# Glucose Generates Coincident Insulin and Somatostatin Pulses and Antisynchronous Glucagon Pulses from Human Pancreatic Islets

Bo Hellman, Albert Salehi, Erik Gylfe, Heléne Dansk, and Eva Grapengiesser

Department of Medical Cell Biology (B.H., E.Gy., H.D., E. Gr.), University of Uppsala, SE-75123 Uppsala, Sweden; and Department of Clinical Science (A.S.), Clinical Research Center, Malmö University Hospital, University of Lund, SE-20502 Malmö, Sweden

The kinetics of insulin, glucagon and somatostatin release was studied in human pancreatic islets. Batches of 10–15 islets were perifused and the hormones measured with RIA in 30-sec fractions. Increase of glucose from 3 to 20 mM resulted in a brief pulse of glucagon coinciding with suppression of basal insulin and somatostatin release. There was a subsequent drop of glucagon release concomitant with the appearance of a pronounced pulse of insulin and a slightly delayed pulse of somatostatin. Continued exposure to 20 mM glucose generated pulsatile release of the three hormones with 7- to 8-min periods accounting for 60–70% of the secreted amounts. Glucose caused pronounced stimulation of average insulin and somatostatin release. However, the nadirs between the glucagon pulses were lower than the secretion at 3 mM glucose, resulting in 18% suppression of average release. The repetitive glucagon pulses were antisynchronous to coincident pulses of insulin and somatostatin. The resulting greater than 20-fold variations of the insulin to glucagon ratio might be essential for minute-to-minute regulation of the hepatic glucose production. **(*Endocrinology* 150: 5334–5340, 2009)**

More than 30 yr ago Goodner *et al.* (1) reported periodic variations of insulin and glucagon in circulating blood of monkeys. Since then, a number of studies have indicated that these hormones and somatostatin oscillate in various species, including man (2–5). The cyclic variations of the hormones reflect pulsatile release triggered by cytoplasmic $Ca^{2+}$ oscillations in the islet cells (6, 7). Within the islets the insulin-producing $\beta$-cells are coordinated both via gap junctions (8) and diffusible messengers (9). Accumulating data suggest that neural activity, with resulting alterations of the membrane potential, locks the $\beta$-cells in different islets into the same oscillatory phase (10–12).

Because the portal vein drains the pancreas, the liver will be the first extrapancreatic target for the islet hormones. The hormone concentrations are reduced by hepatic extraction (13–15). In humans about 80% of circulating insulin is removed by binding to receptors during the first liver passage (16). The resulting attenuation of the pulses in combination with low frequency of sampling may explain why peripheral insulin was initially reported to oscillate with periods of 10–16 min (2–4). Later investigations indicated 5- to 7-min oscillations of insulin in the peripheral blood (17, 18) and the portal vein (14, 19, 20). Perifusion of isolated islets provides an attractive alternative for studying pulsatile secretion of islet hormones. Measurements of insulin in perifusate from human islets indicated pulse periods of 4–10 min (21–25). Up to now there are no reports about pulsatile release of glucagon and somatostatin from isolated human islets.

Insulin and glucagon have opposing effects on glucose production in the liver. It has been suggested that it is the ratio rather than the absolute concentrations of the hormones that is particularly important for the control of liver glucose output (26, 27). Our recent studies with the perfused rat pancreas indicated that the initial pulse of glu-

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cose-stimulated insulin release is preceded by a brief glucagon pulse and that the following pulses of these hormones are 180° out of phase (28). Different opinions have been expressed whether the pulsatile release of insulin and glucagon in man are coupled or independent processes. Besides reports that glucagon oscillates concomitant (4) or in near synchrony (3) with insulin, other studies indicate absence of a relationship (2, 5). A reason for anticipating different relations between the hormone pulses in rodents and man is the cytoarchitecture of the islets. In human islets the $\alpha$-cells are not exclusively located in the periphery but scattered among the $\beta$-cells (29, 30). It has been reported that cytoplasmic $Ca^{2+}$ oscillations are poorly coordinated both among $\alpha$-cells (31) and $\beta$-cells (32) in human islets.

The aim of the present study was to clarify the timing of pulsatile release of insulin, glucagon, and somatostatin from glucose-stimulated human islets. Evidence is provided that a brief glucagon pulse precedes the first pulse of insulin and somatostatin. The subsequent glucagon pulses were antisynchronous to those of insulin resulting in pronounced variations of the insulin/glucagon ratio.

