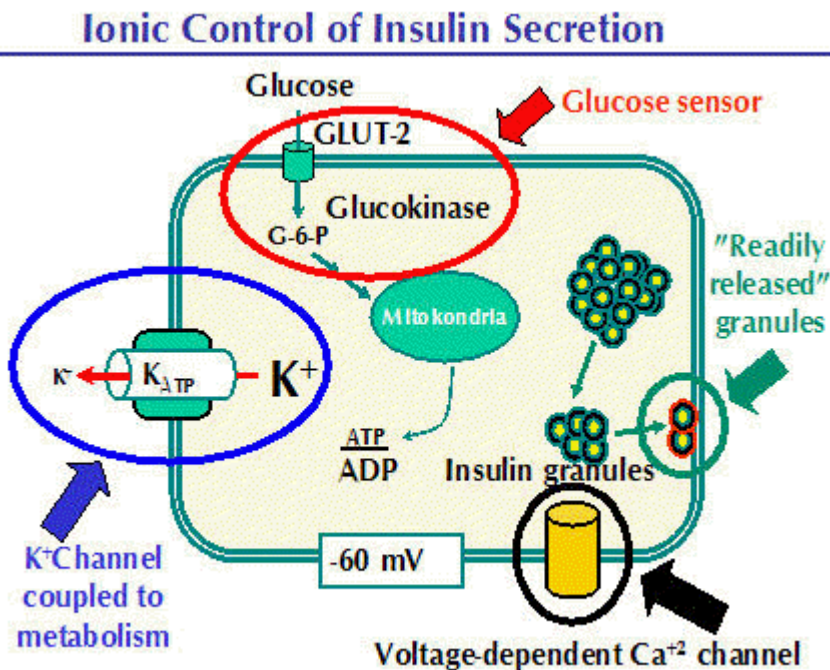# Secretion of Insulin and Glucagon

As I have already mentioned, the pancreas contains clusters of cells known as the Islets of Langerhans. They contain three cell types: alpha cells that produce glucagon, beta cells that produce insulin, and delta cells where somatostatin is synthesized. Together, these cells and their hormone products are responsible for the minute-to-minute regulation of metabolism. Metabolism in this case includes storage and release of carbohydrates and lipids, rates of energy production, protein synthesis and even the regulation of hunger.   Seemingly minor aberrations in function of these cells have large and often devastating effects on an individual's health.

Insulin secretion is stimulated by glucose, some amino acids and fatty acids. Let us take these up individually.

Monitoring Blood Glucose.

The basic functions and physiology of the beta cell are relatively well understood. A model of the beta cell showing the basic components for insulin secretion is presented below. A glucose "sensor" mechanism, a metabolic coupling to potassium channels to control plasma membrane potential and a voltage dependent Ca++ channel are required to link blood glucose levels to insulin secretion. Insulin containing granules are found in a reserve pool and a "readily released" pool.

Ionic Control of Insulin Secretion

Let us look at the "glucose sensor" system first. The beta cell's primary function is to correlate release of insulin with changes in blood glucose concentration. Obviously, these cells must have a sensitive glucose-measuring

device. Nature has achieved this by equipping the beta cell with a glucose transport protein (GLUT2) and a kinase (glucokinase) both of which have low affinities for glucose.   GLUT2 is quite active, but the K_m for glucose is around 5 mmol/l. Therefore, transport of glucose into the beta cell is rapid, but only when the blood glucose concentration exceeds post-meal levels.
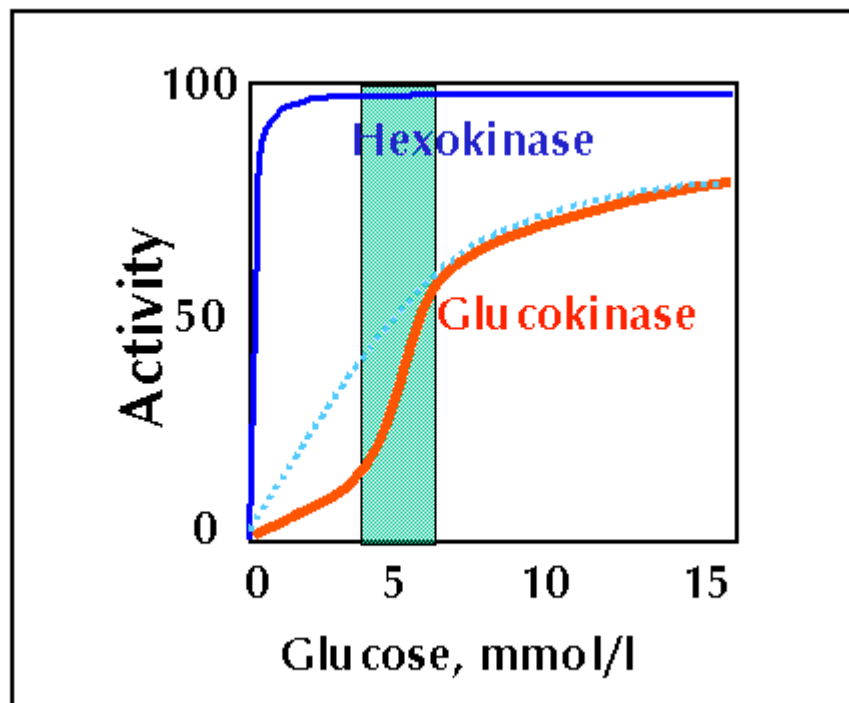
The next component of the glucose sensor is glucokinase, the enzyme that initiates glycolysis. Unlike hexokinase, glucokinase has a low affinity for its substrate.   The K_m for human pancreatic glucokinase is 5.5 mmol/l while the various hexokinase enzymes have K_m values of around 0.01 mmol/l. Glucokinase activity increases and decreases parallel to changes in blood glucose levels within the physiological range (shown in green). Note that glucokinase exhibits sigmoid kinetics (the dotted blue line represents a normal activity curve). Glucokinase activity is, therefore, most sensitive to changes in blood glucose concentration within the physiological range (approximately 4-6 mmol/l).

Consequently, both uptake of glucose by the beta cell and initiation of glycolysis closely follow blood glucose levels.   We have a system that responds to increases in blood glucose with a rapid uptake and metabolism of glucose, but which is rather sluggish at the glucose levels found between meals. The "glucose sensor pair" GLUT2-GK is also found in the liver and hypothalamus and seems to be the universal glucose sensor. Recent findings in the glucagon-releasing alpha cell suggest that there are subclasses of glucose sensors. In the alpha cell, GLUT1 is coupled to glucokinase. This results in a glucose sensor that is quite sensitive for the changes in the lower range of physiological blood glucose levels (< 4 mmol/l), while that found in the liver and pancreas is most responsive to blood glucose alterations over 4 mmol/l.

Activators of Glucokinase Have a Potential Role in Diabetes Therapy (Science 301, 370 (2003).

One of the most exciting observations in the field of diabetes 2 treatment was recently published in Science. Molecular pharmacologists at Hoffman-La Roche screened a library of 120,000 synthetic compounds and found one that specifically activated glucokinase in laboratory animals. RO-28-1675 as it is named, increased insulin levels in rats coupled to a parallel decrease in blood sugar concentrations. The compound appears to increase glucokinase's affinity for its substrate and to boost maximal activity of the enzyme. Both the liver and ß-cell forms of the enzyme were activated by RO-28-1675. This may mark the beginning of a new approach to diabetes type 2 treatment.

Ionic Control of Insulin Release

Beta Cell Depolarization, the K_{ATP} Channel.

The original observation that depolarization of the beta cell membrane was coupled to release of insulin was made by Dean and Matthews in 1968. These authors also observed that waves of depolarization and action potentials were initiated in intact pancreatic islet ß-cells by glucose (Figure to the left).

