Glucagon Release Precedes Insulin Release in Response to Common Secretagogues

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SUMMARY

The dynamics and interrelationships of glucagon and insulin secretion were studied in the isolated perfused rat pancreas by utilizing a series of compounds that stimulate the release of both hormones. Leucine, arginine, prostaglandins $F_{2\alpha}$ and E_2, bovine growth hormone, and isoproterenol were administered individually over 60-second intervals. The release of glucagon preceded that of insulin in response to all compounds tested. The rapidity of glucagon release varied in response to different secretagogues; the time course of insulin release was fairly constant. The timing and the magnitude of glucagon and insulin release did not correlate statistically. Conclusions: (1) pancreatic alpha cells respond more rapidly than beta cells to the same stimulus; (2) antecedent release of glucagon is not the principal mediator of insulin release in response to stimuli common to both hormones; and (3) endogenous glucagon may at best modify the release of insulin evoked by certain secretagogues. DIABETES 25:764-70, September, 1976.

The initial or acute phase of the release of glucagon and insulin occurs within several minutes in response to a variety of secretagogues.[2,3] The dynamics of the acute-phase glucagon and insulin release have not been defined precisely. A series of observations suggests that glucagon and insulin could influence the release of one another. The juxtaposition of the pancreatic alpha and beta cells producing glucagon and insulin respectively makes a direct interaction of these two cell types feasible. Recent electron-microscopic demonstration[4] of gap junctions between these cells, which are thought to be channels of molecular communication, has added strength to this interaction theory. The release of insulin occurring in response to administered glucagon has been demonstrated in vivo[5,6] and in vitro.[7,8] An inhibitory effect of exogenous insulin on in-vivo and in-vitro release of glucagon has been documented as well.[9,10] Yet evidence is lacking that endogenous glucagon may influence the secretion of insulin, and vice versa. A careful analysis of the patterns, sequences, and magnitudes of the acute-phase hormone release at a time both glucagon and insulin secretion occurs may elucidate such possible relationships. Accordingly, we have used a sensitive and precise in-vitro system and studied the dynamics and magnitudes of release of glucagon and insulin from the isolated perfused rat pancreas in response to secretagogues common to both hormones. The results of these studies indicate that the release of glucagon precedes that of insulin in response to a variety of stimuli.

MATERIALS AND METHODS

A perfusion system to study the release of pancreatic islet hormones was used. Pancreases were removed from fasting male Sprague-Dawley rats that weighed 250-300 gm. The surgical technic was similar to that described by Sussman et al.[11] The aorta and the portal vein were cannulated for the inflow and outflow of the perfusion solution, respectively. The isolated organ was placed in a multichannel perfusion apparatus as described previously.[12] The apparatus allows for changing perfusion solutions within a fraction of a second without any alteration in flow rate (2.5 ml./min.) or perfusion pressure (30-50 mm. Hg). When a certain perfusion solution is diverted to the pancreas by switching the manifold valve, the solution takes five seconds to reach the pancreas and an additional seven seconds the distal end of the portal venous

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cannula. The composition of the perfusion medium was 118.5 mM NaCl, 3.5 mM KCl, 1.0 mM $MgSO_4 \cdot 7H_2O$, 1.2 mM KH_2PO_4, 1.25 mM $CaCl_2 \cdot 2H_2O$, 24.9 mM $NaHCO_3$, 5.6 mM D-glucose, 40 gm./L. dextran (average mol. wt. 72,000) and 2 gm./L. bovine serum albumin dissolved in deionized, distilled water.

Shortly before their use in the experiments, individual solutions of the following compounds were prepared as additions to the perfusion medium at the concentrations stated in table 1: methionine-free L-leucine (ICN, L-arginine monohydrochloride (ICN), prostaglandin (PG) $F_{2\alpha}$ (Upjohn Co.), PGE_2 (Upjohn Co.), bovine growth hormone (bGH) (NIH), and isoproterenol hydrochloride (Winthrop). Solutions of the latter compound were protected from light.

