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MINIREVIEW

Release of Newly Synthesized Hormone from the Pancreatic Beta Cell[1] (41833)

R. W. DUDEK

Department of Anatomy, East Carolina University, School of Medicine, Greenville, North Carolina 27834

Work in the pancreatic beta cell and insulin secretion pioneered by Lacy (1, 2) and Orci (3–6) developed the concept of exocytotic release of hormone via secretory granules binding to the cell membrane. This work is well-known and has been adequately reviewed on a number of occasions. However, a number of pulse-labeling studies suggested that newly synthesized insulin (and insulin precursors) was secreted preferentially (7–12). Although little is known about the mechanism by which this occurs, it appears that islets store a portion of their total insulin in a labile compartment that contains newly synthesized hormone and is preferentially secreted in response to glucose. Recently, Halbane (13) and Gold *et al.* (14) concurrently demonstrated a number of conditions where newly synthesized hormone (proinsulin and insulin) is preferentially secreted from the beta cell, thereby providing some insight into the mechanism of preferential secretion. In addition, Gold *et al.* (14) showed that the percentage of radioactive proinsulin in secreted, newly synthesized hormone after 30 and 68 min of stimulation with 20 m*M* glucose was 71.3 and 62.6%, respectively. This means that a large proportion of the secreted, newly synthesized hormone is actually proinsulin and only about 30–40% is insulin. Interestingly, after 162 min of stimulation with 20 m*M* glucose the percentage of proinsulin in the secreted hormone decreased to 15.6%, implying that 85% of the secreted hormone was insulin. In light of this data, the cell biologist faces a dilemma of describing a morphological mechanism that accounts for the preferential release of newly synthesized hormone. A convincing explanation must address three strict requirements: (a) a description of the cellular compartment containing newly synthesized hormone (proinsulin and insulin), (b) a method for its secretion, and (c) a 1:1 quantitative accounting of the frequency of the event (release of newly synthesized hormone) and the suspected morphological correlate (unknown at this time). Such a 1:1 quantitative accounting has only been demonstrated for quantal neurotransmitter release and synaptic vesicle exocytosis in the frog neuromuscular junction (15).

Several possibilities exist for the compartment of newly synthesized hormone that is segregated from the remainder of older, stored insulin. Possible candidates for the compartment are: (i) islets from a particular region of the pancreas, (ii) islets of a particular size, (iii) a subpopulation of beta cell granules, or (iv) a subpopulation of beta cells within an islet. Islets from a particular pancreatic region or size as candidates for the compartment of newly synthesized hormone have been adequately dismissed (14). The possibility that the compartment is a subpopulation of beta cell granules remains viable at this time, but problematic. To conclude that the compartment of newly synthesized hormone is a subpopulation of beta cell granules may be justified based upon the experimental paradigm of the investigator, but this may prove untenable in the long run if the following points can not be adequately explained. First of all, synthesis occurs in the rough endoplasmic reticulum, transport and packaging occurs in the Golgi, and storage in secretory granules. Is not all the hormone that the Golgi packages newly synthesized at the time the Golgi sees it? The answer is yes. Consequently, all the hormone that the Golgi packages is newly synthesized hormone. At present, no evidence exists for two different packaging processes of insulin in the Golgi; one for newly synthesized hormone and another for older, synthesized hormone. If two different packaging processes in the Golgi can not be documented, then the possibility that alteration of the granules occurs within the cytoplasm (post-Golgi) remains

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open. To date, there is no evidence suggesting a cytoplasmic mechanism that segregates two granule populations. Although such a cytoplasmic mechanism may exist, I would suggest that it is difficult to imagine a sophisticated mechanism that could differentiate hormone within a membrane-bound granule based upon when, in time, it was synthesized. Secondly, the idea of two populations of granules, one containing newly synthesized hormone and the other containing older hormone may place an unreasonable capability upon the granule populations. Since 2.8 and 8.3 m*M* glucose preferentially stimulate the release of newly synthesized hormone, one would have to demonstrate that the granules containing newly synthesized hormone recognize the specific glucose level or signal. In contrast, 16.7 m*M* glucose causes the preferential release of older hormone from the beta cell. In this case, the granules containing older hormone would need to recognize the higher level of glucose or signal. In this scenario, we need to propose not simply that the beta cell recognizes glucose and secretes hormone in response to it, but that different beta cell secretory granules can recognize (directly or indirectly) different levels of glucose and respond to them.