Later work of P. Rorsman and others demonstrated that the release of insulin following depolarization of the beta cell is

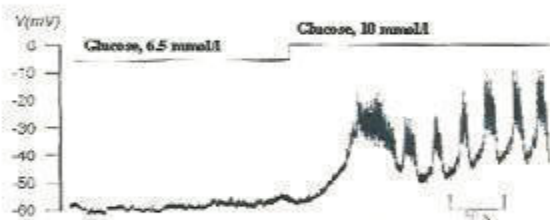Membrane Depolarization is Coupled to Insulin Secretion

Fig. 1. The membrane potential (V) of a single beta cell within an intact pancreatic islet recorded in the presence of 6.5 and 10 mM glucose as indicated by the staircase
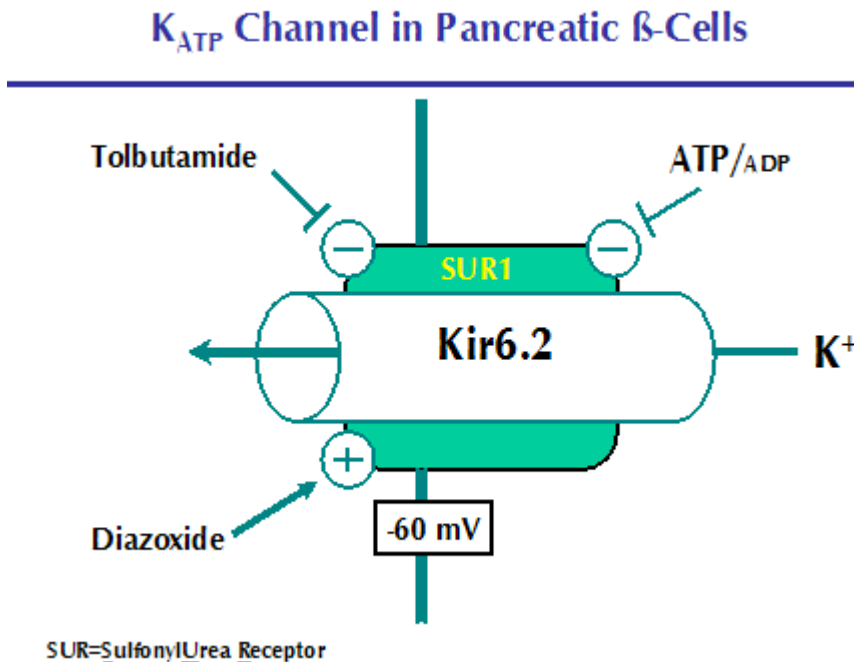
Dean and Matthews, Nature 219, 389, 1968

coupled to entry of extracellular Ca^{++}.

The question that arose was "what is the connection between blood sugar levels and beta cell membrane potential"? Or, more properly, what is the connection between uptake of extracellular Ca^{++} and the beta cell's metabolism. The answer lies in control of the cell's membrane potential.

The resting membrane potential of about -60 mV found in beta cells arises from loss of K^+ ions to the extracellular space. In beta cells the dominant K^+ channel is of the Kir6.2 variety that is also found in several other cell types.

K_{ATP} Channel in Pancreatic ß-Cells

Tolbutamide
ATP/ADP
SUR1
Kir6.2
K^+
Diazoxide
-60 mV
SUR=SulfonylUrea Receptor

The distinguishing characteristic of this ion channel in beta cells is that it is bound to a regulatory protein, known as SUR1. This name comes from the fact that this protein is the receptor for sulfonylurea compounds. These compounds have hypoglycemic actions, first reported by Janbon and coworkers in 1942. Tolbutamide, long used in medical practice to treat diabetes type 2, is one of these sulfonylurea compounds. The mechanism of these effects has only recently been identified.

The Kir6.2-SUR1 complex is now known as the K_{ATP} channel. The complete channel consists of a core of four Kir 6.2 subunits surrounded by four SUR1 subunits. The SUR1 complex acts as a regulator of the K^+ channel, binding ATP as well as sulfonylurea compounds. Both ATP and tolbutamide have inhibitory actions on the K_{ATP} channel and therefore inhibit K^+ efflux. This leads to depolarization of the beta cell, Ca^{++} influx and insulin secretion. Another agent, diazoxide, stimulates the K_{ATP} channel and promotes K^+ efflux, membrane polarization and inhibition of insulin secretion.

4

How does this couple blood glucose levels to membrane potential? The "classical" viewpoint has been that the pancreatic beta cell obtains its energy supply through aerobic glycolysis, using glucose as substrate. However, resting beta cell oxidative phosphorylation may be dependent upon oxidation of fatty acids (discussed later). The rate of fatty acid beta oxidation may limit oxidative phosphorylation.   The ATP/ADP ratio is relatively low in beta cells exposed to fasting blood glucose levels. The accelerated glucose uptake found at glucose levels over 5 mmoles/l augments ATP synthesis. In other words, ATP synthesis is dependent upon the rates of glucose uptake and aerobic glycolysis. We have seen that these events are coupled to blood glucose concentration through GLUT2 and glucokinase. Variations in ATP levels occur parallel to changes in blood glucose concentration. ATP acts as a second messenger in these cells, informing the K_{ATP} channel of variations in blood glucose levels. Stated simply: more glucose, more ATP, increased INHIBITION of K^+ transport, depolarization of the beta cell and then, release of insulin.
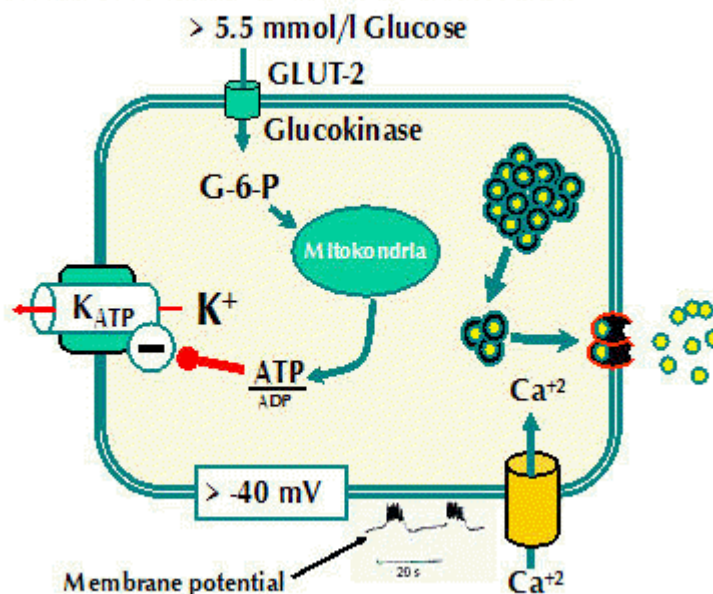
Incidentally, it has been suggested that ADP levels may be just as important as ATP in regulation of K_{ATP}. After all, increases in ATP concentration do signify a fall in ADP.

The final element in the signal system for insulin secretion is the voltage-dependent Ca^{++} (VDCC) channel. This opens when the membrane voltage falls to less than -40 mvolts. The Ca^{++} that then enters the cell is directly involved in the exocytotic process that releases insulin form the "rapidly released pool" of insulin-containing granules.

Insulin Secretion is Controlled by Blood Glucose Levels.

