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Systems of pancreatic beta-cells and glucose regulation

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1. ABSTRACT

We present mathematical models for systems of beta-cells in pancreatic islets. The first topic begins with the effects of noise and coupling strength on bursting action potentials of beta-cells. From the discussion, the regular bursts are produced by a proper amount of noise and coupling strength. Furthermore, the bursting duration and period depend on the cluster size of beta-cells. We also observe the real size of islets mostly consisting of beta-cells and obtain the size distribution of islets. In addition, we derive either *log-normal* or *Weibull* distributions of the islet sizes based on recent observation on islet growth. Islets of Langerhans are composed of several endocrine cells which interact with each other. Considering asymmetric and inhibitory interactions of these endocrine cells, we introduce a simple islet model consisting of alpha-, beta-, and delta-cells. Finally, a whole feedback model for glucose regulation is constructed, connecting the microscopic bursting mechanism and the macroscopic blood glucose regulation of the body. We analyzed these models via numerical simulations based on *in vivo* and *in vitro* experimental data.

2. INTRODUCTION

Islets of Langerhans are embedded in the exocrine acinar cells of a pancreas, and composed of several endocrine cells: alpha-cells (secreting glucagon), beta-cells (insulin), delta-cells (somatostatin), pp-cells (pancreatic polypeptide), and epsilon-cells (ghrelin). These endocrine cells communicate with each other through autocrine and paracrine signaling, in particular, beta-cells are electrically connected to each other. Since German pathological anatomist Paul Langerhans found the pancreatic islets in 1896, they have attracted the attention of both clinical and theoretical investigators. These cells play a central role in maintaining the appropriate level of blood glucose within a narrow range, 3.9~6.1 mmol/l (1) and their dysfunction is closely related to diabetes mellitus. Among these, the beta-cell is of particular interest because it is the only cell in the body that produces and secretes insulin which is crucial for maintenance of glucose homeostasis.

Electrical activity of pancreatic islets has been studied using patch clamp techniques and investigated

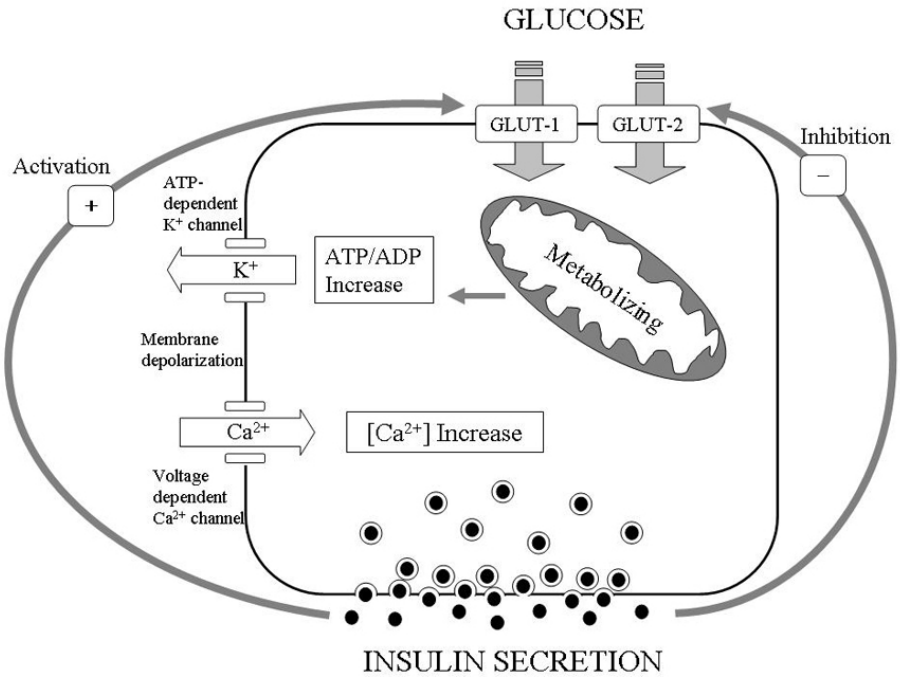

Figure 1. Mechanism of GIIS. Activation and inhibition of glucose uptake via GLUT-1 (glucose transpoter-1) and GLUT-2 (glucose transpoter-2) by secreted insulin are represented by (+) and (-) arrows, respectively. Thick arrows describe the physical transport of materials (glucose and ions).

through the Hodgkin-Huxley-type model (2). A variety of secretory cells display oscillations in the membrane potential which have been correlated with the exocytosis. Particularly, GIIS (Glucose Induced Insulin Secretion) involves a link between energy metabolism and membrane conductance. Pancreatic beta-cells function as glucose sensors to regulate blood glucose homeostasis (see Figure 1); glucose enters into the cytosol and is catabolized through glycolysis (3) to generate ATP (adenosine triphosphate) in the mitochondria. This raises the ratio of ATP (adenosine triphosphate) to ADP (adenosine diphosphate), which promotes closure of ATP-sensitive K^+ channels. Subsequently, this generates membrane depolarization, urging voltage-dependent calcium channels to open. The following increase in free cytosolic Ca^{2+} concentration is well correlated with insulin secretion. Moreover, it is proposed that noise induces heterogeneity on electrical properties of beta-cells, which in turn assists the beta-cells to play a positive and constructive role in bursting when they are coupled (2). In particular, we observe that the cluster larger than a minimal size shows appropriate action potential burst. Furthermore, the bursting duration have a tendency to increase with the size and saturate around some critical size (about 100 cells), beyond which the bursting property appears not to change; it has been reported that the physiological property of beta-cells depends on the size of the cell-cluster (4).