## Materials and Methods

### Islet isolation and culture

The study was approved by the local ethics committees. Human pancreatic islets from six normoglycemic cadaveric donors (five female, one male; aged 33–66 yr) were generously provided by the Nordic Network for Clinical Islet Transplantation. The islets were isolated with semiautomated digestion filtration (33) and purified on a continuous density gradient in a refrigerated cell processor (COBE 2191; COBE Blood Component Technology, Lakewood, CO). After purification the islets were kept for 2–5 d at 37 C in an atmosphere of 5% $CO_2$ in CMRL 1066 culture medium (Mediatech, Herndon, VA) containing 5.6 mM glucose and supplemented with 10 mM nicotinamide, 10 mM HEPES, 0.25 $\mu$g/ml Fungizone, 50 $\mu$g/ml gentamicin, 2 mM glutamine, 10 $\mu$g/ml ciprofloxacin, and 10% heat-inactivated human serum.

### Perifusion of islets

Batches of 10–15 islets (diameter $>$ 200 $\mu$m) were placed in closed 10-$\mu$l chambers of Teflon tubing equipped with a nylon net at the outlet to prevent the islets to escape (34). The perifusate was delivered with a peristaltic pump at a rate of 0.6 ml/min. The experiments were performed with an albumin-containing medium (0.5 or 1 mg/ml BSA) containing (in millimoles per liter) NaCl 125, KCl 4.8, $MgCl_2$ 1.2, $CaCl_2$ 2.6, and HEPES 25 with pH adjusted to 7.4 with NaOH. After 20 min of equilibration the glucose concentration was raised from 3 to 20 mM. The effluent was collected as 30-sec fractions and stored at −20 C.

### Measurements of hormones

Concentrations of insulin, glucagon, and somatostatin were measured in duplicate with RIA. Degradation of glucagon and somatostatin was prevented with aprotinin (80 mg/liter). Glucagon was measured with antibodies (Milab Ltd., Malmö, Sweden) highly selective for pancreatic glucagon. The antiserum for the assay of somatostatin (Euro-Diagnostica AB, Malmö, Sweden) was raised against synthetic cyclic somatostatin-14. The coefficients of intra- and interassay variations were 2.7 and 3.0% for insulin, 3.6 and 6.3% for glucagon, and 3.2 and 9.1% for somatostatin.

### Evaluation of data

Statistically significant hormone pulses were identified with the Cluster Analysis program, a computerized pulse analysis algorithm (35). Each sample was assigned a dose-dependent SD calculated from duplicates. A sliding pooled *t* test was then performed to identify data points within the time series that correspond to statistically significant increases or decreases of the hormone concentrations. Test cluster sizes for the nadir and peak widths were assigned to 2. The minimum *t* statistics was specified to 4.1 for upstrokes and downstrokes, respectively. The settings detected peaks with less than 1% false-positive errors.

The repetitive pulses of each hormone were compared with those of other hormones using median cross correlation analyses. The *r* values were determined in each subject at each of multiple lags. The SD of *r* was estimated by $1/(n-k)^{1/2}$, where n is the number of sample pairs and k is the number of lag units (36).

Results are presented as mean values ± SEM. Differences were statistically evaluated with *t* test.

## Results

### Initial effects of glucose elevation on hormone release

Increase of glucose from 3 to 20 mM generated pulsatile release of insulin, glucagon, and somatostatin from the islets in all of six human donors. A representative experiment is shown in Fig. 1. The initial effect of glucose elevation was a pulse of glucagon with a peak exceeding the release at 3 mM by 122 ± 33% ($P < 0.02$). The rise of glucagon coincided with transient suppression of insulin and somatostatin release with 49 ± 7% ($P < 0.001$) and 68 ± 7% ($P < 0.001$), respectively. Subsequently there was a drop of glucagon secretion below the rate at 3 mM glucose concomitant with an initial pulse of insulin and somatostatin (Figs. 1 and 2). The peak of glucagon preceded the first peak of insulin with 80 ± 13 sec ($P < 0.01$) and somatostatin peaked 50 ± 13 sec ($P < 0.02$) after insulin. The initial insulin pulse exceeded baseline secretion with 1039 ± 178% ($P < 0.01$) and was 75 ± 14% higher ($P < 0.02$) than the mean for the next three pulses. A similar comparison for somatostatin revealed that the initial pulse was 645 ± 131% ($P < 0.01$) above baseline and 34 ± 5% ($P < 0.01$) higher than the following pulses.