Following the attachment of the pancreas to the perfusion system, an equilibration period of 30-40 minutes preceded each experiment, during which the organ was perfused with the basic buffer solution. The perfusions with the solutions of the test compounds were over periods of 60 seconds. Up to three such perfusions with the same test solution were carried out on each pancreas separated by 14-19-minute "rest" periods, with buffer only as the perfusate. When the experiments were completed, the functional integrity of the pancreas was ascertained by assessing the glucagon and insulin secretory response to 60-second perfusions of 10 mM arginine. The portal venous effluent was collected in 12-second fractions with a fraction collector (Isco 1200). The samples were identified according to the elapsed time; they were stored at −20° C. until the time of hormone assays.

The levels of glucagon and insulin in the portal venous effluent were measured by a "double-antibody" radioimmunoassay technic.[13] The anti-glucagon serum G9-I employed in the glucagon assay has a negligible cross-reactivity with glucagon-like compounds present in extracts of intestines.[14] Rat insulin (Novo, Denmark) and bovine-porcine glucagon (Lilly, Indianapolis) were used as standards in the insulin and glucagon assays, respectively.

The results are reported as means ± standard errors of the means (S.E.). The cumulative hormonal secretory responses were expressed as the area under the response curve from 0 to 120 seconds (120-second area). The 120-second area is the sum of the 10 12-second partial areas (A_p), derived by using the formula $A_p = \frac{1}{2}(v_{t-1} + v_t)(t - t_{-1})$, where v_{t-1} is the value at time $t-1$ and v_t the value at time t. The timing of secretion was expressed in three ways: (a) The time of the first significant change (t_Δ) from basal hormone concentrations was obtained by paired analysis of basal versus response levels at each time point. (b) The time of the maximal response (t_{max}). (c) The time of "half-maximal" hormone concentrations ($t_{.5}$) was calculated from a function derived from normalized response areas (*vide infra*). Normalization was achieved by setting the value of the integrated response area equal to 1. Since, by normalization, all response areas are made equal, this allows for the expression of the response as a function of time only. Once normalized, a sequence of partial sums of areas over each of the "k"th 12-second intervals from 0 to 120 seconds can be computed and fit to a quadratic equation by simple linear regression. This function $PSN(t_k)$ (partial sum, normalized, at the "k"th time interval) derived for each set of experiments is plotted on the inserts to figures 1-6. To calculate $t_{.5}$ the

TABLE 1

Magnitudes and times (mean ± S.E.) of glucagon and insulin secretory responses to 60-second perfusions of various compounds

Compound	Conc'n M	N	120-sec Area* Glucagon ng. sec./ml.†	120-sec Area* Insulin mU./sec./ml.†	t_Δ* sec. Glucagon	t_Δ* sec. Insulin	t_{max}* sec. Glucagon	t_{max}* sec. Insulin	$t_{.5}$* sec. Glucagon	$t_{.5}$* sec. Insulin
L-leucine	2×10^{-2}	12	145± 7	13.0±2.4	24	36	36.0±0	76.0±0	29±1	70±2
L-arginine	10^{-2}	14	59±16	15.0±4.5	24	36	44.6±3.2	72.9±2.0	44±2	65±2
PG $F_{2\alpha}$	1.4×10^{-6}	19	160±16	2.7±0.6	24	48	40.6±1.6	78.3±2.3	39±1	69±2
PG E_2	1.4×10^{-6}	6	177±25	0.4±0.1	24	48	48.0±0	76.0±2.5	46±1	70±2
bGH	10^{-7}	10	96±11	1.2±0.4	24	48	36.3±4.1	70.8±6.1	37±2	68±2
bGH	5×10^{-7}	7	102±12	3.9±1.1	24	48	36.0±0	75.4±5.7	34±1	64±3
Isoprot.	1.1×10^{-7}	9	51± 1	0.5±0.1	36	60	61.3±3.7	88.0±5.7	49±2	67±3
Isoprot.	1.1×10^{-6}	7	28± 4	1.3±0.3	36	60	48.0±8.3	72.0±3.7	48±4	65±2

*The calculation of the parameters used and symbols are explained under Methods.