Therefore, in the fourth section, we observe actual sizes of islets mostly consisting of beta-cells and obtain their size distribution. There are a number of experimental studies for the islet size distribution, specifically, in rats (5,6), mice (7-9), pigs (10), and humans (11-14). In mammals, it is of interest that the total number of islets increases with the size of the species and physiological demand, but the islet size remains in the limited range. It was, therefore, suspected that there exists the optimal size of an islet as a functional unit (15). However, there still lacks theoretical elucidation about the optimal size and morphology of islets as well as their developmental changes. We investigate their physiological implications and proposed a theoretical model for islet growth, based on the experimental observations. Obtained results from the model are *log-normal* and *Weibull* distributions depending on how cells in an islet proliferate. Our study of how islets grow to satisfy the demand of growing body shows that each islet in general grows proportionally to the number of cells in the islets, i.e., each islet may grow faithfully to the external demand, independently of others. Our growth model might thus be rather general, applicable to a variety of growth processes in other tissues with constant proliferation rates.

In the fifth section, we move our focus to the role of other endocrine cells, alpha- and delta-cells. Three types of islets cells, alpha-, beta-, and delta-cells, have intercellular interactions, which seem to have beneficial effects in blood glucose regulation. Insulin secreted from beta-cells tends to inhibit alpha-cells, while glucagon secreted from alpha-cells activates beta-cells (16,17) and delta-cells. It is easily comprehended that beta-cells inhibit alpha-cells which play the opposite role. However, it appears rather paradoxical that alpha-cells activate beta-cells. Interestingly, it has been reported recently that there

are similar asymmetric interactions via neurotransmitters. GABA (gamma-aminobutyric acid) released from beta-cell inhibits alpha-cell (18-20), whereas glutamate released from alpha-cells activates beta-cell (21). In addition to the asymmetric interactions between alpha- and beta-cells, it has also been reported that the somatostatin secreted from delta-cells (5~10% of the islet volume) inhibits both alpha- and beta-cells (22-24). Even though these results give qualitative information on the sign of each interaction (positive or negative), they are not sufficient to be used as a reference point of simulations and modeling. There are a number of limitations inherent in current experimental techniques measuring the activities of an intact islet comprehensively and accurately. Under the present circumstances, therefore, it is worthy of theoretical approach unifying only the known qualitative results. In this review, we introduce the simple model only considering the signs discovered from the experiment data to elucidate the roles of asymmetric interactions between alpha- and beta-cells, and the role of delta-cells.

Finally, in the last section, we describe a mathematical whole feedback model of glucose regulation bridging the bursting mechanism and the blood glucose regulation. Insulin secretion is oscillatory with a variety of time scales: fast oscillations with periods of several second, slow oscillations with periods of several minutes and hours, and slower ultradian rhythms. As we will discuss on sections 4 and 5, isolated beta-cells actually show continuous spikes or fast and irregular bursts (24-27). Meanwhile, coupled heterogeneous beta-cells exhibit more pronounced burst (28-31), each of which produces continuous spikes or bursts depending upon such cell parameters as the size, channel density, etc. Extensive numerical simulations lead to a variety of oscillatory behavior of the bursting electrical activity, calcium concentration, and insulin secretion, which are in good agreement with experimental data. However, the secreted insulin at a high glucose level does not continuously reduce the blood glucose concentration because there is a negative feedback. It has been assumed that the glucose transport through GLUT-2 (glucose transporter-2) is inhibited by insulin. Due to the complexity of this system, mathematical modeling and numerical simulations on systems of beta-cells and glucose regulation are desirable for obtaining an insight of the current qualitative knowledge with the development of molecular biology. It may also provide a tool to make meaningful quantitative predictions which contribute to experimental and further clinical studies.

3. BURSTING ACTION POTENTIAL

As the Hodgkin-Huxley model (32) describes the electrical activity on the cell with ion channels, a few mathematical models for beta-cells, based on electrophysiological data (33-35) of ion channels in beta-cells, have been proposed. Although there are simple models using two-dimensional maps (36-38), we consider the Sherman model, which allows direct physical interpretations (2,39).

The model is described by the current balance condition between the membrane potential and ionic current, which includes the fast voltage-dependent Ca^{2+} current, delayed-rectifier K^+ current, ATP-sensitive K^+ current, and very slow inhibitory potassium current. The voltage dependent Ca^{2+} current and the voltage dependent K^+ current are responsible for generating action potentials. The ATP-sensitive K^+ current provides the background current with voltage independent conductance; this determines the plateau fraction, i.e., the ratio of the active phase duration to the burst period. For example, as the conductance of the K (ATP) channel decreases under high glucose concentration, there appears only an active phase without silent phases. The slow inhibitory potassium current is a phenomenological one representing slow dynamics in the bursting action potential. This model thus assumes that single beta-cell originally contain the slow dynamics, which works just under the appropriate condition. Also within an islet, beta-cells are known to be electrically coupled, and bursting is approximately synchronous. Thus the model includes the electrical coupling via gap junctions between neighboring cells in an islet.