The possibility that the compartment of newly synthesized hormone is a subpopulation of beta cells within an islet (perhaps exposed to a different intraislet environment) remains a viable candidate, especially in the light of recent evidence that cell heterogeneity is involved in the preferential release of newly synthesized prolactin from pituitary cells (16). As far as islet physiology if concerned, the question of whether all the beta cells or only a subset of beta cells respond to a particular secretagogue by secretion has never been adequately resolved. The reverse hemolytic plaque assay and the modifications reported by Neill and Frawley (17) may provide a powerful tool to gain insight into this question. However, even if a subpopulation of beta cells within an islet is responsible for the release of newly synthesized hormone, questions concerning the secretory mechanism of this subpopulation would still remain open.

In this review, I would like to raise and explore the possibility that two intracellular routes of secretion may exist within the beta cell, one route for release of newly synthesized hormone and the other route for release of older, stored hormone. In this regard, an alternate mechanism of secretion has been proposed by a number of investigators. Osamura *et al.* (18) reported that secretion of prolactin from cells in the anterior pituitary occurs via two pathways: (a) a long, regulated pathway and (b) a short, accelerated pathway. They suggested that the short, accelerated pathway is prolactin secretion occurring directly from the endoplasmic reticulum without passing through the other cellular compartments. A similar possibility has been suggested by Moriarty and Tobin (19) from their studies on the localization of TSH in pituitaries of thyroidectomized rats. They reported that TSH was primarily localized in the dilated rough endoplasmic reticulum (intracisternal) of hypertrophied TSH cells ("thyroidectomy cells"). They also found some extracisternal staining for TSH in the vicinity of rough endoplasmic reticulum and suggested that this might represent molecules of TSH in the process of secretion via a cytoplasmic route. Although these observations do not fit the classical concept of secretion, other interpretations of the data generated from the experiments of Palade (20) have been raised by Rothman (21). In addition, Farquhar (22) posed several possibilities to explain the increased secretion of TSH from thyroidectomy cells which contain few, if any, secretory granules. She noted that the ER membrane frequently approached the cell membrane in a manner similar to fibroblasts where discharge of glycoprotein occurs directly from the rough endoplasmic reticulum. Along these lines, Gold *et al.* reported that glucose "marks" beta cells thereby diverting newly synthesized hormone for immediate release before transiting the Golgi and packaging into granules (23). In addition, Dr. Boyne and I have reported that in islets stimulated with various levels of glucose followed by quick-freeze fixation and freeze-drying procedures, the endoplasmic reticulum binds orthogonally to the cell membrane of the beta cell and perforates the membrane, thereby forming pores leading to the extracellular space (24, 25). We concluded from this study that beta cells may secrete newly synthesized proinsulin and insulin directly from endoplasmic reticulum synthesis sites via the cortical endoplasmic re-

