 min, reflecting a rise in 7 of the 10 dogs. The change was not statistically significant. Glucose did not change appreciably.

The effect of secretin in alloxan-diabetic dogs. To determine if the suppressive effect of secretin upon the alpha cells was, like that of glucose (23), absent in dogs with alloxan-induced insulin deficiency, secretin was infused via a peripheral vein at a rate of 10 U/min for 20 min in a group of four dogs with moderately severe alloxan diabetes. Inferior vena caval plasma glucagon declined in all four dogs from a preinfusion level of 272 pg/ml (SEM±39) to a nadir of 128 pg/ml (SEM±23) at 15 min; these changes were highly significant ($P < 0.01$) at

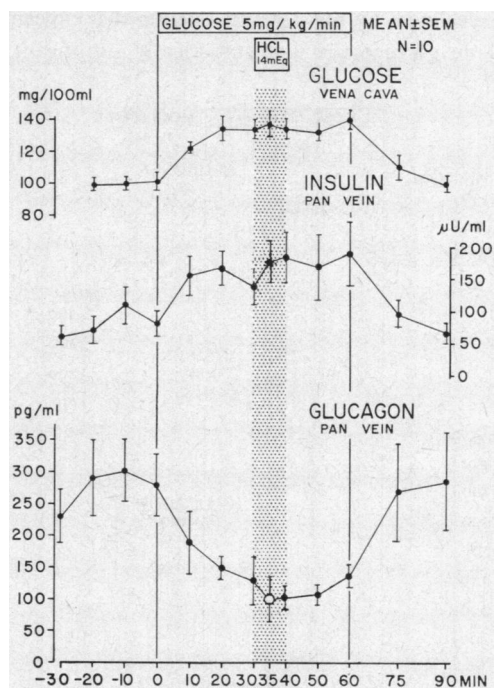

FIGURE 3 The effect of intraduodenal HCl on pancreaticoduodenal vein glucagon and insulin on 10 dogs made hyperglycemic by glucose infusion. The open circles signify a statistically significant difference from the 30 min level ($P < 0.05$), using a t test based on a paired comparison of the two points.

15 and 20 min (Fig. 4). Within 70 min after terminating the infusion, glucagon levels had returned to their original values.

Insulin, which averaged less than 3.0 μU/ml before the infusion, did not change perceptibly. Plasma glucose, which averaged about 290 mg/100 ml, also remained unchanged.

DISCUSSION

The present study constitutes the first evidence that exogenous secretin promptly and profoundly suppresses the secretion of pancreatic glucagon. The effect is even more dramatic when slight hyperglycemia is produced by the intravenous infusion of glucose. Thus, as in the case of pancreozymin, secretin's known target cells in the pancreas must now include not only the acinar cells of the pancreas and the beta cells of the islets of Langerhans, but the alpha cells as well. However, whereas pancreozymin stimulates both alpha and beta cell secretion, secretin stimulates the beta cell and suppresses the alpha cell, which qualifies it as an augmenting hormone of the reciprocal response of these cells to glucose.

This study provides further support for the older view (8–11), recently revived by Dupré (12), that secretin is the gastrointestinal modifier of the islet cell response to ingested glucose. In view of these biological properties, and the report by Chisholm, Young, and Lazarus (24) that plasma immunoreactive secretin rises after the ingestion of glucose, the evidence that secretin is, in fact, a glucose-responsive modifier of the islet cell response to ingested glucose must be regarded as substantial.

However, the physiologic importance of secretin's actions upon glucose homeostasis is not clearly established. Even the unphysiologically high doses of exogenous secretin employed in certain of our studies were associated with a decline in plasma glucose averaging only 13 mg/100 ml. Furthermore, even though stimulation by HCl of endogenous secretin reduced glucagon secretion slightly and elicited a modest rise in insulin (24), it failed to change glucose concentration.