We probe the role of noise and coupling in inducing bursting action potentials in pancreatic beta-cells, and found that they effectively call into action the inherent slow dynamics in individual cells. As candidates for the stimulus of noise, both the (additive) random noise arising from fluctuating currents and the (multiplicative) voltage-dependent noise from the channel gating stochasticity are considered. As a result of the noise effects, fast bursts and irregular spikes or bursts in single beta-cells are observed. In particular, the emergence of regular bursts assisted by an appropriate amount of noise is reminiscent of coherence resonance. In view of physiology, the consecutive firing induced by fluctuations gives rise to relative depolarization for a while, which is followed by the activation of the slow potassium channel lasting until the slow variable reaches the upper threshold. At this time the slow K^+ channel opens fully, and the out flux of cytosolic potassium ions gets very large, thus hindering depolarization. Accordingly, the membrane potential is compelled to stay in the silent phase, and the slow K^+ channel in turn starts to be inactivated. In consequence, the membrane can become depolarized as the out flux of K^+ ions reduces. Finally, firing occurs again, and consecutive firing also happens by the help of appropriate stimulation. Further, it is also found that stronger gating fluctuations from fewer channels give rise to modules of more rapid spikes, compared with the case of more channels. Similar results can be obtained with fluctuations in the Ca^{2+} channels and in the delayed-rectifier K^+ channels although they act somewhat differently from the fluctuations in the ATP-blockable K^+ channels.

In addition to the above electrical effects of single beta-cells with noise, we consider two cells coupled with each other via a gap junction. In consequence of the coupling effects, revealed is the optimal coupling strength for longer bursting periods. The coupling term, proportional to the potential difference between two cells, operates in a similar manner to the voltage-dependent noise: It increases with the potential difference and thus

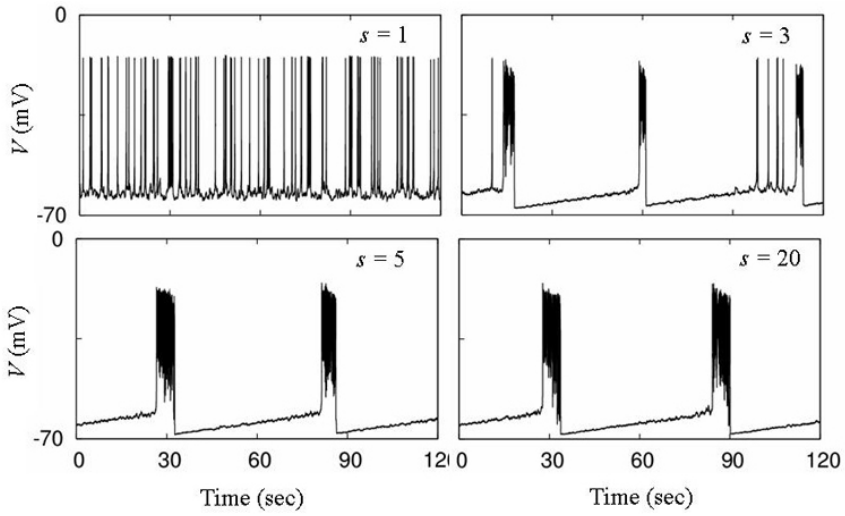

Figure 2. Bursting action potentials depending on the cluster size of beta-cells. The cluster is assumed to form a cube of size s, i.e., an $s\times s\times s$ cubic lattice, consisting of s^3 beta-cells.

becomes large for the cells in active phases, stimulating the cells like noise. On the other hand, it is small for perfectly synchronized cells in silent phases. Robust bursts emerge as a consequence of the competition between heterogeneity and coupling (40). Therefore, heterogeneity is in general important for bursting in coupled cells, no matter whether it is cell-to-cell heterogeneity or induced by noise.

In the analysis, the heterogeneity has been found to play an important role in inducing strong fluctuations during active phases, which may cause robust bursts. Namely, bursting in general results from the interplay of coupling and heterogeneity. This allows us to interpret the fact that a large cell cluster (up to the critical size) shows more regular bursts (2,41): Assuming cubic islets, we have considered beta-cells arranged into an s^3 cube, under free boundary conditions. Adopting physiological gap junction conductance, we find that the bursting period and duration first increases with the size s but tends to saturate beyond s = 5 (Figure 2). Such saturation behavior may be explained as follow: Via the coupling through gap junctions, the number of nearest neighbors in the three-dimensional space is limited, e.g., to six or so; this suggests that the cluster above some critical size can get no more advantage of the heterogeneity from neighboring cells through given coupling strength.

4. SIZE DISTRIBUTION OF MOUSE LANGERHANS ISLETS

The regulation of pancreatic beta-cell mass is a critical issue for understanding diabetes, so that the development and growth of beta-cells have been studied experimentally and theoretically (42). The theoretical approach makes it possible to estimate the size distribution of islets, which are well described with the following assumptions: Neogenesis of new islets are negligible after some development stage, and every cell in a pancreas proliferates with the same rate, regardless of whether the cell belongs to a small islet or to a large islet. Based on these observations, we have proposed a theoretical model for the growth of islets. Depending on whether cells in each islet proliferate coherently or independently, the model results in different distribution patterns for the islet size.