†The amount of hormone secreted during the 120-second period may be derived by multiplying the values by 5 ml., which is the volume of buffer perfused.

t_{max} and $t_{.5}$ values for glucagon are significantly different than the corresponding values for insulin in all series ($2p < 0.05 - 0.001$).

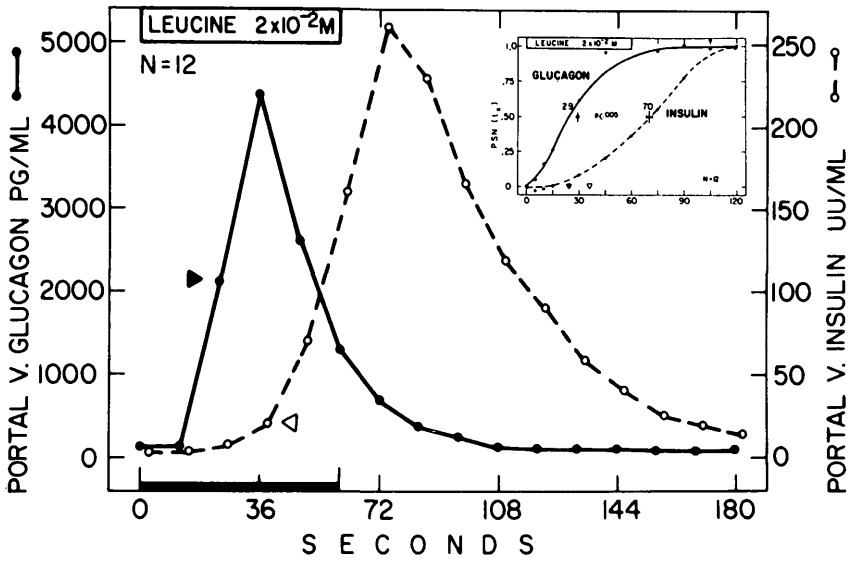

FIGURE 1

Mean portal venous levels of glucagon and insulin in response to 60-second perfusion of 20 mM L-leucine in the presence of 5.6 mM glucose. Insert: The same data expressed as normalized partial sums (PSN); the mean ± S.E. of $t_{.5}$ superimposed on each regression slope; p value refers to significance of difference between $t_{.5}$ for glucagon and insulin. The arrowheads signify the time of first significant increase above basal levels.

function PSN(t_k) is set equal to 0.5 (hence the term "half-maximal") and the root is obtained. Statistical hypotheses were tested by Student's *t*-test or analysis of variance where appropriate.[15]

RESULTS

A total of 84 experiments were performed with 34 pancreases. Six different compounds known to stimulate the release of both glucagon and insulin were administered individually: leucine, arginine, $PGF_{2\alpha}$, PGE_2, bGH, and isoproterenol. The latter two secretagogues were given at two different concentrations. The data obtained from these eight series of experiments are summarized in table 1. The mean levels of glucagon and insulin in the portal venous effluent observed with the six secretagogues are depicted in figures 1-6.

The baseline levels of glucagon and insulin were stable prior to the administration of the secretagogue in all experiments. The mean of basal values ranged from 100±18 to 194 ± 47 pg./ml. for glucagon and from 1.0±0 to 3.6±1.6 μU./ml. for insulin. Significant increases in mean portal venous effluent levels of glucagon and insulin were observed within the first 60 seconds in response to all secretagogues. The hormone levels increased steeply and reached maximal values within 90 seconds in all instances. The mean maximal levels attained were quite variable, depending upon the type and concentration of the secretagogue. They ranged from 370±114 to 4,408±234 pg./ml. for glucagon and from 8±1 to 261±46 μU./ml. for insulin. The mean hormone levels declined more gradually than their respective rise. The levels of glucagon as well as insulin had returned to or toward baseline values by 180 seconds.