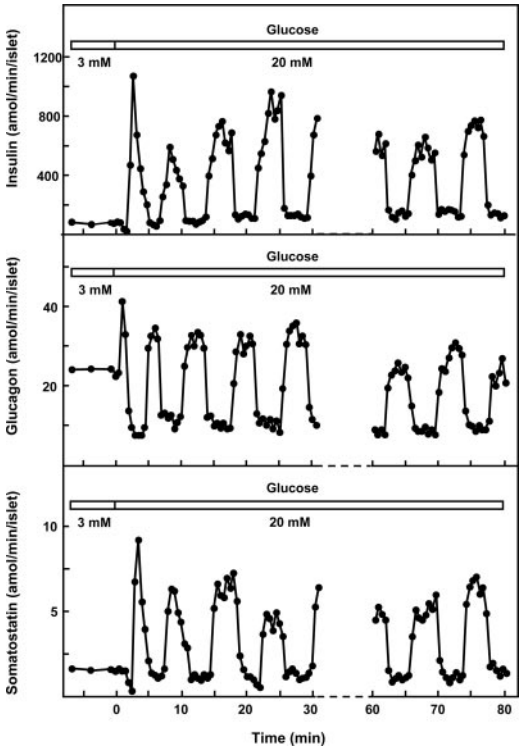


**FIG. 1.** Effects of raising glucose from 3 to 20 mM on the release of insulin, glucagon, and somatostatin from a batch of 15 perifused human islets. The hormones were measured in 30-sec fractions of the perifusate. Sampling was interrupted during 30 min as indicated by the *dashed line*. Prolonged exposure to 20 mM glucose induced pulsatile release with a periodicity of 7–8 min for all three hormones.

### Sustained effects of glucose elevation on hormone release

The initial glucose effect was followed by repetitive pulses of insulin, glucagon, and somatostatin (Fig. 1 and Table 1). Each hormone was released with a periodicity of 7–8 min and the pulsatile component accounted for 60–70% of the amounts secreted. There was no time-dependent potentiation of average hormone release and the shapes of the pulses were maintained during 80 min exposure of the islets to 20 mM glucose. The insulin and somatostatin pulses started from the basal level. However, the glucagon pulses started 70 ± 2% below the release at 3 mM glucose reaching peak values 60 ± 13% above this level. Accordingly, rise of glucose from 3 to 20 mM resulted in 18% suppression of time-average glucagon release ($P < 0.02$).

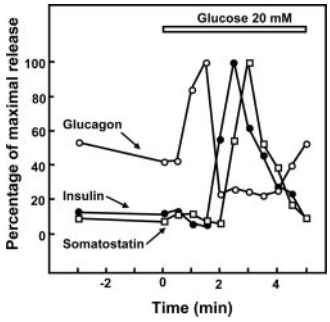


**FIG. 2.** Initial effects on hormone release from human islets after raising glucose from 3 to 20 mM. The hormones were measured in 30-sec fractions from a batch of 15 perifused islets. The release is expressed in percent of maximal release. A brief pulse of glucagon was accompanied by inhibition of insulin and somatostatin release followed by the first peak of insulin and a delayed peak of somatostatin. Representative experiment of six.

**TABLE 1.** Characteristics of sustained pulsatile hormone release from human islets

| | |
|---|---|
| Time-average release (percentage of release at 3 mM glucose) | |
| Insulin | 251 ± 27 |
| Glucagon | 82 ± 5 |
| Somatostatin | 272 ± 45 |
| Pulsatile component (percentage of total release) | |
| Insulin | 70 ± 5 |
| Glucagon | 59 ± 2 |
| Somatostatin | 73 ± 3 |
| Pulse cycle duration (min) | |
| Insulin | 7.6 ± 0.1 |
| Glucagon | 7.8 ± 0.1 |
| Somatostatin | 7.6 ± 0.1 |
| Pulse half-width (min) | |
| Insulin | 3.3 ± 0.1 |
| Glucagon | 3.7 ± 0.1 |
| Somatostatin | 3.8 ± 0.1 |

Mean values ± SEM for islets from six human donors.

Figure 3 presents the time relations between the repetitive hormone pulses. The insulin pulses coincided with those of somatostatin but were antisynchronous to glucagon. Median cross-correlation analyses confirmed that insulin oscillates together with somatostatin but is 180° out of phase compared with glucagon (Fig. 4). Due to the antisynchrony there was greater than 20-fold variations of the insulin/glucagon ratio during the pulse cycles.