In the case of coherent cell proliferation, all cells together replicate or not (see Figure 3 (a)), and if a newborn islet, consisting of one cell, proliferates k (= 0, 1, 2 …) times, the islet contains 2^k cells. Denoting P_k (t) to be the number of islets at the kth proliferation stage, i.e., the number of islets in which cells have proliferated k times, at time step t, the distribution P (k) for the proliferation stage k approximately evolves to a normal (Gaussian) distribution with the mean μ' and the variance σ', if the time t is sufficiently large compared with the initial time t_0 (43). Every preexisting islet grows with the same cell proliferation rate λ and there are no more newborn islets, both of which are based on the experimental results. This process is exactly the same as the `random walk' with given constant transition probability; this is the reason why P (k) ends up in a Gaussian distribution function. From this distribution P (k) for proliferation stage k, it is straightforward to obtain the distribution P (s) for size s.

Defining the effective (dimensionless) islet size s to be the cube root of the number n of cells in the kth proliferating islet, i.e., $s \equiv n^{1/3}$, the normal distribution for the number of islets at given proliferation stage corresponds to a *log-normal* distribution of the islet size. With the relation $s=2^{k/3}$ between the islet size s and the proliferation stage k, it is straightforward to obtain the *log-normal* distribution

$$P(s)=\frac{1}{\sqrt{2\pi}\sigma s}\exp\left[-\frac{(\ln s-\mu)^2}{\sigma^2}\right], \quad (1)$$

where $\mu \equiv \mu'$ (ln 2)/3 and $\sigma \equiv \sigma'$ (ln 2)/3.

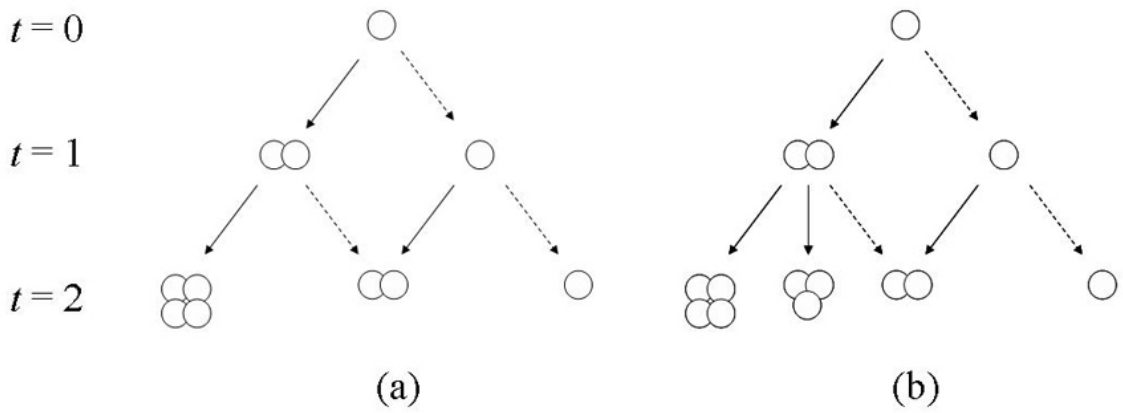

Figure 3. Cell proliferation in an islet. At each replication time *t*, every cell replicates (a) coherently or (b) independently of each other. The growth process is illustrated until the second replications. Solid arrows represent that one or more cells replicate, and dashed arrows represent that all cells do not replicate.

In the case of independent cell proliferation, on the other hand, every cell replicates independently of each other (see Figure 3 (b)), and the possible islet sizes increase geometrically: At replication time t, there are 2^t possible islet sizes. Therefore, it is formidable to estimate the size distribution after many replications (43). To circumvent this difficulty, we resort to Monte Carlo (MC) simulations, which make it possible to estimate the distribution. Namely, we begin with an islet consisting of a progenitor single cell, and allow every cell in the islet to replicate independently, according to the proliferation probability λ at each MC step. Performing such simulations on sufficiently many samples, we can obtain the distribution $P(n)$, where n is the number of cells in an islet, at each replication time. Noting the relation $n = s^3$ between the islet size s and the number n of cells, we obtain the size distribution $P(s)$ from the distribution $P(n)$:

$$P(s) = \frac{\gamma}{\eta}\left(\frac{s}{\eta}\right)^{\gamma-1} \exp\left[-\left(\frac{s}{\eta}\right)^{\gamma}\right] \quad (2)$$

which is the *Weibull* distribution with the shape parameter γ and the scale parameter η.

Observing the image of islets that DTZ (Dithizone) stains only beta-cells, we have measured both the equatorial radius a and the polar radius b of a total of 2,222 islets from eight mice of six weeks old and a total of 2,337 from eight mice of five months old. Assuming that each islet has the shape of a prolate spheroid, the effective islet size $s \equiv n^{1/3} = a^{2/3}b^{1/3}r^{-1}$ corresponds to the size of the core consisting of beta-cells. The resulting (normalized) size distributions of islets are plotted in Figure 4. Note that the size $s = 1$ corresponds roughly to the diameter of a single cell. It is remarkable that in both distributions for the two age groups there arise peaks at s ≈ 5, which appears to give the most optimal size. This is very interesting and indicative, in view of our simulation results that beta-cell clusters in general generate longer bursts than single cells but the duration of bursts tend to saturate above the size s ≈ 5 (44) (see Figure 2).

