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*J Clin Invest.* 1970;**49**(3):524-529. <https://doi.org/10.1172/JCI106262>.

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To further study the role of secretin in insulin release in normal subjects, two consecutive 20 min intravenous glucose infusions were administered 150 min apart with and without an intervening secretin infusion (10 U) given to approximate serum secretin levels seen after oral glucose ingestion. A highly significant ( $P < 0.01$ ) potentiation of the insulin response to the post-secretin glucose infusion was observed. This occurred both when secretin was given 7 min or 25 min before glucose. In the latter case, serum secretin was undetectable during the glucose infusion. These studies demonstrate that secretin potentiates the glycemic release of insulin.

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# The Gastrointestinal Stimulus to Insulin Release

## II. A DUAL ACTION OF SECRETIN

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**ABSTRACT** The insulin release after an oral glucose load is both earlier and greater than would be expected from the glycemic stimulus. This augmentation of insulin release has been attributed to humoral factors from the gut.

It has been previously demonstrated that secretin is released very rapidly after oral glucose and postulated that it acts as an early trigger to insulin release. This effect would not explain the magnitude of the peak insulin response which occurs about 30 min after peak secretin levels. The present studies, however, demonstrate an additional action of secretin which may explain this.

To further study the role of secretin in insulin release in normal subjects, two consecutive 20 min intravenous glucose infusions were administered 150 min apart with and without an intervening secretin infusion (10 U) given to approximate serum secretin levels seen after oral glucose ingestion. A highly significant ( $P < 0.01$ ) potentiation of the insulin response to the post-secretin glucose infusion was observed. This occurred both when secretin was given 7 min or 25 min before glucose. In the latter case, serum secretin was undetectable during the glucose infusion. These studies demonstrate that secretin potentiates the glycemic release of insulin.

Despite the augmented insulin response, no consistent change in blood glucose variation was observed. This is consistent with the suggestion that the facilitated disposal of an alimentary glucose load is not dependent solely on enhanced insulin secretion.

Secretin appears to have a dual role in insulin release, an early direct stimulation followed by a prolonged potentiation of the glycemic stimulus. The potentiating effect is of such magnitude to suggest that secretin is the dominant factor in the enteric component of insulin release after an oral glucose load.

*Received for publication 10 August 1969 and in revised form 6 November 1969.*

## INTRODUCTION

It is now generally accepted that the gut hormones contribute to the insulin release observed after the oral ingestion of glucose or protein (1-4). Perley and Kipnis (5) have estimated that this contribution results in both an earlier and greater release of insulin, accounting for approximately 50% of the insulin response after a 100 g glucose load.

Using a radioimmunoassay for secretin, studies were reported previously which demonstrated the response of this hormone to oral glucose (6). The major response to a moderate (50 g) load occurred within 15 min of glucose ingestion. Because the insulin response to small amounts of exogenous secretin is modest, immediate, and transient (7, 8), it was suggested that this hormone acts as an early trigger to insulin release after oral glucose. However, the amount of insulin directly released by secretin would be quantitatively small in relation to the peak insulin response which approximately coincides with the peak blood glucose level and occurs some 30 min after the secretin response.

Is secretin then a major factor in the enteric release of insulin? We report here observations of the insulin response to consecutive intravenous glucose infusions with and without intervening secretin administration to demonstrate that secretin possesses an additional prolonged potentiating effect on the glycemic release of immunoreactive insulin.

## METHODS

Nine normal informed male student volunteers aged 18-23 yr were studied in the morning after a fast of at least 8 hr. They were ambulant, nonobese, had normal dietary habits, no family history of diabetes mellitus, and fasting plasma glucose levels below 90 mg/100 ml. Venous blood samples were obtained via an indwelling polyethylene catheter inserted into an antecubital vein. Glucose was infused at a constant rate for 20 min into a superficial vein in the opposite forearm using a constant infusion pump. An