In all series of experiments the mean 120-second response areas for glucagon and insulin (table 1) were significantly greater than the corresponding basal areas extended over 120 seconds. The magnitude of the secretory response (120-second areas) of glucagon and insulin correlated for only two series of experiments: $PGF_{2\alpha}$ ($r = 0.531$, $p < 0.05$) and 1.1×10^{-7} M isoproterenol ($r = 0.926$, $p < 0.01$). The correlation between the average glucagon and insulin response areas for all experiments was not significant ($r = -0.017$).

With all secretagogues, the time course of secretion of glucagon preceded that of insulin during the entire 120-second period (figures 1-6). The time of the first significant change above basal levels (t_Δ) for glucagon was 12-24 seconds earlier than that for insulin in all series of experiments (table 1). Among the total of 84 experiments a rise in the level of glucagon was observed earlier than that of insulin in 72 experiments, synchronous with insulin in 11, and by 12 seconds later than insulin in one experiment. The mean time of the maximal levels (t_{max}) in glucagon also occurred earlier than that in insulin in the eight series of experiments (table 1). The maximal level in glucagon was attained earlier than in insulin in all but two of the 84 experiments. The mean levels of glucagon started to decline before the 60-second time point, at a time the perfusion of the secretagogue was still in progress (figures 1-5). The experiments using 10^{-7} M isoproterenol constituted the only exception to this observation (figure 6). The decreases in insulin always

commenced after the termination of the perfusions of the secretagogues. The mean times of the "half-maximal" hormone concentrations ($t_{.5}$) are given in the figure inserts and in table 1. In each series of experiments the $t_{.5}$ for glucagon occurred significantly earlier than the corresponding value for insulin. The mean $t_{.5}$ for the responses in glucagon varied over a considerable range (29 to 49 seconds). On the other hand the mean $t_{.5}$ for insulin occurred within a narrow time range of six seconds (64 to 70). The analysis of variance for the eight series of experiments revealed that the variations in $t_{.5}$ were statistically significant for glucagon ($F = 18.8$, $p < 0.01$), but not for insulin ($F = 0.96$). No correlation could be demonstrated between the means of $t_{.5}$ for glucagon and insulin ($r = -0.013$).

DISCUSSION

The study was designed to elucidate the time course of secretion of glucagon and insulin when the release of both hormones was being stimulated. The following compounds were used, which are secretagogues common to both hormones: arginine, leucine, prostaglandins $F_{2\alpha}$ and E_2, growth hormone, and isoproterenol. Arginine is the most widely used common secretagogue. Using the isolated perfused rat pancreas, we also have identified leucine as a common secretagogue,[16] which is contrary to our earlier observations in man and dog that this amino acid stimulates insulin but not glucagon release.[17,18] Again using the isolated rat pancreas we have demonstrated that prostaglandins[12,19] and bovine growth hormone[20] consistently stimulate the release of glucagon and insulin. Finally, isoproterenol was used in our experiments as we and others have shown that beta adrenergic stimulation augments the release of both hormones.[21,22]

The release of glucagon in response to these secretagogues occurred very rapidly as manifested by the significant increases in its concentration above the basal effluent levels within 24 seconds after the beginning of the perfusions. This response period is actually 12 seconds, since five seconds elapse for a new perfusate to reach the pancreas and another seven seconds for it to travel from the pancreas to the collection tube. The time course of glucagon release varied considerably in response to different kinds of secretagogues, as reflected by the wide range of "half-maximal" response time ($t_{.5}$) (table 1). The timing of the release appeared to be more dependent on the nature of a secretagogue rather than its concentration, as suggested by the experiments with bGH and isoproterenol. The release of insulin consistently lagged behind that of glucagon. Unlike that of glucagon, the time course of release of insulin was fairly constant for all series of experiments, with mean $t_{.5}$ ranging from 64 to 70 seconds. The release time for either hormone was not related to the magnitude of the hormone being released. The earlier release of glucagon might be attributable to possible difference in the microcirculation to the alpha and the beta cells of the pancreatic islets, allowing the secretagogue to reach the alpha cell faster than the beta cell. This is unlikely, since the response times for glucagon varied over a wide range and those for insulin remained fairly constant. For the same reason, a faster transit of glucagon to the portal