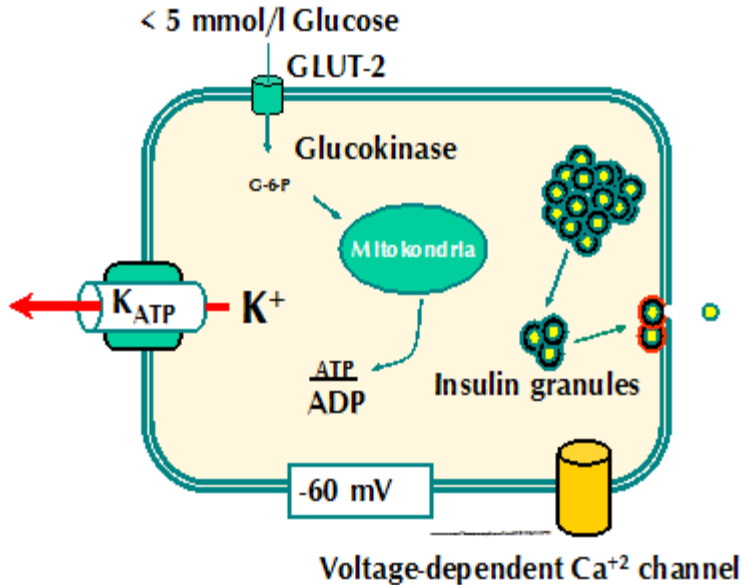This process becomes clearer when we examine some examples. Let us look at glucose levels that lead to release of insulin first. GLUT2 and glucokinase are activated when blood glucose increase to about 5.5 mmol/l. Aerobic glycolysis will drive the ATP/ADP ratio upwards. The ATP produced inhibits the K_{ATP} channel, thus reducing the flow of potassium ions from the beta

Ionic Control of Insulin Secretion

> 5.5 mmol/l Glucose
GLUT-2
Glucokinase
G-6-P
Mitokondria
K_{ATP} — K^+
ATP/ADP
> -40 mV
Membrane potential
20 s
Ca^{+2}
Ca^{+2}

cell. As a result, the cell becomes increasingly depolarized. Look at the recording of membrane potential. Slow depolarization waves are initiated as the membrane potential falls.   Action potentials occur at the tops of these waves. Insulin secretion is pulsatile, the hormone being secreted in bursts that occur simultaneously with the action potentials. Calcium causes exocytose from the "rapidly released pool" and migration of insulin-containing granules from the "reserve pool" to the cell membrane where they are "docked" and energized.

What happens when blood glucose falls below approximately 5 mmol/l? Remember that the activity curve for glucokinase is steep within the physiological range. A fall in blood glucose to between 4 and 5 mmol/l is quite enough to reduce GLUT2 transport of this sugar into the beta cell. Glucokinase activity falls as a result of the reduction in GLUT2 activity (remember, the K_m is about 5.5 mM). This will lead to a cutback in the rates of aerobic glycolysis and ATP production. The inhibitory effect of ATP on the K_{ATP} channel will be reduced as a consequence of this, and the membrane potential will once again approach -60 mV. The Ca^{++} channel will close and the rate of insulin release will fall.


Ionic Control of Insulin Secretion

< 5 mmol/l Glucose

GLUT-2

Glucokinase

G-6-P

Mitokondria

K_{ATP} — K$^+$

$\frac{ATP}{ADP}$

Insulin granules

-60 mV

Voltage-dependent Ca^{+2} channel

Note that insulin secretion is a continuous process. About one half of the daily secretion of insulin is not associated with meals.   A basal secretion and level of insulin in serum is maintained at all times. The metabolic effects of insulin secretion are always balanced by those of glucagon. Minute-to-minute metabolism is directed by the sum of the effects of these two hormones.

Mutations of K$_{ATP}$ Can Cause Neonatal Diabetes.

A recent article in a Norwegian newspaper reported that "the last prick" for Silius was soon a fact. Silius, a boy about four yeas old, had a rather seldom form of diabetes and was insulin-dependent. However, a recent study indicated that some such patients (with neonatal diabetes) have a fail in the coupling of the K$_{ATP}$ channel to metabolism. The channel does not respond to ATP. These patients produce insulin. However, they do not secrete insulin in respond to increased blood glucose. They can respond to sulfonylurea compounds. That is, the K$_{ATP}$ is not inhibited by increases in the ATP/ADP ratio while the SUR1 moiety can still block K$^+$ flux. In these patients tolbutamide tablets just might be able to replace insulin in these patients. This is now being tested.
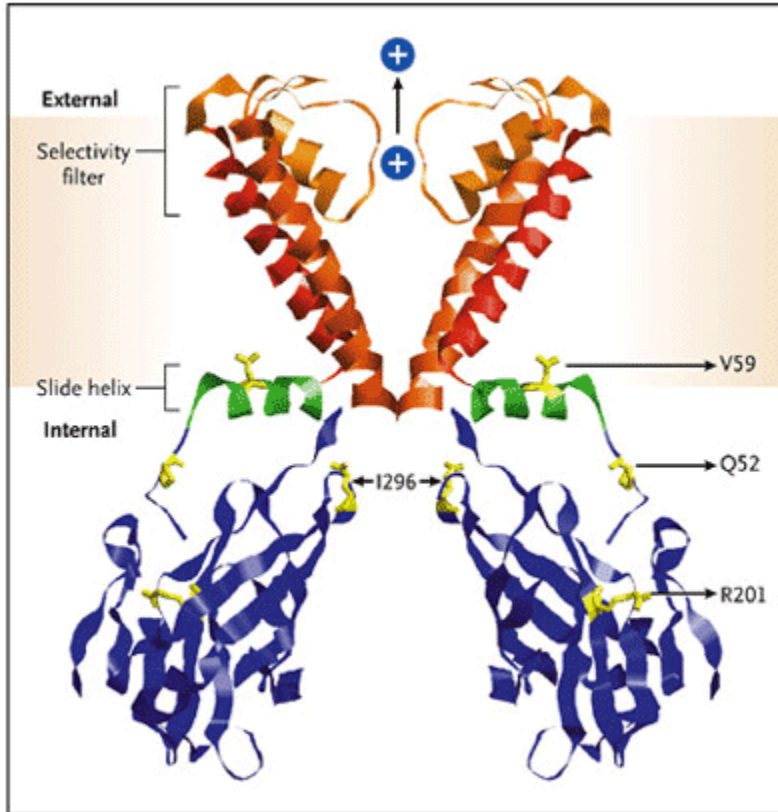
The newspaper article was based on an extensive recent paper published in the <u>New England Journal of Medicine</u> (N Engl J Med 2004; 350:1838-49). (Click on the title if you have library connections). Neonatal diabetes is very seldom, but genetic studies of Kir6.2 revealed 6 different mutations in 10 of 29 patients. Two figures from the article presenting the structure of the K$^+$ channel and mutations in Kir6.2 are shown below.
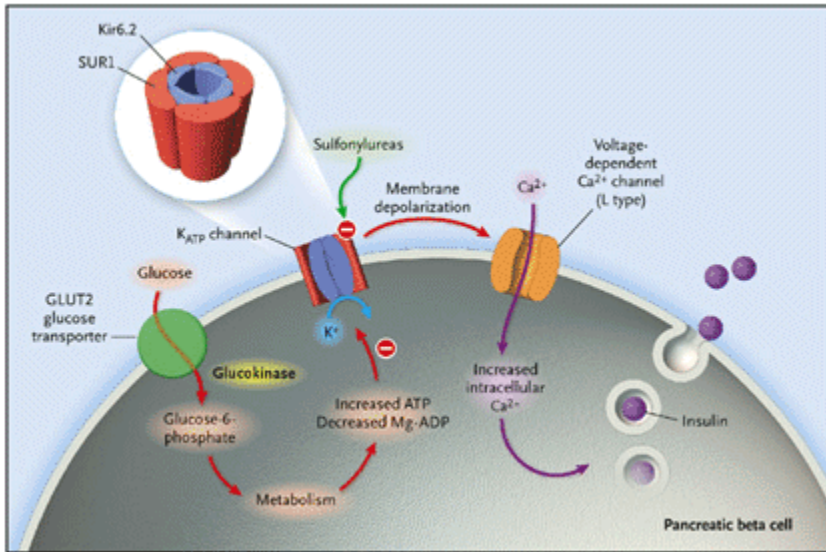
The first repeats the explanation of the structure of the pancreatic beta cell. Here, the authors show the detailed structure of the K$_{ATP}$ channel. You can see that there are four repeats of each protein; 4 potassium channeling elements and four sulfonylurea-binding elements.

The second figure presents the tree-dimensional structure of the Kir6.2 protein. Mutations, found in several children with neonatal diabetes, are indicated in yellow. These may affect binding of SUR1 or ligands such as ATP and ADP. Altered coupling between glucose and ATP levels and coupling to K^+ transport was noted in these children. Treatment with sulfonylurea compounds could, in some cases, increase insulin secretion and better the child's glucose tolerance. Please go to the original article if you want more information.

As you can see, the Norwegian boy, Silius, really looked forward to escaping "the insulin needle"!