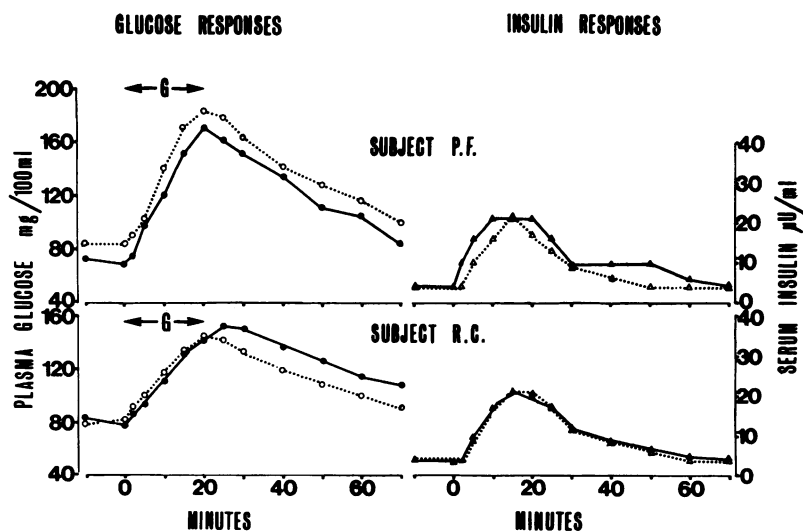


FIGURE 1 Plasma glucose and serum insulin responses of two normal subjects to consecutive intravenous glucose infusions (G). Responses to the second infusion (solid lines) are superimposed on those to the first infusion.

identical infusion of glucose was commenced 150 min after commencement of the first infusion. The rate of glucose infusion varied from 0.75 to 0.82 g/min in the group of subjects studied, but for each subject, the infusion rates differed by less than 0.02 g/min.

An intravenous dose of 10 U purified secretin<sup>1</sup> was administered 7 min or 25 min before the second glucose infusion. This was given as a 5 U priming dose, followed by 1 U/min for 5 min to approximate peripheral serum secretin levels previously observed after a 50 g oral glucose load (6). Two subjects were studied with secretin at each of these times (Figs. 2 and 3, Table I A). A control study without secretin was carried out on one subject from each pair, and also on three other subjects.

To detect any quantitative difference due to the timing of the secretin pulse, glucose-secretin-glucose infusions were performed on two additional subjects (Table I B) on consecutive days using a 7 min time interval and a 25 min interval alternately.

In addition, the responses of blood glucose, serum secretin, and serum insulin after a 50 g oral glucose load in a normal subject aged 20 have been added to the group of normal subjects reported previously (6).

Blood and plasma glucose were measured by the Auto-Analyzer modification of the Hoffman ferricyanide technique (9). Serum immunoreactive insulin (10) and secretin (11, 6) were measured by radioimmunoassay. Insulin results from the infusion studies were determined in three assay batches where the within-assay standard deviation for duplicate estimation was respectively  $\pm 2.0$ ,  $\pm 2.5$ , and  $\pm 2.5$   $\mu\text{U}/\text{ml}$ . The experimental design is such that comparisons are only subject to within-assay errors.

## RESULTS

The plasma glucose and serum insulin responses for two of the five subjects given consecutive infusions of

<sup>1</sup> Potency approximately 4000 clinical U/mg. Obtained from Professor E. Jorpes, Karolinska Institute, Stockholm, Sweden.

TABLE I  
Integrated Insulin Response

Subject	First glucose infusion	Secretin* infusion	Second glucose infusion†
<i><math>\mu\text{U} \cdot \text{min per ml}</math></i>			
(A) Initial study			
No secretin			
P. F.	490	—	850
R. C.	640	—	660
G. P.	640	—	680
S. C.	590	—	460
J. H.	450	—	580
Secretin, -7 min			
P. F.	230	100	1500
B. W.	190	70	1240
Secretin, -25 min			
R. C.	190	470	2080
D. H.	280	140	1800
(B) Comparative study			
Secretin, -7 min			
P. C.	320	280	3980
P. T.	910	340	2840
Secretin, -25 min			
P. C.	1310	580	1770
P. T.	690	340	2080

Integrated insulin responses are shown to two consecutive glucose infusions with and without an intervening secretin infusion.

\* Calculated to the nadir.

† Calculated from the nadir in the postsecretin studies.