## Discussion

The present study shows that a brief glucagon pulse precedes the first phase of glucose-induced release of insulin and somatostatin from human islets. Initial glucose stimulation of glucagon release has been observed also from the perfused rat pancreas (28, 37). It is likely that the early flash of glucagon amplifies the following firsts peaks of insulin and somatostatin. Indeed, the initial pulse of each of the latter hormones was more prominent than the following ones. This finding illustrates the cAMP sensitivity

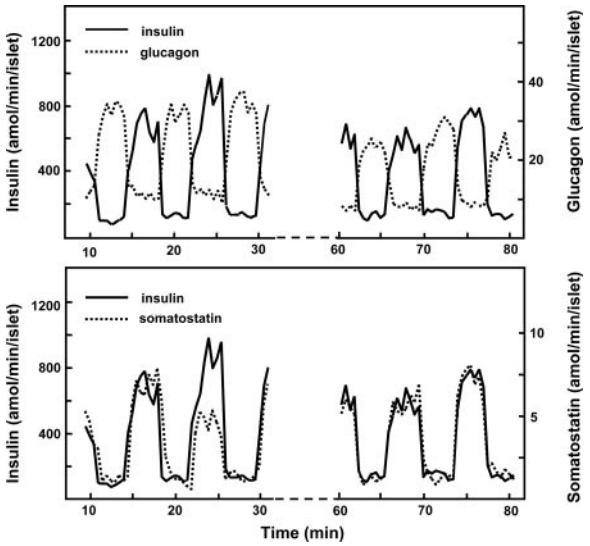


**FIG. 3.** Temporal relationships between repetitive islet hormone pulses. The superimposed traces show that insulin and glucagon pulses are antisynchronous (*upper panel*) and that insulin and somatostatin pulses coincide (*lower panel*). Data from the experiment shown in Fig. 1.

of initial insulin release, evident from early reports that infusion of glucagon, or other means of raising cAMP, restores the first phase of glucose-induced insulin release in human prediabetes (38, 39). We now observed that the repetitive pulses of insulin and somatostatin coincide. Nevertheless, the initial peak of inhibitory somatostatin was significantly delayed compared with the first insulin peak. This delay may contribute to the pronounced initial response of insulin. Indeed, immunoneutralization of somatostatin with monoclonal antibodies has been found to potentiate the glucose-stimulated insulin release from the isolated perfused human pancreas (40).

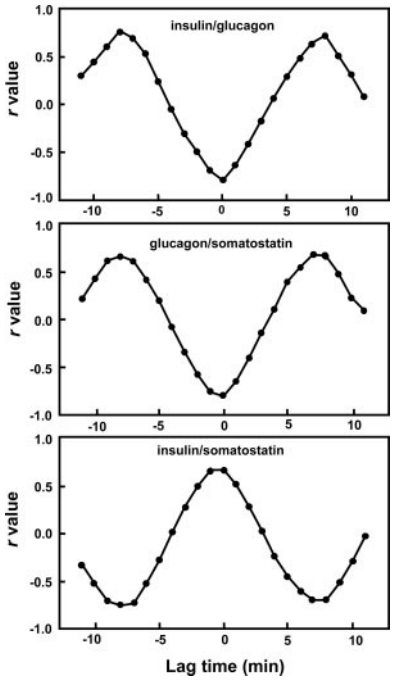


**FIG. 4.** Median cross-correlation coefficients for the repetitive pulses of insulin, glucagon, and somatostatin in islets from six human donors. The results confirm that insulin pulses are locked in the same phase as somatostatin pulses ($P < 0.001$) but antisynchronous to glucagon ($P < 0.001$).

Glucose stimulates both the entry of $Ca^{2+}$ into the $\beta$-cells and the removal of the ion from the cytoplasm by intracellular sequestration and outward transport. The initial effect of raising glucose is lowering of cytoplasmic $Ca^{2+}$ observed both in isolated $\beta$-cells (41) and whole islets (42). Intravenous injections of glucose often result in brief decrease of circulating insulin before the subsequent rise (41). The present study provides further evidence for early lowering of cytoplasmic $Ca^{2+}$ in human islets, indicating transient suppression both of insulin and somatostatin release. Glucose promotes $Ca^{2+}$ sequestration in the endoplasmic reticulum also in glucagon-secreting $\alpha$-cells (43, 44). The reason that the rise of glucose to 20 mM did not result in transient inhibition of glucagon release may be that $Ca^{2+}$ sequestration in $\alpha$-cells is close to maximal already at 3 mM glucose (44).