The size distribution, skewed to smaller size, fits well to the *log-normal* function or the *Weibull* function derived theoretically. We first consider the *log-normal* function in Eq. (1). The least-square fit of the experimental data yields values of the mean and variance $(\mu, \sigma) = (1.89 \pm 0.04, 0.68 \pm 0.05)$ for six-week-old mice and $(2.10 \pm 0.04, 0.64 \pm 0.05)$ for five-month-old mice. The resulting distributions are represented by the dotted lines in Figures 4 (a) and (b). We then fit the experimental data to the *Weibull* function in Eq. (2), to obtain, from the least-square fit, the shape parameter and the scale parameter $(\gamma, \eta) = (1.64 \pm 0.07, 8.9 \pm 0.3)$ for six-week-old mice and $(1.70 \pm 0.08, 10.2 \pm 0.4)$ for five-month-old mice, and plot the resulting distributions with the solid lines in Figures 4 (a) and (b).

Comparing the two fitting function, in both cases of six-week and five-month old mice, the mean square deviation of the *log-normal* distribution is smaller than that of the *Weibull* distribution. It thus appears that the *log-normal* distribution provides a better fit than the *Weibull* distribution. However, it should be noted that in the regime of large islets the experimental data fit much better to the *Weibull* distribution. Furthermore, such statistical characteristics of the experimental data as the mean islet size $\int sP(s)ds$ and the mean cell number in an islet $\int s^3P(s)ds$ are more consistent with the results of the *Weibull* function. As for the mean-square deviations and fitting around the optimal size, the *log-normal* distribution appears better, whereas the mean values and large-size data are more consistent with the *Weibull* distribution.

As for the resulting increase of the islet size, we examine the lower and upper limits of the islet size. Figure 2 shows bursting action potentials above the size $s = 3$, in contrast with single cells generating spiking action potentials, which means that coupled beta-cells secret insulin more effectively than single beta-cell do. The bursting duration first increases with the size s and tends to

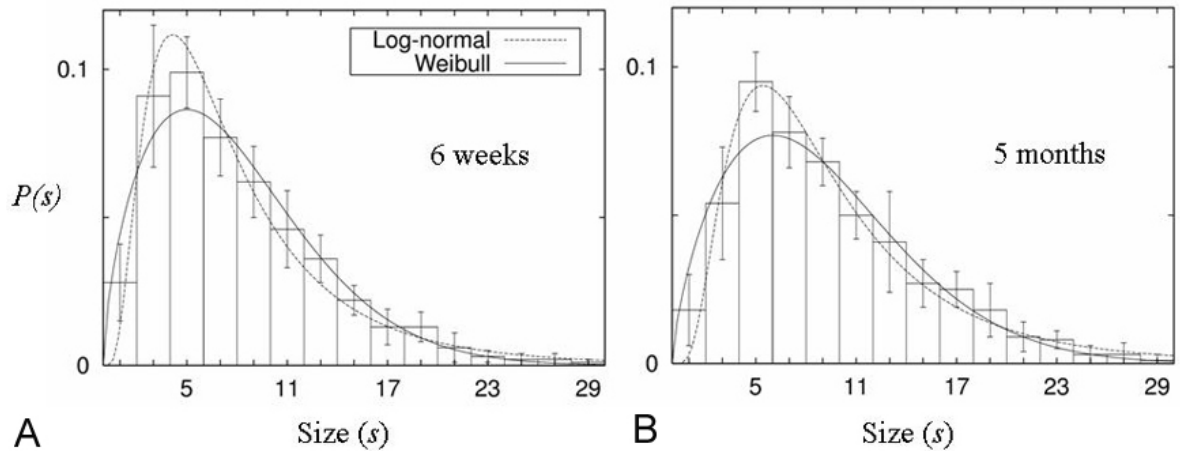

Figure 4. Islet size distribution $P(s)$ obtained from eight mice of (a) six weeks old and (b) five month old. Dotted lines represent the *log-normal* distribution with (μ, σ) = (a) (1.89, 0.68) and (b) (2.10, 0.64). Solid lines represent the two-parameter *Weibull* distribution with (γ, η) = (a) (1.64, 8.89) and (b) (1.70, 10.15).

saturate around s = 5, beyond which the bursting property appears to remain unchanged. As for the upper limit of the islet size, Figure 4 shows that there are no islets above s = 30. One possible explanation of the upper limit is that the relative populations of cells of different types determine the paracrine interactions between them. Since non-beta-cells exist mostly in the mantle of islets (45), it is very difficult to maintain the relative populations as the area-to-volume ratio keeps decreasing with the increase of the islet size; the elongate shape of a large islet helps to circumvent this difficulty. Beyond the upper limit of the islet size, the relative population of beta-cells should become too high, concerning the local paracrine effects in an islet. This may give constraint on the assumption of random cell proliferation in too large islets. The effects of intercellular interactions will be discussed more in the following section.

5. BENEFICIAL EFFECTS OF INTERCELLULAR INTERACTIONS IN BLOOD GLUCOSE REGULATION

The Langerhans islet is a precise system which controls the glucose level with the help of mainly three types of endocrine cells: alpha-, beta-, and delta-cells. As a first approximation, alpha- and beta-cells are sufficient for glucose control because alpha-cells tend to increase the glucose level whereas beta-cells decrease the level. However, it should be noted that endocrine cells in the islet interact with each other rather than act independently. Moreover, there are some phenomena such as hyperglucagonemia which can be explained with cellular interactions (46). Among the interactions, it has been recently reported that the chemical interactions between neighboring cells through hormones (16,23,25,47-50) and neurotransmitters (18-21,51,52), termed "paracrine interactions", affect glucose regulation. Those complex interactions between different coexisting cell-types working in different conditions can be analyzed by means of a mathematical model (53).