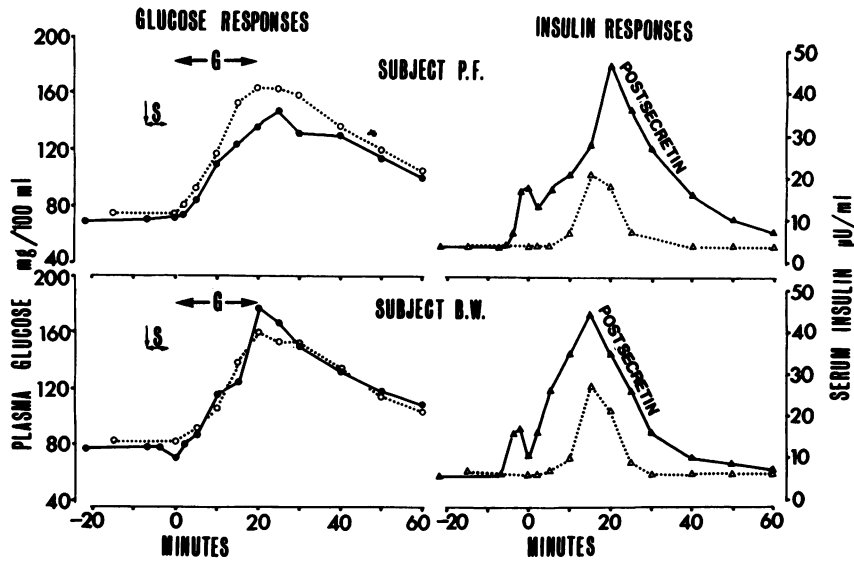


FIGURE 2 Plasma glucose and serum insulin responses of two normal subjects to consecutive intravenous glucose infusions (G). Secretin (S) was administered 7 min before the second glucose infusion (solid lines).

glucose without secretin are shown in Fig. 1. Figs. 2 and 3 show responses when secretin was given intravenously 7 min and 25 min, respectively, before the second glucose infusion. For ease of comparison, the responses to the first and second glucose infusions are superimposed in these figures. Table I compares inte-

grated insulin responses (area under insulin rise) for all subjects in these three studies.

There was only a small difference in the insulin responses to the glucose infusions in the control studies without secretin (Fig. 1, Table IA, and Table II). Thus it appeared valid to use this experimental design

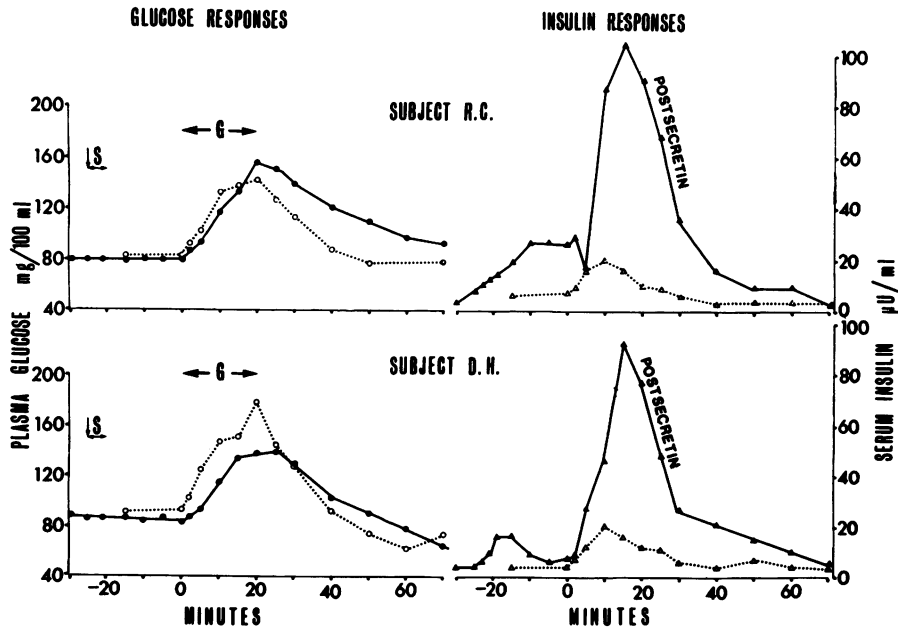


FIGURE 3 Plasma glucose and serum insulin responses of two normal subjects to consecutive intravenous glucose infusions (G). Secretin (S) was administered 25 min before the second glucose infusion (solid lines).

to examine the effect of an additional variable on insulin response.

The insulin response after secretin was biphasic, with a nadir significantly ( $P < 0.01$ ) below the first peak in all subjects, and occurred between  $-5$  and  $+5$  min. This was most evident in subject D. H. (Fig. 3). The first phase of insulin elevation commenced immediately after secretin administration and fell at or about the time glucose was infused. This phase was considered to represent the direct insulinogenic action of secretin and was of modest proportions when calculated as an integrated insulin response (Table I A).

However, the second phase of insulin elevation coincident with the glucose infusion in the postsecretin studies was considerably greater than that produced by the first glucose infusion. The integrated insulin response calculated from the nadir shows an increase of from 500 to 900% of that produced by the respective preceding glucose infusions. This phase is attributed to potentiation of the glycemic stimulus to insulin release.

This potentiation initially appeared greater in the two subjects where secretin was given 25 min rather than 7 min before the second glucose infusion (Table I A). In a further comparative investigation (Table I B) using identical subjects on consecutive days for the two infusion sequences this difference in potentiation was not apparent. All subjects showed a similar pattern of insulin response after secretin, namely a small rise after secretin, a clearly defined nadir, and a potentiation of the insulin response to the second glucose infusion. The potentiation however appeared approximately equal in the two studies on subject P. T. and was greater with the shorter time interval in subject P. C. Subject P. C. showed an unexplained but wide disparity in insulin response to the first glucose infusion on the 2 days.

