Pharmacological Agents That Directly Modulate Insulin Secretion

MÁIRE E. DOYLE AND JOSEPHINE M. EGAN

Diabetes Section, National Institute on Aging, National Institutes of Health, Baltimore, Maryland

Abstract .................................................................................. 106

I. Introduction ................................................................................ 106

II. Insulin synthesis and secretion ....................................................... 107

A. Stimulus secretion coupling/the metabolism of glucose .................. 107

1. The basic mechanism of glucose-induced insulin secretion ........ 107

2. Mitochondria—calcium effects and metabolism ......................... 108

B. Components of the insulin secretory pathway ......................... 109

1. Ion channels ........................................................................ 109

a. The potassium channels .................................................. 109

b. The voltage-dependent Ca$^{2+}$ channels ......................... 109

2. Second messengers ......................................................... 110

a. G-protein-coupled receptor systems .................................. 110

b. G-protein-coupled receptors on the β-cell .................. 112

C. Insulin synthesis ............................................................... 113

1. Transcriptional and translational regulation ......................... 113

2. Endoplasmic reticulum, insulin secretory vesicles and transportation, and exocytosis ........................................... 114

III. Pharmaceutical agents active in the treatment of disorders of glucose homeostasis .................................................. 115

A. Insulinotropic agents ............................................................. 115

B. Thiazolidinediones .............................................................. 117

C. Agents used in the treatment of hyperinsulinemia ..................... 117

D. The potential agents and targets for future treatment of diabetes ............................................................ 117

1. Agonists at the glucagon-like peptide-1 receptor .................. 117

2. Agonists at the purinergic 2 receptor .................................. 118

3. Imidazolines ................................................................. 118

IV. Drugs administered in the treatment of disorders other than diabetes that have effects on pancreatic insulin secretion and β-cell function .................................................. 118

A. Drugs implicated in post-transplant diabetes mellitus .................. 118

1. Calcineurin inhibitors ..................................................... 118

2. Antiproliferative agents ................................................... 120

B. Quinolines ....................................................................... 120

C. Somatostatin receptor agonists ........................................... 120

D. Drugs used mainly to treat hypertension .................................. 121

E. Methylxanthines ............................................................. 123

F. Phosphodiesterase inhibitors .............................................. 123

G. Diamidines .................................................................... 124

H. Colchicine ...................................................................... 124

I. Acetylcholine and cholinesterase inhibitors ................................. 125

J. Miscellaneous .................................................................... 125

1. Anesthetics ...................................................................... 125

2. Oral contraceptives .......................................................... 125

3. Anti-psychotic drugs ......................................................... 126

4. Glucosamine .................................................................... 126

Address correspondence to: Dr. Josephine M. Egan, Diabetes Section, #23, NIA/NIH, 5600 Nathan Shock Drive, Baltimore, MD 21224.
E-mail: eganj@vax.gcc.nia.nih.gov

Article, publication date, and citation information can be found at http://pharmrev.aspetjournals.org.
DOI: 10.1124/pr.55.1.7.

105
Abstract—Blood glucose levels are sensed and controlled by the release of hormones from the islets of Langerhans in the pancreas. The β-cell, the insulin-secreting cell in the islet, can detect subtle increases in circulating glucose levels and a cascade of molecular events spanning the initial depolarization of the β-cell membrane culminates in exocytosis and optimal insulin secretion. Here we review these processes in the context of pharmacological agents that have been shown to directly interact with any stage of insulin secretion. Drugs that modulate insulin secretion do so by opening the K_{ATP} channels, by interacting with cell-surface receptors, by altering second-messenger responses, by disrupting the β-cell cytoskeletal framework, by influencing the molecular reactions at the stages of transcription and translation of insulin, and/or by perturbing exocytosis of the insulin secretory vesicles. Drugs acting primarily at the K_{ATP} channels are the sulfonylureas, the benzoic acid derivatives, the imidazolines, and the quinolines, which are channel openers, and finally diazoxide, which closes these channels. Methylxanthines also work at the cell membrane level by antagonizing the purinergic receptors and thus increase insulin secretion. Other drugs have effects at multiple levels, such as the calcineurin inhibitors and somatostatin. Some drugs used extensively in research, e.g., colchicine, which is used to study vesicular transport, have no effect at the pharmacological doses used in clinical practice. We also briefly discuss those drugs that have been shown to disrupt β-cell function in a clinical setting but for which there is scant information on their mechanism of action.