Newer Aspects of Insulin Secretion: Several Potassium Channels Determine Membrane Potential and Steer Insulin Secretion.
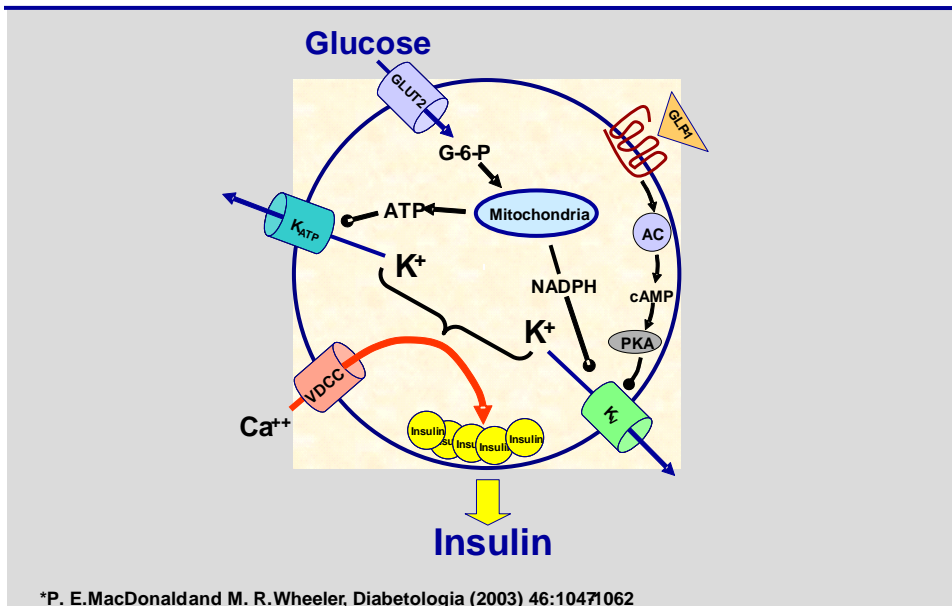
The Voltage-Dependent K$^+$ Channel (K$_v$ channel).

Insulin release from beta cells is graded, controlled by the intracellular K$^+$ concentration. As described above, flux of K$^+$ <u>from</u> these cells is believed to be controlled by the K$_{ATP}$ channel. However, there are several other potassium- carrying pores in beta cells and the intracellular concentration of K$^+$ reflects the balance between these.

The most interesting today is perhaps the K$_v$ channel. This pore system is voltage-dependent. It opens following depolarization of the cell membrane, in a manner similar to the voltage dependent Ca^{++} channel (VDCC channel).

While depolarization of the cell membrane follows inhibition of the potassium releasing channel, repolarization is the result of activation of a channel which ships potassium out of the cell! The potassium (K$^+$) level in the beta cell (and, therefore, the membrane potential) is dependent upon the balance between these two port systems. <u>The rate of insulin secretion is, therefore, also dependent upon the balance between these K$^+$ transport mechanisms.</u>

An Electrogenic Model for Glucose –Stimulated Insulin Secretion*

*P. E. MacDonald and M. R. Wheeler, Diabetologia (2003) 46:1047–1062

Activity of the K$_v$ channel is subject to control by hormones acting through G-proteins, adenyl cyclase and protein kinase A. Phosphorylation of the K$_v$ channel inhibits K$^+$ transport. K$_v$ is also inhibited by NADPH which is formed through glucose

metabolism. Glucagon-like protein 1 (GLP-1), which stimulates insulin secretion, seems to act through K_v. The figure above, slightly modified from MacDonald's review article, summarizes the potassium channel theory.

The work lying behind these conclusions is presented in a review article by P. E. MacDonald and Wheeler. Click here for that article. (Voltage-dependent K+ channels in pancreatic beta cells: Role, regulation and potential as therapeutic targets, P. E: MacDonald and M. B. Wheeler, Diabetologia 48, 1046 2003). This is especially interesting work and may provide a basis for new treatment regimes for diabetes type 2.

Recent developments in incretin hormone treatment of type 2 diabetes i discussed in depth in CME "Changing the Course of Disease: Gastrointestinal Hormones and Tomorrow's Treatment of Type 2 Diabetes (November 2004). Click on the title to open the CME and to hear the lectures.
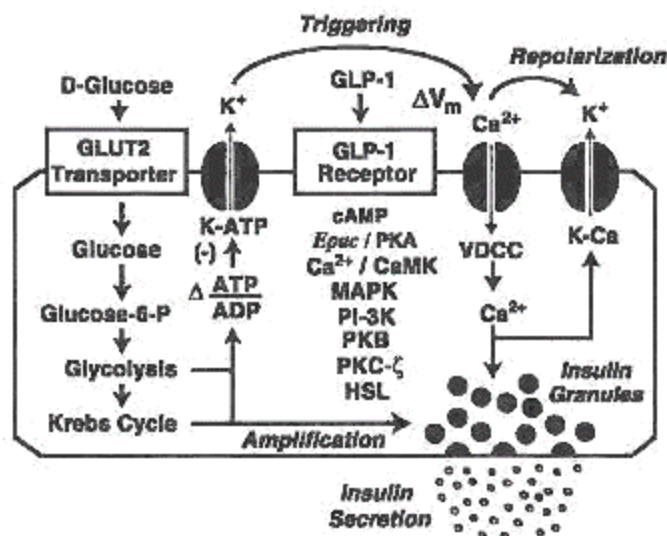
Recent Developments in Intretin Stimulation of Insulin Secretion (November 2005).

Protein Kinase Independent Actions of Cyclic AMP; Epac.

Recent work with the actions of incretins on the ß-cell indicates that some of the actions of the cAMP-linked hormones are not dependent upon protein kinases. Cyclic AMP has been shown to bind to cAMP receptor proteins known as Epac1 and Epac2. These are also known as "cAMP-regulated guanine nucleotide exchange factors" (cAMPGEFs). The cAMP-activated Epacs stimulate exchange of GDP for GTP on Rap1, thus activating this GTP-binding protein. Intracellular Ca^{++} levels are partially controlled by Epacs as they have been shown to regulate release of Ca^{++} from intracellular stores. See the next section for more about this.

It is important to remember that insulin secretion is the result of changes in the ß-cell's membrane potential and calcium-dependent action potentials. All cellular modifications can influence these processes and, therefore, the beta cell's rate of insulin secretion. The system is quite sensitive to a wide variety of physiological factors. These are

Factors Influencing Insulin Secretion*

* Holz, G., Diabetes 2004, 53, 5-13

summarized in the figure taken from the work of Professor George G. Holz, New York University School of Medicine. Here we can see that a whole host of factors including cyclic AMP, many protein kinases, Ca^{++} kinase and phosphatidylinositol are involved in amplification of the initialing signal for insulin release. Do these play a role in development of glucose intolerance? Are they involved in the eventual loss of ß-cell activity seen in type 2 diabetes? Further work may give answers to these questions and establish the basis for new medications for treatment of type 2 diabetes.   Please go to the original paper for a more detailed analysis of the role of Epac in insulin secretion and references to further work (click here for that publication).

## Intracellular Sources of Ca^{++} Influence Ca^{++} Influx and Insulin Release.

Part of the actions of incretins appear to follow cAMP initiated liberation of intracellular Ca^{++} which, in turn, triggers a further "Ca^{++} -induced Ca^{++} release" or "CICR". The point to note is that insulin release from ß-cells depends on Ca^{++} initiated action potentials and that <u>the amount of insulin released is dependent upon the magnitude and frequency of these potentials.</u> The careful and precise regulation of blood glucose levels found in most people is absolutely dependent upon integration of a large number of factors; glucose sensing, mitochondrial metabolism, cyclic AMP levels, and the levels of K$^+$ and Ca^{++}. These points are discussed in detail by Patrick Rorsman, George Holz (1) , George Holz (2) in a series of publications. (Click on their names to come further). The precise mechanisms which lie back of their findings are yet to be well described. However, it appears that genetic variations play a major role in this strict multifactorial coordination. It would appear that a certain degree of disparity is acceptable. However, the metabolic consequences of overweight and aging can throw a genetically stressed situation out of balance and lead to development of glucose intolerance and type 2 diabetes. New approaches to control of diabetes may be expected from these studies.