The interactions between alpha- and beta-cells are asymmetric: While glucagon secreted from alpha-cells enhances insulin secretion of beta-cells (22,50), insulin inhibits glucagon secretion (16,22,47-49). The former positive interaction to the counterpart cells may seem strange, but it eventually contributes to the construction of a negative feedback loop for both cells. At low glucose levels, alpha-cells secrete glucagon, which enhances insulin secretion. In turn, insulin inhibits glucagon secretion of alpha-cells. These mutual interactions constitute a negative feedback loop for alpha-cells. Therefore, their interactions as a whole tend to suppress glucagon secretion from alpha-cells. Similar negative feedback operates when beta-cells are activated by high glucose concentration. In general, a negative feedback gives system stability because it attenuates the over-action of the system such as overshoot or undershoot. The negative feedbacks in an islet system contribute to the stable recovery to the normal glucose level, when the system is externally perturbed by stimuli such as a glucose dose. If alpha- and beta-cells would inhibit each other, how should the result change? As suggested by Saunders *et al.* (54), the bi-directionally inhibitory interactions seem to be optimal in view of that alpha- and beta-cells play opposite roles in glucose regulation. Remarkably, however, such symmetric interactions can result in dynamically unstable responses. If this were the case, glucagon secreted by alpha-cells at low glucose levels would suppress beta-cells from secreting insulin. As the secretion of insulin would reduce, so would the inhibitory effects of insulin on the glucagon secretion. It should thus follow that glucagon secretion enhances, implying that glucagon would end in enhancement of glucagon secretion. Such an apparent positive feedback loop, enhancing hormone secretion, gives rise to instability of the islet system.

There is basal hormone secretion from alpha- and beta-cells even at the normal glucose level (47), where it is not necessary to change the blood glucose concentration with the help of glucagon or insulin. Obviously, the simultaneous secretion of glucagon and insulin at the

normal level should be inefficient because the opposite effects of the two would cancel out, nullifying the net effects on the glucose level. Such wasteful co-secretion of counteracting hormones can be prevented by delta-cells secreting somatostatin, which inhibits secretion of both glucagon and insulin.

Another consequence of the inhibitory interaction of delta-cells is the shift of glucose dose-responses for insulin secretion, associated with the increased control of beta-cells by delta-cells at high glucose levels. In general beta-cells are coupled with each other through gap-junction channels, which assist the cells to synchronize their behaviors (41). A beta-cell cluster thus tends to produce all-or-none glucose responses (22). In the real islet, on the other hand, delta-cells, with their inhibitory interaction depending on the glucose level, can regulate accurately insulin secretion and enlarge the glucose dose-responses of beta-cells.

6. GLUCOSE REGULATION

Finally, a whole feedback model of glucose regulation is proposed to connect the microscopic bursting mechanism and the macroscopic blood glucose regulation of the body. To describe the glucose regulation of beta-cells, we modify the model for a perifusion system (55). In view of the perifusion, we consider conservation of the insulin concentration around the beta-cell, which increases by its secretion and decreases proportionally to the flow rate of the perifusion system. Experimental observations indicate that insulin secretion from beta-cells grows with the intracellular calcium concentration and with the amount of insulin stored for rapid secretion (56). In particular, the secretion rate is known to depend on the free intracellular Ca^{2+} concentration quartically. We thus assume that the insulin secretion rate is a quartic function of the intracellular Ca^{2+} concentration (57), which increases by the influx through the Ca^{2+} channel and decreases by calcium clearance. The calcium clearance considers the flux through sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps, plasma-membrane Ca^{2+} ATPase, plasma-membrane Na/Ca^{2+} exchangers (58), and endoplasmic reticulum (ER) (59); the complete description of the calcium clearance is referred to Ref. 57.

We consider the dynamics of the intracellular glucose concentration in the beta-cell. Glucose is taken up into the beta-cell through GLUT-1 (glucose transpoter-1) and GLUT-2 (glucose transporter-2), with GLUT-2 playing a dominant role, and metabolized to increase the ATP/ADP ratio, which in turn reduces the conductance of the ATP dependent K^+ channel (see Figure 1). We thus take the rates through GLUT-1 and GLUT-2 to be simple increasing functions of the extracellular glucose concentration and choose an increasing function of the intracellular glucose concentration for the metabolism rate. Note that the rate through GLUT-1 has been taken to be an increasing function of the insulin concentration, which models the recruitment of GLUT-1 by insulin and results in positive feedback. On the other hand, to take into account indirect effects of insulin on the conductance of K (ATP) channel, we choose the rate through GLUT-2 to account for inhibitory effects of insulin on its own release via the inhibition of GLUT-2 (60); this assumption is made as a possible interpretation of recent observation of the inhibiting role of insulin (61-63). Accordingly, the rate through GLUT-2 tends to decrease as the insulin concentration is raised. To complete the model, we assume the two-step process in a beta-cell, which includes manifestation of the inhibitory signal from insulin receptors in the presence of insulin (first step) and the inhibition of GLUT-2 by that signal (second step). In addition, it has been known that the conductance of ATP-sensitive K^+ channel depends on the glucose concentration. In general, an increase in the intracellular glucose concentration due to injection of glucose raises the ATP/ADP ratio after being metabolized; this in turn reduces the conductance of ATP-sensitive K^+ channel and induces depolarization of the membrane potential. Thus we assume that the conductance of ATP-sensitive K^+ channel is a decreasing function of intracellular glucose concentration G_{in} in the Hill equation form.