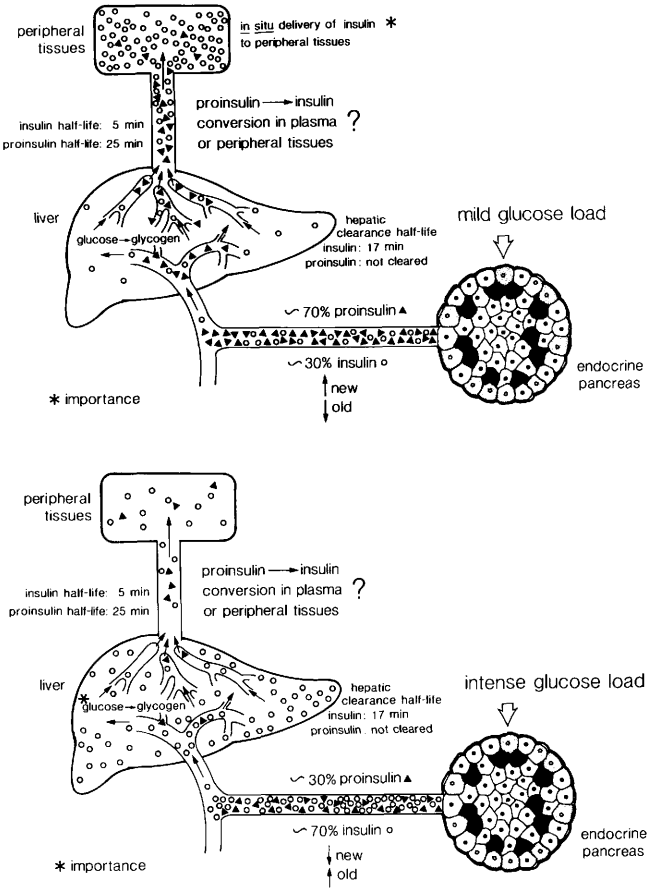
ticulum and pores perforating the cell membrane. This mechanism eliminates the need for two populations of secretory granules and indicates that the compartment where newly synthesized hormone (proinsulin and insulin) resides is the lumen of the endoplasmic reticulum. That newly synthesized insulin may exist with the endoplasmic reticulum is compatible with current concepts of protein synthesis indicating that conversion of proinsulin to insulin *begins* in the endoplasmic reticulum and continues in the secretory granules (26, 27). We also observed granule discharge via exocytosis which would explain the release of older, stored hormone. Although the idea of beta cell secretion occurring directly from the endoplasmic reticulum needs substantiation by further critical tests and resurrects a controversy from long ago, I would like to suggest that this may be a viable possibility to explain the preferential release of newly synthesized hormone.

Since the beta cell secretes newly synthesized hormone and large amounts of proinsulin under certain circumstances and an adequate morphological explanation has been suggested, the question is whether this has any physiological significance. The routine use of 16.7 m*M* glucose as a stimulatory agent stems from the dose–response curve of insulin release indicating that at 16.7 m*M* glucose the insulin response is maximal and plateaus (28). However, the *in vivo* human situation indicates that the beta cell generally sees a range of glucose from 2.8 to 8.3 m*M*. At this range of glucose, newly synthesized hormone is preferentially secreted from rat islets. Therefore, if the data in the rat can be extrapolated to the human, the secretion of newly synthesized hormone with large amounts of proinsulin may be a common occurrence and one would assume has an important biological function in the human. In this regard, human portal blood values of proinsulin have been reported to range from a fasting level of 0.31 ± 0.11 to 2.12 ± 0.72 ng/ml during a glucose infusion of normal healthy subjects (29). We know the following about the half-life of insulin and proinsulin: (i) in an isolated perfused liver system, the clearance half-life for insulin is 17 min, whereas proinsulin is not cleared (30); (ii) the immunological half-life for insulin is 6–8 min and for proinsulin it is 18–20 min (31); (iii) the metabolic clearance half-life for insulin is 5 min, and for proinsulin it is 25 min (32); (iv) the half-disappearance time for insulin is 3–4 min, whereas for proinsulin it is 18–25 min (33). In all the above cases, the clearance half-life for proinsulin is significantly increased. Since the beta cell secretes newly synthesized hormone with significant amounts of proinsulin under certain circumstances and the proinsulin half-life is significantly elevated, proinsulin exists in the peripheral circulation. Human peripheral blood proinsulin values range from a fasting level of 0.19 ± 0.08 to 0.72 ± 0.04 ng/ml during a glucose infusion of normal, healthy subjects (29). In addition, other investigators have reported values of fasting peripheral proinsulin in normal healthy subjects of 0.16 ± 0.2 ng/ml (34) and a range of 0.05–0.4 ng/ml (35). This begs the question of whether proinsulin has any functional importance in the peripheral circulation. The evidence for biological activity of proinsulin has been reviewed by Rubenstein *et al.* (36) which indicates the proinsulin does indeed effect various metabolic parameters in fat cells and hemidiaphragm. More recently, Tompkins *et al.* (37) studied the mechanism of action of insulin and insulin analogues, including proinsulin, on hepatic and peripheral glucose turnover (rate of appearance and rate of disappearance) in dogs. They indicated that when comparisons between insulin and proinsulin are made at low levels of glucose, proinsulin behaves in a manner very similar to insulin. The problem concerning the biological activity of proinsulin centers around its possible conversion to insulin *in situ* at peripheral tissues. Although the available evidence is indirect and highly variable (36), Shaw and Chance (38) studied the *in vitro* effects of proinsulin on adipose tissue and diaphragm muscle. They concluded that proinsulin enhances the glucose metabolism of both tissues by being converted through enzymes present in these tissues (presumably at the cell membrane) to a molecule which is similar to or identical with insulin. Consequently, this study suggests that *in situ* conversion of proinsulin to insulin at peripheral tissues may occur. In addition, Rubenstein *et al.* (39) excluded the possibility of a large amount of conversion of proinsulin to insulin in the peripheral circulation, but their studies did not eliminate the possibility