Three Amino Acids Cause Insulin Release.

Only three of the twenty amino acids found in our food lead to secretion of insulin. They do this through the same basic mechanism as glucose. That is, their entry into the beta cell leads to ionic changes that depolarize the beta cell, trigger Ca^{++} uptake and stimulate exocytose of insulin-containing granules. However, the K$_{ATP}$ channel is <u>not</u> involved in this process.


Glycine, Alanine and Arginine
are Insulin Secretagogues
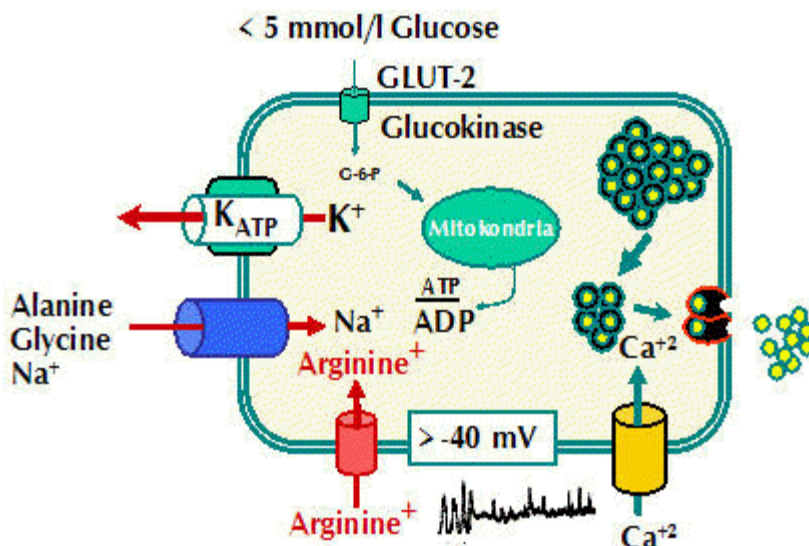
How can these amino acids lead to depolarization of the beta cell without involving the K$_{ATP}$ channel?

Alanine and glycine share a symport that also transports Na$^+$ into the beta cell. The influx of Na$^+$ is sufficient to depolarize the cell, activating

the Ca^{++} channel with ensuing Ca^{++} uptake and insulin release.

A dedicated arginine transport protein is also present in the beta cell plasma membrane. Arginine is a cation at physiological pH and can directly depolarize the beta cell. Arginine is, in fact, the strongest insulin secretagogue, measured on a mole for mole basis. It is often used to initiate insulin secretion in clinical testing of beta cell capacity.

# Glycine, Alanine and Arginine increase Cytoplasmic Ca^{++}
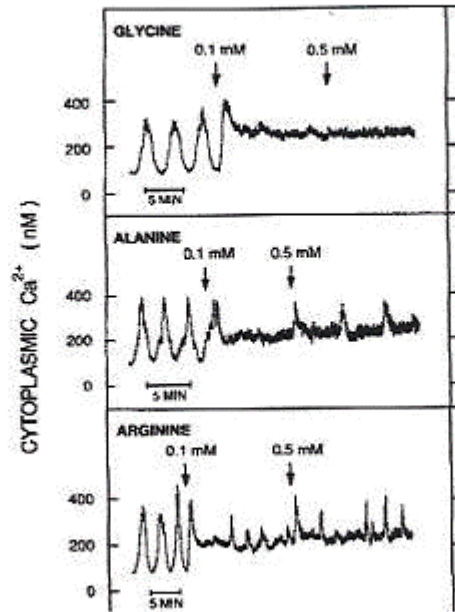
Ahmed, Grapengiesser and Hellman, J. Endocrinology 160, 191, 1999

The effects of these three amino acids on Ca^{++} influx can be seen in the next figure. The Ca^{++} waves were initiated by incubation with 10 mM glucose. The three named amino acids caused further and sustained Ca^{++} influx.

Protein Meals Stimulate Both Insulin and Glucagon Secretion.

Secretion of insulin and glucagon are most often opposed; ingestion of a balanced meal leads to increased insulin secretion and a fall in glucagon secretion. The opposite occurs in the post-adsorptive period or when exercising. However, a striking exception to this "rule" is intake of a protein meal (a steak, a salad and a glass of good red wine).   In the following figure you can see that levels of both

Insulin and Glucagon Secretion is Stimulated by Arginine or a Protein Meal
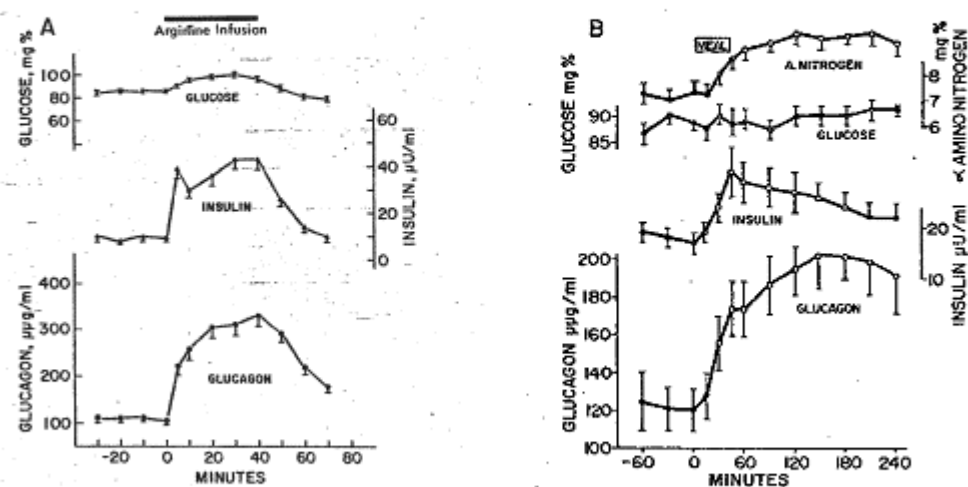
Müller et al, New Engl. J. Med 285, 1450-1454 (1971)

insulin and glucagon rise after an arginine infusion or a protein-rich meal. What is the logic behind this?

Amino acids obtained from degradation of muscle proteins are the body's largest reserve of precursors for gluconeogenesis. During periods without food glucagon secretion increases and insulin levels fall. One of the major metabolic effects of this is stimulation of hepatic gluconeogenesis. After a protein meal we absorb large quantities of amino acids. There is no storage form for these. After requirements for amino acids for protein synthesis are covered, the remaining amino acids are either converted to glucose or lost to the urine. Conversion of amino acids to glucose requires activation of gluconeogenesis. The increased levels of glucagon that follow a protein meal do just this, leading to synthesis of glucose from the ingested amino acids. However, this does not lead to marked changes in blood glucose. You can see from panel B in this figure that blood glucose levels are relatively stable after

a protein meal. This is due to the balance between insulin and glucagon secretion. Glucagon stimulates conversion of amino acids to glucose in the liver, insulin stimulates uptake of this glucose in muscle tissue and storage as glycogen. We can see in panel A that arginine infusion has a strong and similar effect.

## Insulin Secretion is Biphasic.

Ingestion of sugar is followed by a biphasic insulin release. A very rapid rise in insulin secretion is seen within minutes after administration of a glucose load. A rapid fall in secretion follows, then pursued by a long-lasting slow release of the hormone. The first top results from exocytose of granules from the "readily released" pool and comprises 5-10% of the insulin stored in the beta cells. The sustained slow release is comprised of granules from the "reserve pool". Closer examination of this phenomenon has greatly increased knowledge about insulin secretion. Let us examine these steps closely.

> 1: A fast phase requiring ATP and Ca^{++} in which granules in the "readily released pool" are already "docked" and "energized". This is the rapid first phase of insulin release and is depicted in the next figure. This phase of insulin secretion accounts for only about 5% on the total released after a meal.