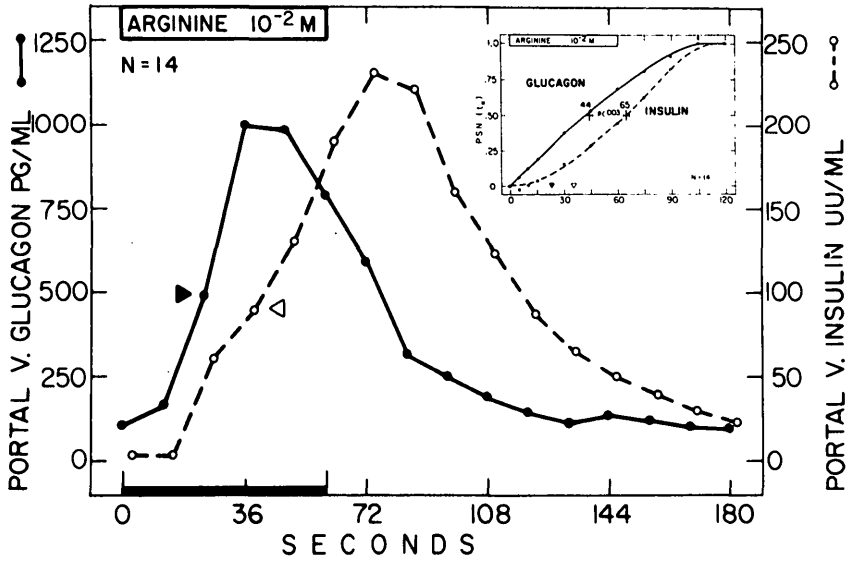

FIGURE 2

See legend to figure 1. In these experiments 10 mM arginine is the secretagogue.

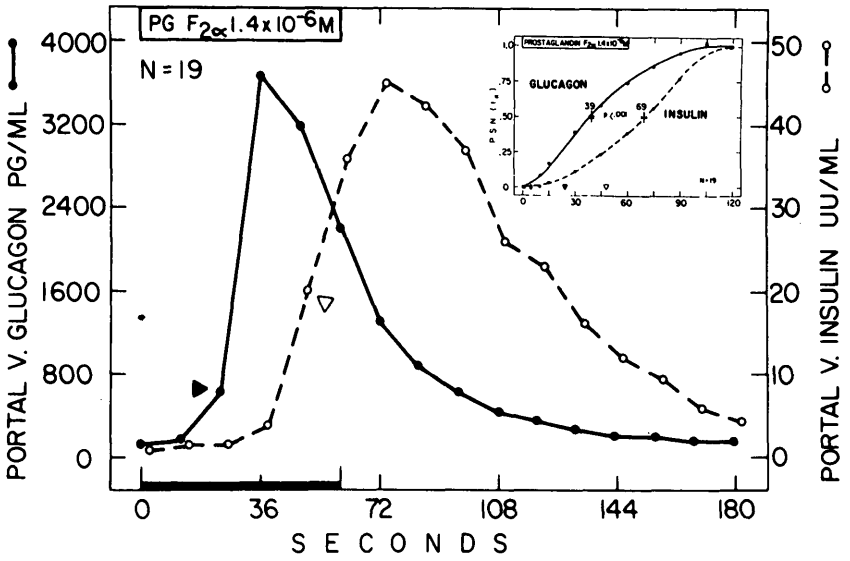

FIGURE 3

See legend to figure 1. In these experiments 1.4 × 10^{-6} M prostaglandin $F_{2\alpha}$ is the secretagogue.

venous cannula is not likely. The present data suggest that with the administration of a common secretagogue the secretory response of the alpha cell consistently occurs faster than that of the beta cell, because of either a more expeditious activation of the secretory process or a more efficient mechanism of discharge.