Yet, the possible importance of the secretin system in glucose homeostasis cannot necessarily be ruled out by these results. First, Dupré and Chisholm, using doses of secretin which they regard as within the physiologic range, have observed improvement in glucose tolerance (25) and glucagon suppression in man.[2] Second, failure of HCl-stimulated endogenous secretin to augment the alpha and beta cell responses to hyperglycemia to the same degree that ingested glucose does, could represent quantitative and/or qualitative differences between HCl-induced and glucose-induced release of intestinal hormones. Third, secretin may be less important in the dog than in man. Finally, the fact that even large doses of exogenous secretin caused only a small reduction of the hyperglycemia may be a consequence of the fact that the hyperglycemia resulted from peripheral rather than portal venous glucose administration. It has been suggested previously (26) that the enterohumoral signal to the islets of Langerhans may serve to provide early "preabsorptive" preparation of the liver to increase the extraction fraction of the absorbed glucose; if this is correct, the peripheral venous route of glucose administration employed in these experiments would deprive the liver of its customary access to all incoming glucose and thereby reduce the discernible impact of the enteroinsular system upon glucose homeostasis.

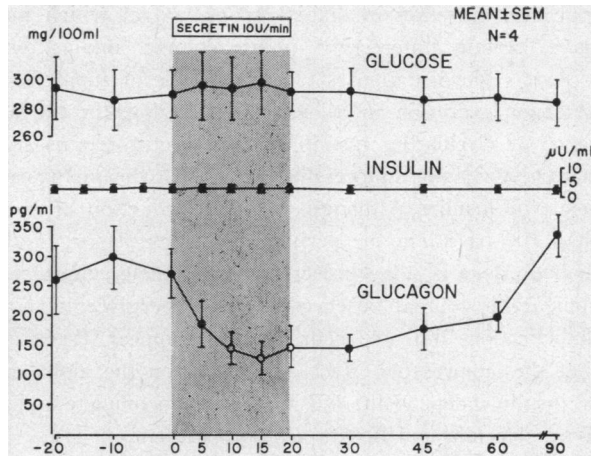

FIGURE 4 The effect of peripheral vein secretin infusion on vena caval glucagon and insulin in four alloxan-diabetic dogs. The open circles represent statistically significant differences from the mean of the three base line values ($P < 0.02$).

In the present study, the mean insulin response to secretin infusion was unimpressive (Fig. 1) and in this sense fails to confirm previous work (16). However, in every experiment of Fig. 1 insulin rose from the zero time value immediately after the start of the secretin infusion, but elevated preinfusion base line values in two of the five experiments were responsible for the apparent lack of an insulin rise. Consequently, these experiments should not be interpreted as necessarily refuting the earlier work from this laboratory.

Irrespective of the incompletely resolved issue of its physiologic importance, it is clear that, at least at the high dose level, secretin is an effective suppressor of

[2] Personal communication.

glucagon secretion in diabetic dogs, a fact which may have certain interesting pharmacologic implications. Whereas glucose appears to be incapable of suppressing glucagon secretion in alloxan-diabetic dogs in the absence of circulating insulin (23), secretin retains substantial glucagon suppressing activity in the virtual absence of insulin. Although the 50% reduction of more than 100 pg/ml in the peripheral venous plasma of the diabetic dogs is a large decrement, complete suppression comparable to that observed in the hyperglycemic nondiabetic dogs did not occur. It would appear, therefore, that the suppressive effect of secretin on the alpha cell is insulin-independent, but that it is incomplete unless hyperglycemia and insulin are both present.

Despite the fact that in these animals with virtually complete insulin deficiency the transient reduction in plasma glucagon by secretin did not lower plasma glucose, these findings may have possible therapeutic implications for human diabetics. Recent studies from this laboratory reveal that in patients with diabetes mellitus the alpha cell is not normally suppressed by hyperglycemia, even when massive amounts of insulin are provided together with administered glucose.[3] Inasmuch as the inappropriately high level of circulating glucagon apparently is biologically active (27) and, therefore, presumably detrimental to diabetic control, the possibility that secretin might suppress alpha cell secretion in human diabetes and thereby enhance the effectiveness of insulin warrants consideration. It is of interest, therapeutically as well as historically, that Moore, Edie, and Abram (7) in 1906 conducted a therapeutic trial of secretin-containing extracts of hog duodenum in the treatment of human diabetes and reported initially gratifying results. If future studies indicate that secretin is capable of suppressing glucagon in human diabetes, a reevaluation of their remarkable report might be worthwhile.

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