TABLE II  
A Comparison of the Mean Integrated Insulin Responses in Each Group of Studies

Study	Integrated insulin response*		Significance of increase
	First glucose infusion	Second glucose infusion†	
	$\mu\text{U} \cdot \text{min per ml}$		
No secretin‡	560 $\pm$ 40	650 $\pm$ 35	0.05 < $P$ < 0.1
Secretin, -7 min	410 $\pm$ 140	2390 $\pm$ 550	$P$ < 0.01
Secretin, -25 min	620 $\pm$ 220	1930 $\pm$ 75	$P$ < 0.01

\* Mean  $\pm$  SEM.

† Calculated from the nadir in insulin response for postsecretin studies.

‡ Five subjects.

|| Four subjects.

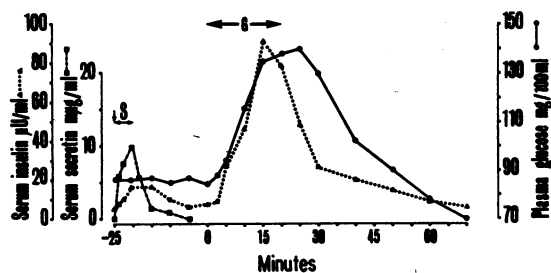


FIGURE 4 Plasma glucose, serum secretin, and serum insulin levels produced in subject D. H. by parenteral administration.

Table II shows the mean insulin responses to all studies. There was a highly significant but approximately comparable potentiation after secretin at 7 and 25 min.

Plasma glucose levels showed some variation between first and second infusion in all subjects, but there was no consistent change either in maximum levels reached nor in rate of fall of glucose concentration in the post-secretin studies.

The relationship these studies bear to oral glucose responses is shown in Figs. 4 and 5. Fig. 4 demonstrates the glucose, secretin, and insulin levels of subject D. H. Fig. 5 represents the glucose, secretin, and insulin response (mean  $\pm$  SEM) of seven normal subjects to a 50 g oral glucose load. There is a similarity in the time relationship of these parameters in both studies. Responses are however more prolonged after oral glucose.

Serum secretin levels were estimated in two of the six subjects after intravenous administration of the hormone. Both showed a similar pattern with peak levels of 10 and 8  $\mu\text{g}/\text{ml}$ . These figures are slightly below the mean peak level after oral glucose (Fig. 5) but fall well within the normal range. There is a possibility that the intravenous glucose infusions may have released endogenous secretin. We have found small but inconstant elevations of serum secretin with intravenously induced blood glucose elevations above 250 mg/100 ml.<sup>3</sup> Although plasma glucose levels did not reach this figure, serum secretin was measured at the time of maximum glycemia in four subjects. There was no detectable response.

Serum secretin therefore was only present as a transient pulse during the glucose-secretin-glucose infusion studies, levels declining rapidly after cessation of parenteral secretin administration. When this was commenced 7 min before the second glucose infusion, secretin would only be present in very small amounts during the first 10 min of glucose infusion, and when commenced 25 min before the second glucose infusion, secretin would be negligible throughout the glucose infusion.

<sup>3</sup> Unpublished observations.

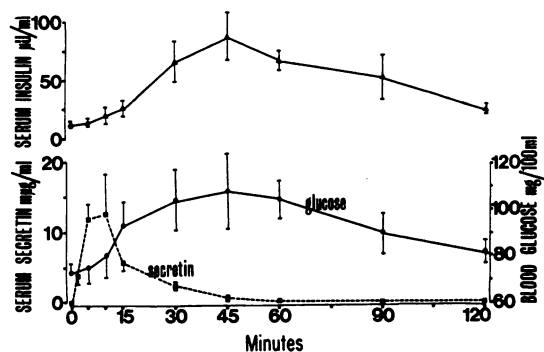


FIGURE 5 Mean levels  $\pm$ SEM of blood glucose, serum secretin, and serum insulin of seven normal subjects after oral administration of 50 g glucose.

## DISCUSSION

The studies reported here confirm that a brief elevation of secretin causes an immediate though modest rise in serum insulin levels (7, 8) but also demonstrate that secretin has a prolonged potentiating effect on the glycemic stimulus to insulin release.