2: A slow second phase requiring ATP.   Here, granules are moved from the "reserve pool" to the "readily released" pool of insulin granules. Docking and energizing of the granules are part of this slow second phase. This slow phase is the major contributor to insulin

Insulin Secretion is Biphasic
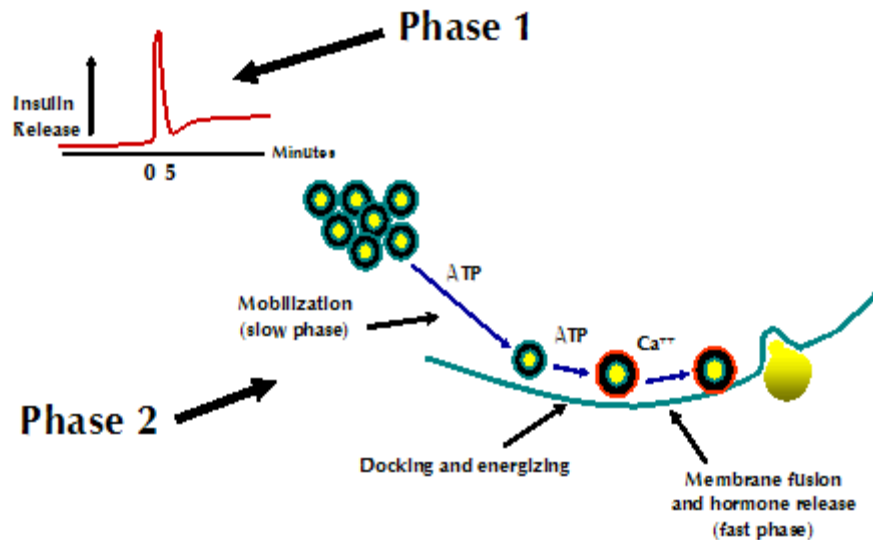
Modified from F. Komman, Diabetologia 40, 487-495, 1997

release. Maximal quantities of insulin are released after about 60 minutes.

Please note that this discussion is merely an introduction to control of insulin release. For more and very up-to-date information please go to Insulin Granule Dynamics in Pancreatic Beta Cells, P. Rorsman and E. Renstrõm, Diabetologia 46, 1029 2003. Just click on the title.

The Rate of Insulin Secretion in Impaired Glucose Tolerance (IGT) and Diabetes Type 2.

An understanding of these processes has clinical importance. It is well known that patients with diabetes type 2 and those with impaired glucose tolerance exhibit a delayed response to a glucose load. While these patients produce apparently sufficient quantities of insulin in response to a challenge, <u>secretion is not properly</u>

Peak Insulin Response after Oral Glucose
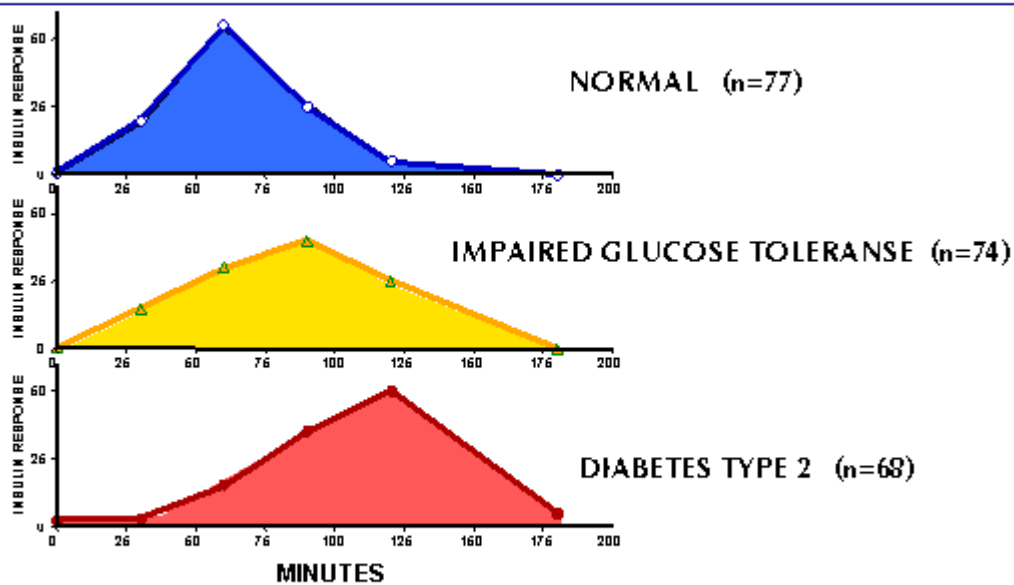
R.W.BERGSTRØM, J. CLIN. ENDOCRINOL. METAB. 71: 1447-1453, 1990

<u>timed</u>. The initial rapid rise in blood glucose does not illicit the usual "insulin spike"; the rapid first phase is reduced or lacking.   Because of the lack of correlation between carbohydrate uptake from meals and timing of insulin secretion, these patients experience hyperglycemic periods after meals.

Medication and dietary treatment of patients can in many cases provide adequate adjustment of the <u>amount of insulin </u>released from beta cells i diabetic and IGT patients. However, loss of the rapid first phase of insulin release remains a major problem. To date, treatment does not restore correct timing. This can lead to glucose "tops" after meals in excess of acceptable levels. These periods of increased blood glucose levels are thought to lead to the development of diabetic complications.

Once again, What is Wrong with Glucose "Overshoots"?

Two pathological processes seem to follow hyperglycemia.

1. **Conversion of glucose to sorbitol with ensuing osmotic disturbance.**

The Polyol Pathway
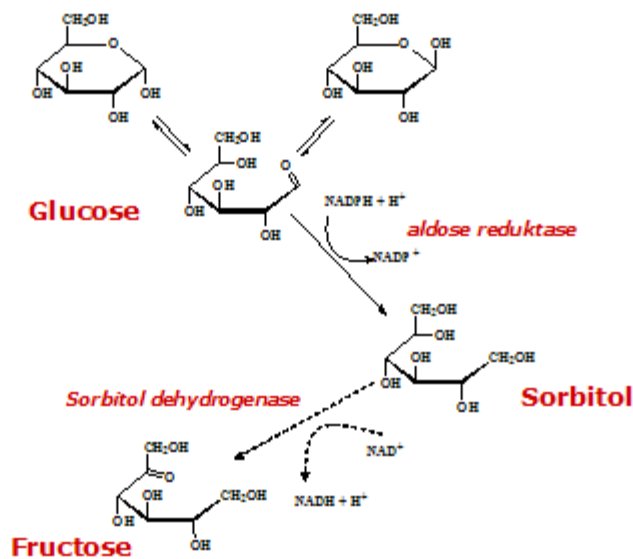
This occurs through the "polyol pathway", a normal reaction sequence in testes but not other tissues. In the first step, Glucose is converted to sorbitol by aldose reductase. The sorbitol formed is then oxidized to fructose.

A problem arises when sorbitol production occurs in tissues other than testes. At high glucose levels, aldose reductase activity occurs in other organs which often lack sorbitol dehydrogenase. This leads to an accumulation of sorbitol in these tissues, notably in the lens of the eye, leading to osmotic damage with following cataract formation.
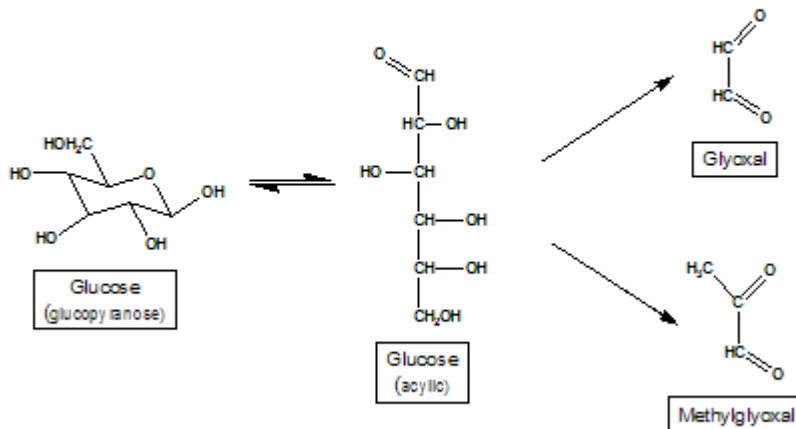
2. Spontaneous non-enzymatic glycation of proteins (Maillard Reaction).