of *in situ* conversion at the peripheral tissue sites. I would like to emphasize that the *in situ* conversion of proinsulin to insulin is not the only available explanation for the biological activity of proinsulin. First of all, it is reasonable that proinsulin may have some affinity for the insulin receptor in the same way that proinsulin cross reacts with insulin antibodies. Secondly, proinsulin is structurally similar to a class of polypeptides called insulin-like growth factors (IGFs) or somatomedins (40). In addition to the insulin receptor (41), it is now well established that there are two distinct receptors for IGFs, Type 1 and Type 2 (42). The Type 1 receptor consists of three disulfide linked forms structurally similar to the insulin receptor and exhibits relative affinities for ligands in the following order: IGF-1 > IGF-2 > insulin. The Type 2 receptor consists of one single labeled species with the following relative affinities for ligands: IGF-2 > IGF-1, and no significant for insulin (43). With the growing body of evidence indicating a large number of heterogeneous receptor structures and the structural similarity of proinsulin to IGFs, it may be possible that proinsulin could exert its biological action by binding to one of the IGF receptors.

A theory based upon the presently available data as indicated in the above discussion is shown in Figs. 1 and 2. Figure 1 indicates the islet response to a mild glucose load in the range of 2.8–8.3 m*M* glucose. Under these conditions the beta cell secretes predominantly newly synthesized hormone, but the exact percentages of proinsulin and insulin released are not known. Since this data is not available, a critical assumption must be made that the percentage of proinsulin released under this condition is high. The values shown in Fig. 1 (70 and 30%) are taken from the work of Gold *et al.* (14) for the release of hormone during the *early* time point after stimulation with 20 m*M* glucose, namely, 30 min. All the secreted proinsulin passes through the liver unaltered into the peripheral circulation. Proinsulin is then delivered to the peripheral tissues where *in situ* conversion of proinsulin to insulin or receptor binding may occur to enhance glucose utilization. Consequently, secretion of proinsulin under certain conditions may be an interesting biological mechanism that allows passage of potentially active hormone through the liver without clearance for delivery to the peripheral tissues. It would seem that much more work is needed to document beta cell function during physiological levels of glucose (2.8–8.3 m*M*). Figure 2 indicates the islet response to intense stimulation which occurs with exposure to 16.7 m*M* glucose for a long duration as, for example, 162 min as shown by Gold *et al.* (14). This situation may occur *in vivo* immediately after a meal where the local concentration of glucose at the beta cell is high. In this case, the secreted hormone is older stored hormone containing approximately 70% insulin and 30% proinsulin (in this case, the exact percentages of proinsulin and insulin are known). Insulin delivered directly to the liver via the portal circulation activates the enzymes necessary for conversion of glucose to glycogen. This rapidly reduces the blood glucose level to a normal range. When the blood glucose level is lowered, the percentage of proinsulin secretion increases for delivery to the peripheral tissues.