In most of our experiments the effluent levels of glucagon started to decline at a time the perfusion of the secretagogue was still in progress. This observation suggests that the readily releasable compartment of the stored hormone is emptied almost instantaneously as soon as the stimulus has activated the release process and that the repletion of this labile compartment[23] requires a time period longer than our experimental period. A similar conclusion for the insulin secretory process cannot be based on the present data, since the levels of insulin reached their maxima after the perfusions of the secretagogues had come to an end. Nevertheless the rate of increase in insulin levels closely paralleled that of glucagon in most experiments, thus suggesting a discharge process of similar rapidity.

Since administered glucagon stimulates the release of insulin,[5-8] the antecedent release of glucagon seen in these experiments would support the possibility that endogenous glucagon contributes to the activation of secretion of insulin. As depicted in figures 1-6, the first significant increase in mean levels of insulin occurred synchronously with or 12 seconds subsequent to the mean maximal levels of glucagon. This observation suggests that the attainment of a certain "permis-

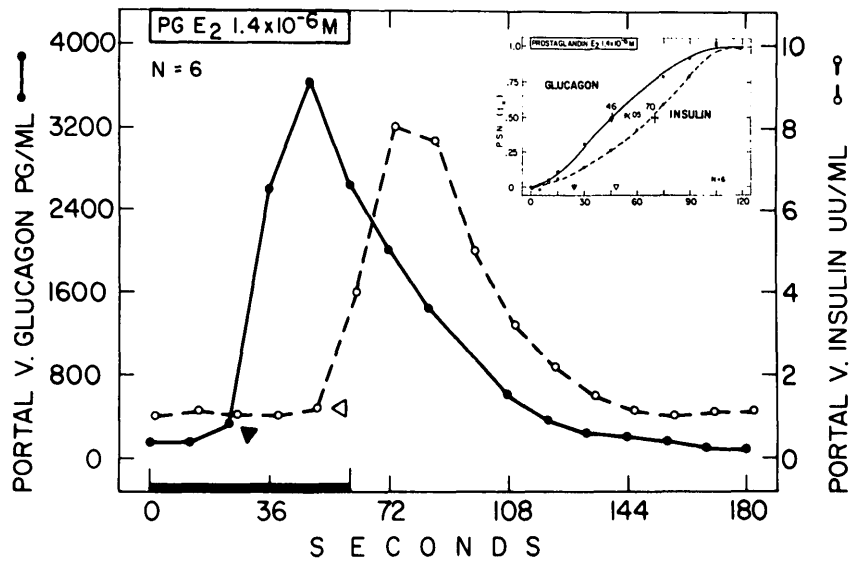

FIGURE 4

See legend to figure 1. In these experiments 1.4 × 10^{-6} M prostaglandin E_2 is the secretagogue.