The whole feedback model should be equipped to incorporate the bursting mechanism into the glucose regulation, with the glucose-dependent conductance of ATP-dependent K^+ channel and the calcium-dependent rate of insulin secretion as well as the insulin-dependent rate of glucose uptake playing the role of a bridge between the two. Simulating this model on 8×8 lattices, we obtain various oscillations depending on the external glucose concentrations: oscillatory behaviors of membrane potential V, average intracellular calcium concentration C, plasma glucose concentration G_{in}, and secreted insulin R_S per minute, obtained at external glucose concentrations 5, 8, and 15mM (see Figure 5). In particular, clusters of bursts and slow and fast calcium oscillations are also observed. Note the assumption that oscillatory behavior in the beta-cell population brings three main physiological advantages: resetting of the secretory mechanism, resetting of peripheral effector systems, and fine-tune modulation (in frequency and duration) instead of amplitude modulation (22). The first burst persists longest, while following ones last for progressively shorter times, similar to experimental observations (56). The total duration time of such a regularly bursting cluster, namely, the duration that the membrane potential is higher than V = -55 mV, grows with the glucose concentration. Note that oscillations of the average calcium concentration have periods similar to those of repetitive activation of the membrane potential; this reflects that most of the cells in an islet are well synchronized except for a slight phase shift (64). The insulin release occurs in the form of sharp peaks as soon as the Ca^{2+} concentration increases in the cell. After an appropriate rest period, the secretion rate of insulin reaches the maximum value, then diminishes at the next burst, as the amount of insulin stored for rapid secretion reduces.

To confirm the assertion that insulin inhibits bursting of the membrane potential in the low-insulin feedback condition with a sufficiently high flow rate, we examine the behavior of the membrane potential in

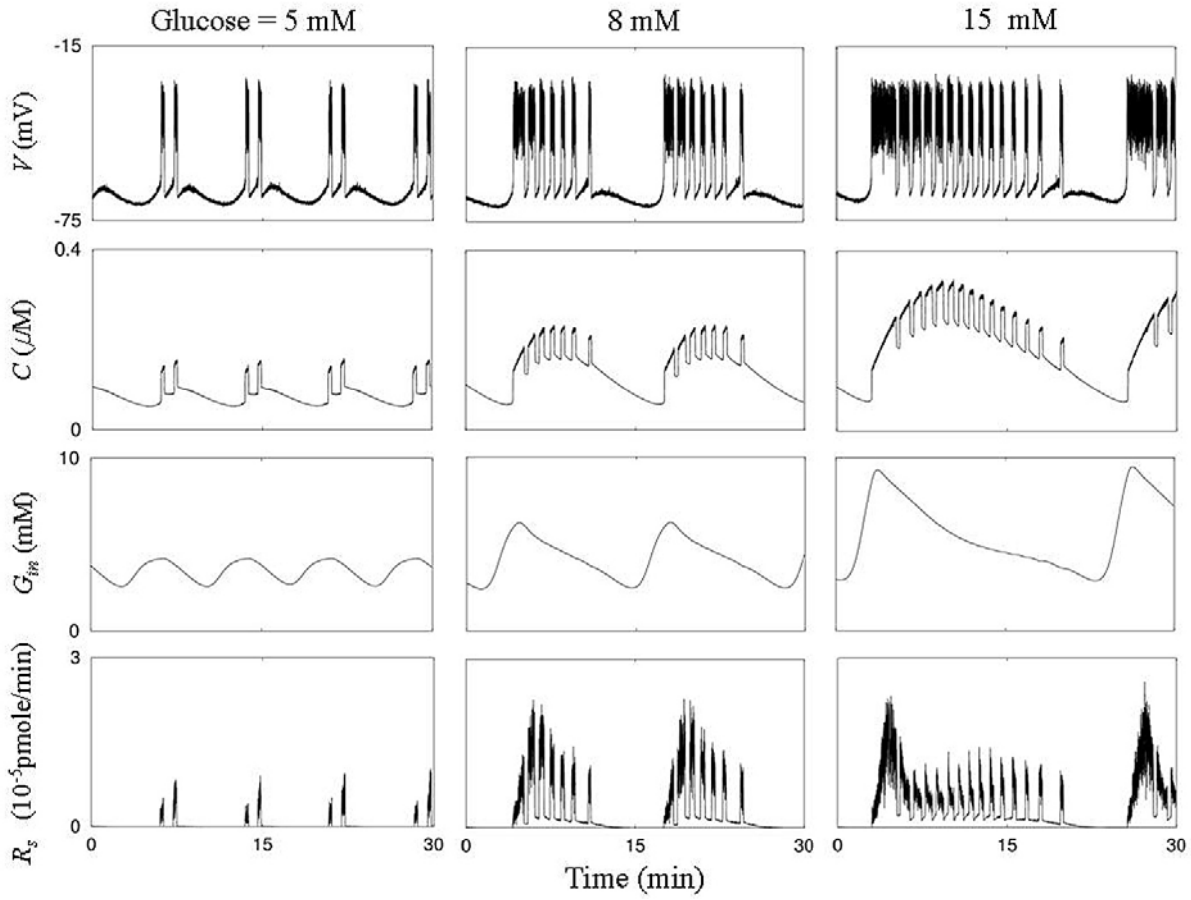

Figure 5. Fast and slow oscillations of membrane potential V, average intracellular calcium concentration C, plasma glucose concentration G_{in}, and secreted insulin R_S per minute, depending on the extra-cellular glucose concentration G_0.