Does this mechanism of hormonal release from the pancreatic beta cell offer any new clinical insight? If the mechanism as indicated in Figs. 1 and 2 adequately represents the human *in vivo* situation, then one wonders about some aspects of the insulin regime that insulin-dependent diabetics presently received. Between meals, that is mild glucose load, delivery of proinsulin into the portal circulation with subsequent conversion of proinsulin to "active" insulin by enzymes occurring on the peripheral tissues or binding of proinsulin to receptor sites would seem important. However, insulin-dependent diabetics are not treated with proinsulin. Is this why secondary complications of diabetes persist despite the regulation of blood glucose levels by subcutaneous injections of insulin? Perhaps, the nonphysiological insulin regime diabetics presently use keeps peripheral tissues in a insulinopenic state, thereby causing secondary complications. With recent and forthcoming developments in implantable insulin pumps and glucose sensors, perhaps both the form of hormone delivered (proinsulin or insulin) and timing of hormone delivery (pre- or postprandial) should be taken into account. In addition, one of the major justifications for islet cell transplantation as a potential cure of insulin-dependent diabetes is that the trans-



FIGS. 1 AND 2. These figures are diagrams of the events that may take place upon mild stimulation (Fig. 1) or intense stimulation (Fig. 2) of an islet. In Fig. 1, a low glucose load mildly stimulates the islet to secrete preferentially newly synthesized hormone containing a large percentage of proinsulin (we assume the percentage of proinsulin released is 70% as indicated in the text). Since proinsulin is not cleared by the liver it passes into the peripheral circulation unaltered for delivery to the insulin-dependent peripheral tissues. The critical step at this point is conversion of proinsulin into insulin. Although one could postulate that this conversion may occur in the plasma, evidence indicates that the conversion is *in situ* at the cell membrane of the peripheral tissue. During mild stimulation, the relative importance of events (as indicated by the asterisk) is to maintain the peripheral tissues in a normal functioning state by providing enough insulin for glucose metabolism to occur. The necessary amounts of insulin are provided by the secretion of proinsulin from the islet which is not cleared by the liver and which has a 25-min half-life for *in situ* conversion to insulin. Upon intense stimulation with a high glucose load as indicated in Fig. 2, the islet secretes predominantly older, stored hormone containing 70% insulin and 30% proinsulin. The secreted insulin is rapidly utilized by the liver to convert glucose into glycogen, thereby reducing blood glucose levels. The 30% of proinsulin released is delivered to the peripheral tissues for *in situ* conversion to insulin. Upon intense stimulation, the relative importance of events is the glucose to glycogen conversion in the liver (indicated by the asterisk) to reduce blood glucose levels.

plant exquisitely regulates the circulating blood glucose levels. Several laboratories have shown that normalization of glucose levels by transplantation of whole pancreas or islets minimizes or prevents the formation of diabetic lesions in the eye, kidney, and nerve (44–46). However, when blood glucose levels are strictly controlled in diabetic patients (47–49) by insulin infusion pumps or a strict regimen of insulin injections, the frequency of

secondary complications does not decrease. This indicates that normalization of blood glucose levels may not be the only factor in ameliorating the secondary complications of diabetes. Interpreting these results with the latest information concerning insulin secretion, one could suggest that the observed beneficial effects of islet transplantation on the secondary complications of diabetes occur because the islet transplants are capable of secreting proinsulin for delivery to the peripheral tissues. Indeed, since the islet transplants more than likely secrete hormone in a physiological fashion as reported by Halban (13) and Gold *et al.* (14) the amounts of proinsulin and insulin secreted under various conditions (mild and intense stimulation), not only provide insulin for the liver to convert glucose to glycogen for the normalization of blood glucose levels, but also provide proinsulin for delivery to the peripheral tissues.

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