I. Introduction

Diabetes mellitus (DM\textsuperscript{1}) is a chronic condition that is diagnosed by a blood test and requires life-long management (American Diabetes Association, 2002a; The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2002). It stands apart because of the importance of patient education (Nicollerat, 2000). For a detailed description of the etiological classification of the disease we refer the reader to Table 1 of the report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2002). The more patients understand about the disease the better they are enabled to make good decisions on its management. Dietary therapy and exercise are critical both in preventing and managing DM, and the results of the Diabetes Prevention Program Research Group indicate that changes in lifestyle (7% weight loss and 150 min of physical activity per week) reduced the incidence of diabetes by 58% (Knowler et al., 2002). Smoking should be avoided as it not only increases the prevalence of diabetes, regardless of exercise, strict diet, and low body mass index, but it greatly increases the probability of patients developing large vessel disease in the presence of DM (Wannamethee et al., 2001; American Diabetes Association, 2002b). In type 1 DM, where there is an absolute deficiency of insulin, insulin replacement forms a major component of treatment. In type 2 DM, insulin release from the pancreas is altered and may also be absolutely deficient in amount, and therefore its replacement also plays a part in management, especially when DM has been present for a long time.

Many new pharmacological agents have been added to our armamentarium of treatments for DM in the last decade. The goal of all treatments is the same irrespective of the cause of the DM: namely, to normalize blood glucose. Treating DM is not at all as simple as treating hypothyroidism, where replacing the missing hormone by the correct amount so that thyroid hormone and thyroid stimulating hormone levels are in the physiological range is all that is required. For normal metabolism insulin must be released from the pancreas in an exquisitely exact amount, at the correct time and in a correct pattern. The normal pancreas also senses the fasting and fed state as well as the energy content of the meals eaten. So far, no pharmacological agent can take the place of or restore this exquisite sensing capacity when it is diseased, and no agent can restore the exact pattern of insulin kinetics. The presence of so many types of insulin available to treat type 1 DM, and the availability of so many compounds to treat type 2 DM, is a testament to the complexity involved in normalizing blood glucose.

\textsuperscript{1}Abbreviations: DM, diabetes mellitus; GLUT2, glucose transporter 2; TCA, tricarboxylic acid; PKA, protein kinase A; PKC, protein kinase C; PLC-β, phospholipase C-β; PI, phosphatidylinositol; PIP, phosphatidylinositol phosphate; AC, adenylyl cyclase; DAG, diacylglycerol; PDE, phosphodiesterase; GIP, glucose-dependent insulinotropic factor; GLP-1, glucagon-like peptide 1; SST, somatostatin; IBMX, isobutyl methylxanthine; AKAP, A-kinase anchor protein; ACh, acetylcholine; CRE, cAMP response element; CREB, cAMP response element binding protein; NFAT, nuclear factor of activated T-cells; RRP, ready releasable pool; OGTT, oral glucose tolerance test; SNARE, soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein; SNAP, soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptor; IVGTT, intravenous glucose tolerance test; PTDM, post-transplant diabetes mellitus; ACE, angiotensin-converting enzyme; CCF, congestive cardiac failure; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; FMF, familial Mediterranean fever; GFAT, glucosamine:fructose-6-phosphate aminotransferase; IP_3, 1,4,5-trisphosphate.
At any one time glucose homeostasis is maintained by a balance between insulin secretion and insulin action. Because of robustness within the system in nondiabetic subjects, alteration in one of these will lead to compensation by the other. As a general concept, type 2 DM occurs because insulin secretion no longer compensates for insulin resistance due to increasing obesity, aging, illness, etc. (Elahi et al., 2002). Before the onset of biochemical type 2 DM, prediabetes exists in that the pancreas is secreting an increasing amount of insulin, in the face of increasing insulin resistance, to maintain nondiabetic levels of blood glucose; at that point in the maintenance of glucose homeostasis, the balance is fragile. Therefore, any pharmaceutical agent that has a negative impact on this fragile balance can cause type 2 DM to occur. An example is the use of the immunosuppressant tacrolimus, which inhibits calcineurin, decreases insulin secretion, and causes β-cell damage. Similarly, in the presence of known type 2 DM, the introduction of any agent that has a negative impact on glucose homeostasis could increase blood glucose and require adjustments to the diabetes treatment regimen. Therefore, a knowledge of possible or even theoretical interactions of pharmacologic agents that have an impact on glucose homeostasis would be beneficial in managing patients.