Glycation of proteins is a normal process which follows reaction of the carbonyl group in glucose with amino groups in proteins. The Maillard reaction, which we all enjoy as the browning of meats and development of good smells from the kitchen, is based on this non-enzymatic reactions between carbohydrates and the proteins in meat. The carbonyl group in glucose makes it a reactive compound. Glycation proceeds at a rate which is proportional to the concentration of glucose in the blood.

Formation of reactive α-oxoaldehydes from glucose; glyoxal and methylglyoxal.

Several studies published during the past few years have shown that reactive aldehydes are normally formed in the body from glucose. These are found at rather low levels at normal blood sugar levels, but increase with age and in type 2 diabetes. Reaction with glucose or these breakdown products leads to increased glycation and formation of so-called "advanced glycation end-products" or AGE. These are stable proteins formed from those found normally in the body. Unfortunately, these often lose their physiological functions when glycated. It seems that damage to t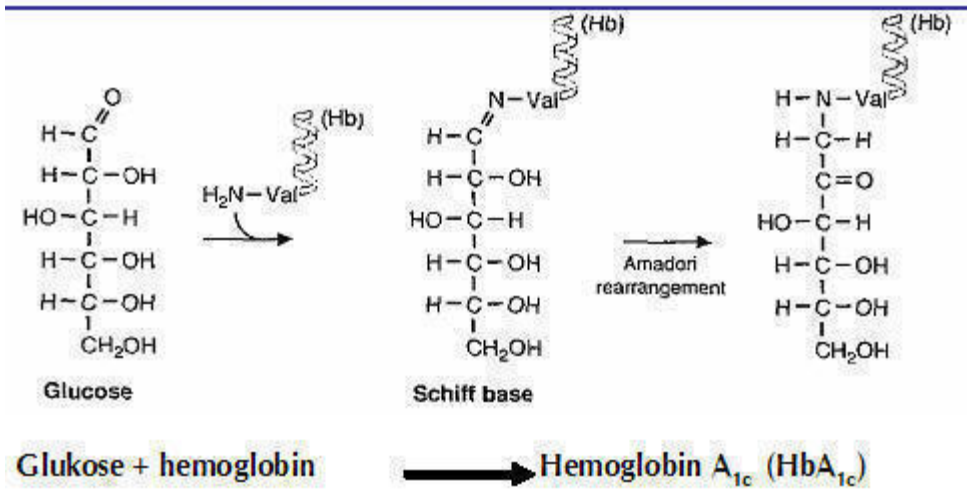he retina (retinopathy) and lens of the eye (cataracts), nerves (neuropathy) and the kidneys (nephropathy) at least partially due to glycation of cellular proteins.

Two of the reactive α-oxoaldehydes formed from glucose are glyoxal and methylglyoxal. These are synthesized from normal intermediates in glycolysis, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. These substances are present at about 0.3% of the blood glucose concentration. Long-lasting increases in glucose levels leads to accumulation of methyl glyoxal in various tissues with ensuing glycation and damage to cellular proteins. Check the following references for more information: Ramasamy et al, Cell (2006) 124, 258-260; Thornally et.al., Biochem. J. 344(1999) 109-166; Ahmed et. al., Invest Ophthalmol Vis Sci (2003); 44: 5287-5292.

Hemoglobin A1c (HbA$_{1c}$).

As I mentioned above, the degree of glycation of the body's proteins is related to blood glucose levels. Hemoglobin has a half-live of about 100 days. The degree of glycation of hemoglobin gives, therefore, a picture of average blood glucose levels for the previous three month period. Glycated hemoglobin is known as HbA$_{1c}$. Normally, HbA$_{1c}$ accounts for approximately 5-6 % of the total hemoglobin. Diabetic patients often have HbA$_{1c}$ in excess of 8-10%. It has been usually assumed that glycation of hemoglobin followed reaction with glucose, as

Ikke-enzymatisk glukosylering av proteiner

Glucose   Schiff base

Amadori rearrangement

Glukose + hemoglobin ⟶ Hemoglobin A$_{1c}$ (HbA$_{1c}$)

Marks, Marks, and Smith, s. 88

shown in the next figure. Current studies have, as noted above, indicated that other reactive species, especially methylglyoxal, may be involved in this process. Note that the degree of glycation of hemoglobin is an indication of the extent of glycation of many of the body's proteins and possible ensuing cellular damage.

Successful Treatment of Diabetes Lowers HbA$_{1c}$ levels.

Treatment of diabetes type 2 is often monitored by follow alterations of HbA$_{1c}$ levels. Marked improvements are seen with small changes in this parameter. In the following table we can see that a 1% fall in HbA$_{1c}$ resulted in improvement in retinopathy and kidney, nerve and cardiac function in the studies quoted here.

Effect of Improved Glycemic Control on Complications of Diabetes

Study	N	Follow-up, years	Risk reduction per 1% fall in HbA$_{1c}$			
			Retino-pathy	Renal	Neural	CV
1	739	6.5	33%	25%	32%	40%
2	55	6	33%	50%	↑NCV*	25%
3	4209	9	19%	26%	18%	18%

* Neural conduction velocity

Redrawn from Medscape CME, "Diabetes Management in the 21 st Century: Multiple Therapeutic Options for Achieving Glycemic Control", (03.2001)

Important Updates!

The past year has seen important advances in knowledge around insulin secretion and control of metabolism. I will soon rewrite this section and bring it up-to-date. In the mean time, you can go to:

1. Science 307, 21 January 2005 for a special section on insulin and diabetes (Click on the title).

2. Acta Physiol Scand 2005, 183, 1-130 where you will find a special issue on diabetes and insulin.

3. Insulin Granule Kinetics.

As previously explained, insulin secretion from beta cells goes in at least two steps, a rapid release following increased glucose levels, and a slower sustained secretion. An understanding of the kinetics of insulin secretion is necessary to understand normal and diabetic metabolism. Go to the review article to go deeper into this topic

An important up-to-date review of the kinetics of insulin secretion can be found by clicking here: Insulin Granule Dynamics in Pancreatic Beta Cells, P. Rorsman and E. Renstrõm, Diabetologia 46, 1029 2003.

4. Fatty Acids Control Beta Cell Insulin Secretion and Insulin Sensitivity at Target Organs

The global obesity epidemic has been suggested to be a major factor in the rapid spreading of diabetes type 2. Non-esterified fatty acids (NEFA) stimulate glucose-dependent insulin release and dampen target tissue response (so-called insulin resistance). The pronounced rise in insulin levels seen in "impaired glucose tolerance" and early type 2 diabetes may well follow the increased serum lipids seen in these individuals.

A discussion of these effects would be to encompassing for MedBio at this time. However, you can pick up a top review article by clicking here: "Fatty Acid Metabolism and Insulin Secretion in Pancreatic Beta Cells" , G. C. Yancy and B.E. Corkey, Diabetologia (2003) 46:1297-1312.

Glucagon Secretion.

Control of glucagon secretion is not as well understood as that of insulin.   Secretion of glucagon is clearly linked to the alpha cell's metabolism. Lack of substrate, anoxia and metabolic poisons lead to release of glucagon from these cells. In short, they release their hormone in response to "metabolic stress". As is the case of the beta cell's release of insulin, it has become clear that regulation of the membrane potential is decisive for control of glucagon secretion.

We can begin by examining the glucose sensor of the alpha cell. In contrast to the beta cell, this "sensor" is comprised of GLUT1 and glucokinase. This implies that glucose entry into the alpha cell will occur at lower levels than in the beta cell. (Recall that GLUT1 has a K_m of about 1mM and that the glucokinase's K_m for glucose is around 5.5 mM). Accordingly, uptake of glucose and initiation of glycolysis will start at lower blood sugar levels. The glucose sensor in the alpha cell is, therefore, responsive to changes in blood glucose concentration in the lower physiological range. Expressed simply: the beta cell glucose sensor responds to increases in blood glucose, the alpha cell's sensor to declining blood glucose levels.