response to the injection of insulin corresponding to 100 nM during the time t = 10 to 25 min under the extra-cellular glucose concentration of 10 mM. Figure 6 shows that the bursting action potential disappears after about 6 min from the beginning of the insulin injection and appears again about 3 min after the cease of injection. This is consistent with the experimental result (62).

7. SUMMARY

We have probed noise and coupling effects on the bursting action potential in pancreatic beta-cells, and found that regular bursts would be produced by an appropriate amount of noise and coupling strength. In particular, starting with the condition that single cells produce spiking action potentials, the bursting duration and period increase as the cell cluster grows until some critical size (s = 5), above which enhancing effects of the size saturate. To confirm the functional importance of the cluster size, we have observed sizes of mice islets. Further, we have proposed a theoretical model for the growth of pancreatic islets, and compared their size distributions with experimental results. Our model has predicted either *log-normal* or *Weibull* distributions of the islet size, depending on whether cells in an islet proliferate coherently or independently. The observed size distributions, skewed to smaller size, fit well to the *log-normal* function or the *Weibull* function derived theoretically. In addition, it has been observed that there are no islets above s = 30. As an explanation of the upper limit of the islet size, we have considered that the relative populations of such different cell types as alpha-, beta-, and delta-cells determine the paracrine interactions between them. In particular, alpha- and beta-cells interact with each other in such as way that glucagon enhances insulin secretion whereas insulin inhibits glucagon secretion. Such asymmetric interactions constitute negative feed back loops which contribute to the stable recovery to the normal glucose level, when the system is externally perturbed by stimuli such as glucose dose. Delta-cells, secreting somatostatin, prevent wasteful co-secretion of both glucagon and insulin at the normal glucose level, and enlarge the glucose dose-responses of beta-cells at high glucose levels.

Finally, we have described oscillations with variable time scales in clusters of beta-cells. Bursting action potentials with periods of several seconds have been observed, induced by coupling and heterogeneity between cells. Then, we have considered clusters containing beta-cells enough for robust bursts and glucose regulation in

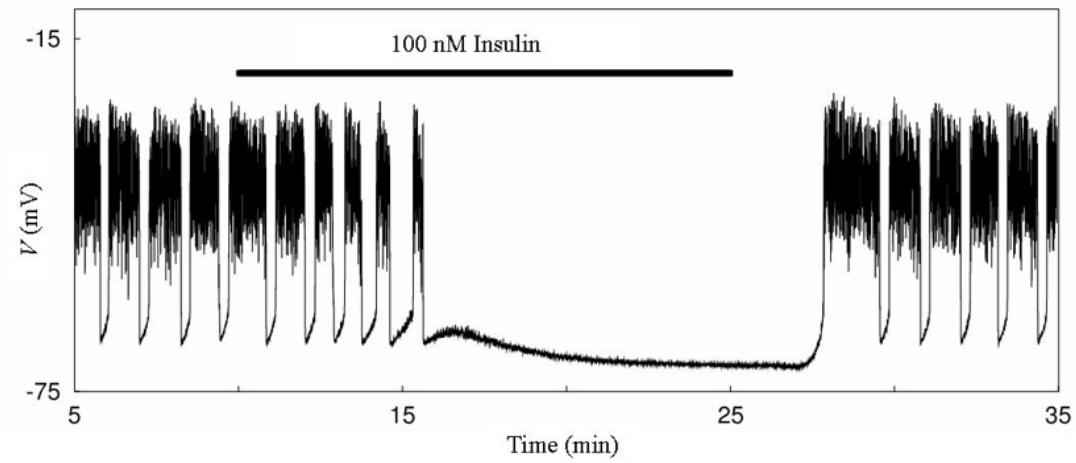

Figure 6. Behavior of the membrane potential V under the insulin injection in a low-insulin feedback condition. 100 nM insulin is injected during time t = 10 to 25 min when the extra-cellular glucose concentration is 10 mM.

each cell. The model possessing fast dynamics of bursts and slow dynamics of glucose-insulin feedback has been demonstrated to generate complex oscillations, clusters of bursts, and fast and slow calcium oscillations. Such mathematical modeling and simulations on the whole feedback glucose regulation are necessary for understanding glucose homeostasis at the system level based on molecular-level knowledge and provide a scientific method of quantitative research into diabetes mellitus, allowing to make quantitative predictions in experimental and clinical studies.

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Abbreviations: GIIS: glucose induced insulin secretion; GLUT-1: glucose transporter-1; GLUT-2: glucose transporter-2; DTZ: dithizone

Key Words: Pancreatic Islet, Beta-Cell, Bursting Action Potential, Size Distribution, Intercellular Interaction, Whole Feedback Model, Glucose Regulation, Review

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