Pharmacological agents that are known to directly influence insulin secretion can be divided into two groups: 1) those prescribed because of their insulinotropic (i.e., insulin-releasing) properties are used in treating type 2 DM; and 2) those used for nondiabetes-related indications but have as their side effects direct negative or positive modulating effects on insulin release from the β-cells of the pancreas. A number of agents, including those used in the treatment of type 2 DM, have an impact on insulin action at the insulin receptor level, thus indirectly influencing the amount of insulin secreted. We will not address such indirect mechanisms, but will focus instead on agents proven to influence insulin secretion at the receptor level, thus indirectly influencing the amount of insulin, in the face of increasing insulin resistance, to maintain nondiabetic levels of blood glucose; at that point in the maintenance of glucose homeostasis, the balance is fragile. Therefore, any pharmaceutical agent that has a negative impact on this fragile balance can cause type 2 DM to occur. An example is the use of the immunosuppressant tacrolimus, which inhibits calcineurin, decreases insulin secretion, and causes β-cell damage. Similarly, in the presence of known type 2 DM, the introduction of any agent that has a negative impact on glucose homeostasis could increase blood glucose and require adjustments to the diabetes treatment regimen. Therefore, a knowledge of possible or even theoretical interactions of pharmacologic agents that have an impact on glucose homeostasis would be beneficial in managing patients.

Pharmacological agents that are known to directly influence insulin secretion can be divided into two groups: 1) those prescribed because of their insulinotropic (i.e., insulin-releasing) properties are used in treating type 2 DM; and 2) those used for nondiabetes-related indications but have as their side effects direct negative or positive modulating effects on insulin release from the β-cells of the pancreas. A number of agents, including those used in the treatment of type 2 DM, have an impact on insulin action at the insulin receptor level, thus indirectly influencing the amount of insulin secreted. We will not address such indirect mechanisms, but will focus instead on agents proven to have direct actions on the β-cell. Similarly, insulin itself, when used as a pharmacological agent in the treatment of type 2 DM, has an impact on the release of endogenous insulin from β-cells, but we refer the reader to a past review in this journal for further information on exogenous insulins (Vajo and Duckworth, 2000).

Pharmacological agents may alter insulin secretion by influencing the myriad of regulated physiologic molecular processes in the β-cell or modifying insulin secretion by cytolytic or cytotoxic means. At the molecular level a drug may influence insulin secretion by 1) acting primarily on the ion channels of the β-cell, and/or 2) by influencing the variety of second messenger pathways and the secretory machinery in the β-cell. For example, in the case of the sulfonylureas the perturbation occurs only on the β-cell K_{ATP} channel of the plasma membrane and mitochondrial membrane, but some receptor ligands, such as acetylcholine, have pleiotropic effects ranging from stimulation of the G-protein-coupled path-way, to activation of serine/threonine kinases, to changes in intracellular calcium levels, to growth effects, to effects on many systems besides β-cells. We begin with an outline of the known mechanisms and molecular processes involved in insulin secretion. We then categorize drugs in terms of their primary mode of therapeutic use or class, discussing how their pharmacological action can modify insulin secretion from the β-cell.