Ionic Control of Glucagon Secretion

Cöpel et al, J. Physiology 528, 509-520 (2000)
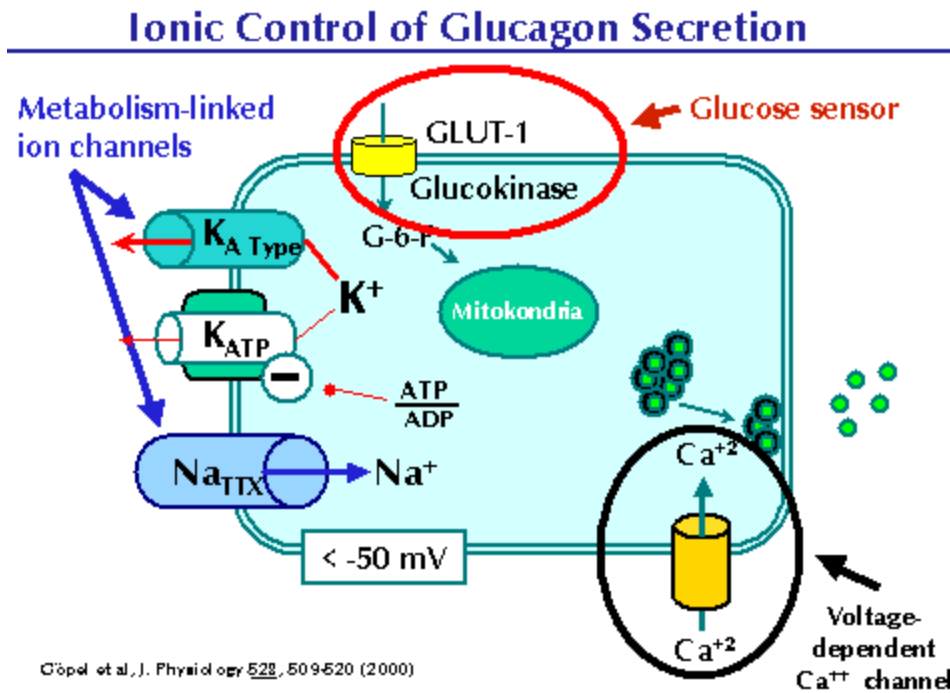
Recent studies by Göpel and coworkers in mouse alpha cells have determined the ion channel composition of these cells. Two ion channels determine the membrane potential of the alpha cell, a potassium channel of the K_A type and the tetrodotoxin-sensitive Na^+ channel. Although K_{ATP} channels were observed in small numbers in alpha cells, the authors concluded that these do not appear to determine the alpha cell's membrane potential.   These cells also differ from beta cells in that they
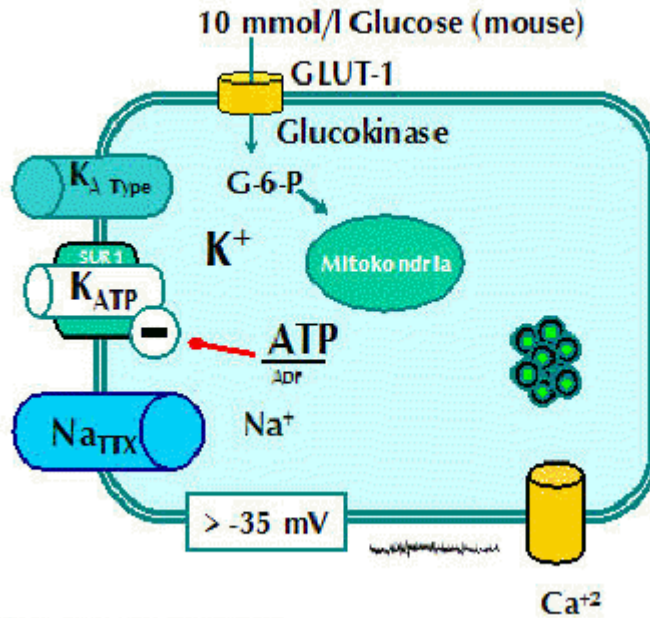
Ionic Control of Glucagon Secretion

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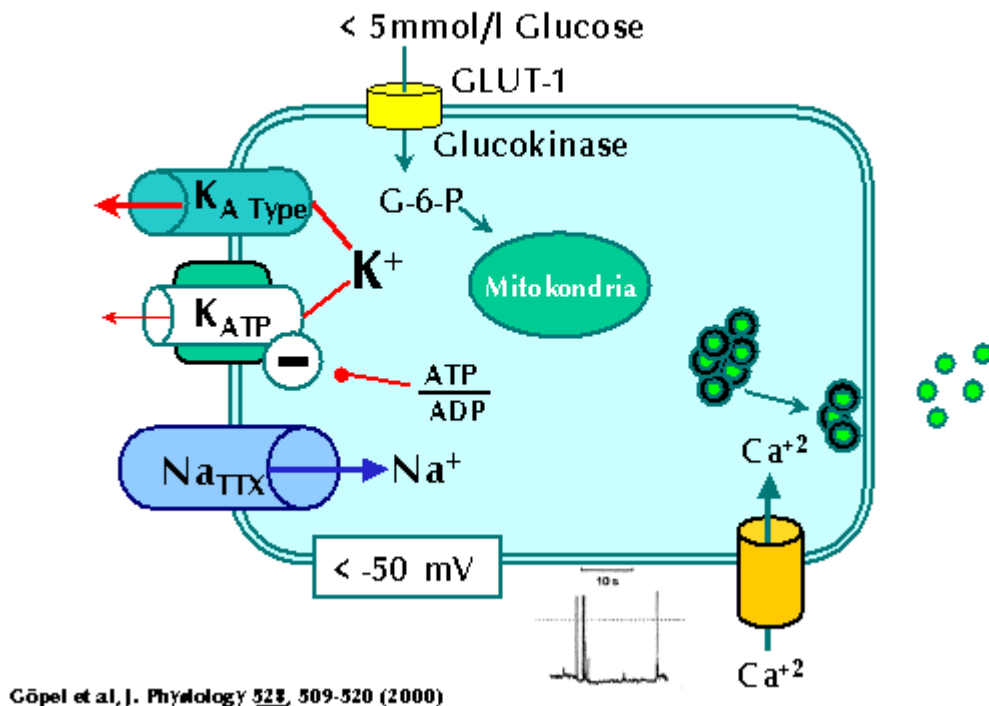
become electrically active with increasing (rather than decreasing) membrane polarization. Action potentials occur when the membrane potential is lower than 50 mvolts. The authors postulate that these arise from the influx of Na^+ and Ca^{++} ions and are coupled to secretion of glucagon. Action potentials in alpha cells appear to be initiated by an influx of Na^+ and Ca^{++} and to be terminated by voltage-dependent K+ channels. Release of glucagon from the "readily released pool" is a function of Ca++ entry.

Increased glucose levels lead to quiescence in the alpha cell. The K_A channel, the Na_{TTX} channel and the Ca^{++} channel become inactive at higher glucose concentrations. Secretion of glucagon falls to basal levels.

Although mouse alpha cells do metabolize glucose, the link between control of the ion channels and metabolism

Ionic Control of Glucagon Secretion

10 mmol/l Glucose (mouse)

GLUT-1

Glucokinase

$K_{A \, Type}$

G-6-P

SUR 1

K^+

K_{ATP}

Mitokondria

ATP
ADP

Na_{TTX}

Na^+

> -35 mV

Ca^{+2}

Göpel et al. J. Physiology 528, 509-520 (2000)

has not yet been identified.

As you can understand now, secretion of insulin and glucagon are extremely complicated processes. Many elements play a role in determining the sensitivity of alpha and beta cells to plasma signal substances. The review articles mentioned above give insight into current knowledge. You will have to follow the literature to keep up with advances in this clinically important field.