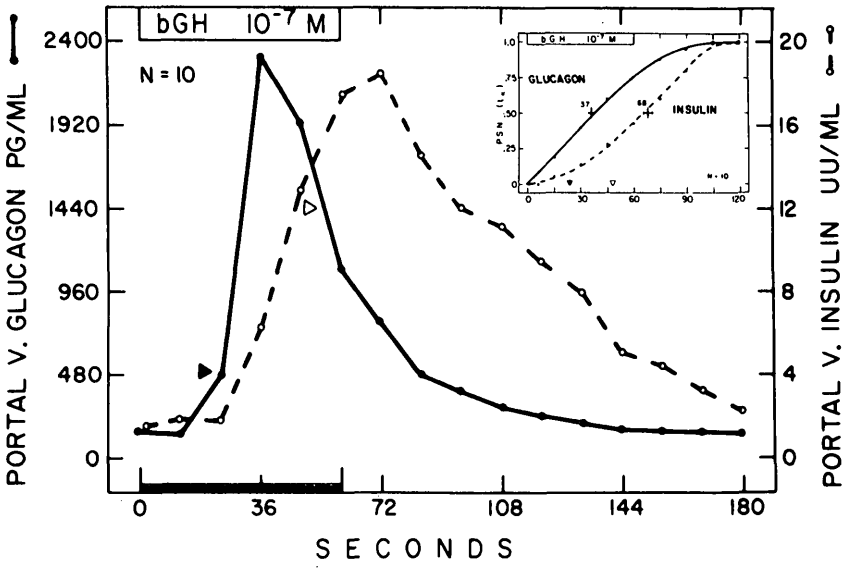

FIGURE 5

See legend to figure 1. In these experiments 10^{-7} M bovine growth hormone is the secretagogue.

sive" concentration of glucagon in the extracellular space plays a role in the initiation of secretion of insulin. On the other hand, the following observations provide evidence that the antecedent release of glucagon is not the principal mediator of insulin release in respone to stimuli common to both hormones: For the eight series of experiments there was no correlation between the magnitudes of glucagon and insulin release. In fact, one of the two lowest mean responses in insulin occurred in association with the highest (PGE_2) and the other with the second lowest (10^{-7} M isoproterenol) mean glucagon response. Secondly, the mean $t_{.5}$ for glucagon varied over a wide range while that for insulin remained fairly constant. Had endogenous glucagon been the mediator of the stimulus for insulin release, the response times for the two hormones should have correlated. Finally, at high concentrations of glucose, in response to some of the secretagogues employed in this study the release of insulin can occur without a preceding release of glucagon (unpublished observations).

In conclusion, our findings indicate that glucagon secretion occurs more rapidly than does insulin secretion in response to a variety of stimuli common to both hormones. The antecedent release of glucagon could have a modifying influence on the release of insulin evoked by a common secretagogue, but the primary signal for the release of insulin appears to be the secretagogue itself.

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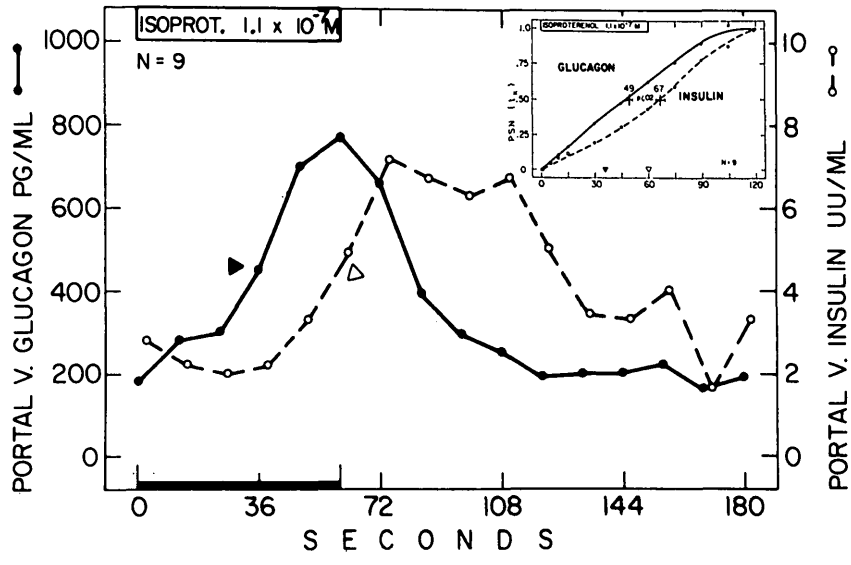

FIGURE 6

See legend to figure 1. In these experiments 1.1 × 10^{-7} M isoproterenol is the secretagogue.

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