Several studies have shown that pulsatile insulin release from batches of human islets has a periodicity of 4–10 min (21–25). We now report 7- to 8-min periods for the release of insulin, glucagon, and somatostatin from 10–15 islets, the number required to obtain sufficient amounts of glucagon and somatostatin for analysis. However, perifusion of individual human islets with a similar technique (22) and measurements in the portal vein (14, 19, 20) indicate insulin pulse periods of 4 and 5 min, respectively. The longer pulse periods observed with perifused batches of islets probably reflect lack of coordinating neurons. It is likely that diffusion of synchronizing factors between perifused islets is slower than neural signaling in the intact pancreas.

The major component of hormone release was pulsatile irrespective of whether the rhythmicity induced by 20 mM glucose resulted in increase (insulin and somatostatin) or decrease (glucagon) of the average secretory rate. The pulses of insulin and somatostatin started from the baseline. In contrast, the glucagon pulses had nadirs below the release at 3 mM glucose and peaks above this rate, indicating inhibitory and stimulatory components in the action of glucose. As reported for insulin (23, 24, 45), the alterations of glucagon release are reflected in the pulse amplitude (46). Amplification of the pulses may well explain why overall release of glucagon from batch-incubated mouse islets is gradually diminished in the 7- to 20-mM range and paradoxically stimulated by higher glucose concentrations (47). The modest inhibition of time-average glucagon secretion now observed after raising glu-

cose is consistent with dose-response data for glucose-regulated glucagon secretion from mouse islets (47, 48).

Reports of species differences in the cytoarchitecture of the islets (29, 30) raise the question whether previous observations of islet hormone pulses in rodents are relevant to man. The oscillations of cytoplasmic $Ca^{2+}$ in $\beta$-cells are considered to be poorly synchronized in the human islet (32). There is also a report of a defect coordination of the $Ca^{2+}$ rhythmicity among $\alpha$-cells present in human islets (31). However, we now observed that human islets release both insulin and glucagon as distinct pulses. There are different opinions whether the insulin oscillations in man are coupled to periodic variations of other islet hormones. Some studies report that glucagon varies almost in phase with insulin (3, 4), whereas other did not show any relationship (2, 5). Although perfusion of pancreas have failed to demonstrate temporally related pulses of insulin and somatostatin in rhesus monkeys (49), these hormones are locked in the same oscillatory phase in dogs (50) and rats (51). The present study indicates coordinated secretory activity among human islet cells by demonstrating repetitive glucagon pulses antisynchronous to pulses of insulin and somatostatin.

The effects of insulin are opposite to those of glucagon in almost all respects. In liver, insulin promotes the conversion of glucose to glycogen and inhibits glycogenolysis. In contrast, glucagon stimulates mobilization rather than storage of fuels, especially glucose. Because pancreas is drained by the portal vein, the islet hormones will reach the liver at much higher concentrations than found in the systemic circulation. The pulsatile component of insulin released into the portal vein amounts to 75% (20), which is similar to that now found for each of the hormones in the islet perifusate. There is evidence that pulsatile delivery of insulin (52) and glucagon (52, 53) is more efficient than continuous exposure to regulate the glucose handling of human hepatocytes. Most (54–56), but not all (57), *in vivo* studies confirm that pulsatile delivery amplifies the insulin and glucagon effects on endogenous glucose production in man. A major advantage of intermittent exposure to insulin and glucagon is to prevent down-regulation of surface receptor responses. The liver expresses the highest concentration of insulin receptors of any organ (58). Most of these receptors are internalized within a few minutes, making the turnover to a rapid process (59–61).

The presence of antisynchronous pulsatility with greater than 20-fold variations of the insulin to glucagon ratio should be essential for minute-to-minute regulation of the hepatic glucose production. Apart from alterations of overall release, disorder of the temporal relation between the insulin and glucagon pulses may contribute to the development of the metabolic abnormalities of diabetes.

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Address all correspondence and requests for reprints to: Professor Bo Hellman, Department of Medical Cell Biology, Biomedicum Box 571, University of Uppsala, SE-75123 Uppsala, Sweden. E-mail: bo.hellman@mcb.uu.se.

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