The potentiating action of secretin persists well after peripheral secretin levels have declined. In all eight studies where secretin was given before the infusion of glucose, there was a profound augmentation of the insulin response to hyperglycemia when compared with a prior control glucose infusion. This effect was seen whether secretin was given 7 min or 25 min before the glucose infusion. The total duration and optimum time of secretin potentiation was not defined in these studies. However, the potentiation persisted after secretin was removed from the circulation, suggesting that this hormone may influence a  $\beta$ -cell enzyme system governing synthesis or release of insulin.

In view of the increased insulin response in the post-secretin studies, the absence of any consistent change in the time course of glucose concentration is surprising. Several possible reasons could be offered, firstly that an undetermined hyperglycemic factor may have countered the extra insulin release during the second glucose infusion, secondly that the rise in serum insulin does not represent biologically active insulin but an immunoreactive precursor, "proinsulin" (12), or thirdly that secretin may in some way alter the hepatic extraction of insulin. These reasons are similar to those previously suggested for an observed lack of suppression of blood glucose from a normoglycemic level after a moderate endogenous elevation of serum insulin (6). They are however not supported by any definite evidence. While not excluding these possibilities, the influence of portal hyperglycemia deserves further consideration.

The importance of the liver in glucose utilization has recently been reviewed by Madison (13). This evidence

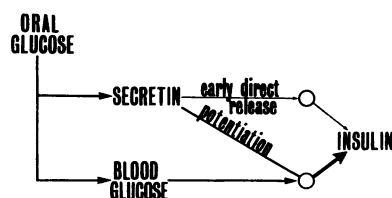


FIGURE 6 A schematic illustration of the dual action of secretin in insulin release after oral glucose.

and the studies of Perley and Kipnis (5) indicate that simultaneous portal glucose and insulin elevations favor net hepatic glucose uptake. In the dog, net hepatic glucose uptake increases progressively with rising portal hyperglycemia above a threshold level (13).

In man, after an alimentary glucose load where there would be considerable portal hyperglycemia, the liver disposes of 60–70% of the glucose (5). It thus seems possible that the parenteral infusion of glucose would minimize the role of this most important organ of glucose uptake unless the degree of hyperglycemia approximated the portal blood sugar levels after an alimentary load. The fact that Dupre, Curtis, Unger, Waddell, and Beck (14) found increased glucose disposal at the end of (but not during) a 40 min parenteral infusion of glucose and secretin, at a point where hyperglycemia was much greater than in our studies, would be consistent with this idea. Our studies thus support this group's suggestion that the increased efficiency of disposal of alimentary glucose does not depend solely on enhanced insulin secretion.

It is not possible from these studies to infer what effects relatively higher portal glucose, insulin, and secretin concentrations have on over-all blood glucose regulation. We can, however, compare the pattern of insulin response produced by an oral glucose load with that produced by the parenteral studies described here. The latter experimental design firstly separates the dual actions of secretin and secondly approximates the secretin response observed after a moderate (50 g) oral glucose load, both in magnitude and time relationship to the subsequent hyperglycemia (Figs. 4 and 5). From this comparison it seems likely that both actions of secretin would contribute to the insulin response to oral glucose (Fig. 6).

The early direct release of secretin would provide an initial trigger to insulin release, as previously suggested (6). The second potentiating effect of secretin on the glycemic stimulus to insulin release appears to be quantitatively more important and is in accord with the close time coincidence observed in maximum blood glucose and serum insulin levels (Fig. 5). This potentiation is of sufficient duration to contribute to the

insulin response for at least 1 hr after an oral glucose load.

The lack of a biphasic insulin response after oral glucose is in accord with the greater overlap of secretin and glucose elevations (Fig. 5). We could thus expect a transition period where both actions of secretin are operative. This is supported by studies of simultaneous glucose and secretin elevations by Dupre et al. (14).

After oral ingestion of larger glucose loads, protein, and mixed nutrients, it appears likely that the enteric stimulus to insulin release also involves other hormones (4). However as serum glucagon-like immunoreactivity and serum pancreatico-cholecystokinin do not show a positive response after a moderate (50 g) oral glucose load (10, 15, 16, 17), it is suggested that the dual effect of secretin might be sufficient to account for the gastrointestinal component of insulin release in this case.

#### ACKNOWLEDGMENTS

We are indebted to Professor Erik Jorpes and Dr. Victor Mutt of the Karolinska Institute, Stockholm, Sweden and to Dr. M. A. Ondetti of the Squibb Institute for Medical Research, New Brunswick, N. J. for their generosity in supplying synthetic secretin which was used in the secretin radioimmunoassay, to Professor Jorpes for further supplies of highly purified secretin used in the clinical studies, and to Professor E. P. George for his helpful advice and criticism.

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