II. Insulin Synthesis and Secretion

A. Stimulus Secretion Coupling / the Metabolism of Glucose

1. The Basic Mechanism of Glucose-Induced Insulin Secretion. Blood glucose levels are very tightly controlled by rapid pulsatile release of insulin from β-cells (Fig. 1). Glucose equilibrates through the GLUT2 transporter across the plasma membrane of the β-cell. It is rapidly phosphorylated to glucose 6-phosphate by glucokinase, which thereafter determines the rate of glycolysis, i.e., acts as the glucose sensor and pyruvate generation for entry into the tricarboxylic acid (TCA) cycle in mitochondria. Subsequent oxidative metabolism provides the link between the products of glucose metabolism and insulin secretion. The resultant increase in the ATP/ADP ratio in the cytosol causes depolarization of the plasma membrane by closure of the ATP-sensitive K⁺ channels. This permits opening of voltage-dependent Ca²⁺ channels and an increase in cytosolic Ca²⁺, which then triggers fusion of insulin-containing secretory vesicles to the plasma membrane, and exocytosis of insulin follows rapidly. Besides activating K⁺ channels, ATP appears to be a major permissive factor for movement of insulin vesicles toward the plasma membrane and for priming of exocytosis (Eliasson et al., 1997) and, as will be discussed under Section II.B.2.a.i., it provides the phosphate for protein kinase A (PKA)-mediated phosphorylation of proteins important in exocytosis.

Insulinotropic agents may act either by direct stimulation of insulin secretion or by amplifying insulin secretion induced by other means. Initiators of insulin secretion include glucose and the plethora of sulfonylurea drugs that bind to and effect closure of the K_{ATP} channels. Glucose stimulation of the β-cell is permissive of further stimulation with a variety of insulinotropic agents. This makes perfect teleological sense, as hypoglycemia would result from insulin secretion unless blood glucose was also rising. This is particularly true of potentiatators such as the gut hormones that stimulate intracellular cAMP production and agents that activate phospholipase C-β (PLC-β), and so their ability to increase insulin secretion is referred to as glucose-dependent (see Section II.B.2.b.). The resultant activation of PKA and protein kinase C (PKC), in turn, can phosphorylate and activate the K_{ATP} channels and mobilize the secretory vesicles. Therefore, we discuss both the ion
channels and the second messenger pathways involved in these processes.

2. Mitochondria—Calcium Effects and Metabolism. The mitochondria, specifically the TCA cycle and electron transport that occur within them, are extremely important in the regulation of insulin secretion. Electron microscopy of the rat β-cell reveals that the mitochondria are in close proximity to the insulin secretory vesicles. They essentially act as fuel sensors coupling the nutrient metabolism to the process of exocytosis (see Section II.C.2.). They are the primary source of energy within the β-cell-regulating recovery from membrane depolarization, protein synthesis, and vesicle transport. In the 1970s it was demonstrated that mitochondrial poisons inhibit glucose-induced insulin secretion (Hellman, 1970). In 1992 a form of type 2 DM, maternally inherited diabetes and deafness, was linked to mutations in the mitochondrial genome (Ballinger et al., 1992). Both of these facts point to the importance of the mitochondria in the process of insulin secretion. The TCA cycle metabolizes the products of glycolysis and provides the reducing equivalents that activate the electron transport chain, resulting in hyperpolarization of the mitochondrial membrane and generation of ATP, which is exported to the cytosol, thus increasing the ATP/ADP ratio.

Calcium signaling is associated with a significant mitochondrial uptake of Ca^{2+}. Intracellular calcium controls the key rate-limiting steps in the TCA cycle through activation of pyruvate dehydrogenase and at least two TCA cycle enzymes: isocitrate dehydrogenase and α-ketoglutarate dehydrogenase (reviewed by Duchen, 1999). As the initial mitochondrial response precedes the elevation in cytosolic calcium it is likely that calcium is involved in the maintenance rather than in the initiation of glucose metabolism-secretion coupling.
and calcium signaling alone is not sufficient to maintain secretion. There has been much speculation as to the mitochondrial factor that couples glucose metabolism with insulin secretion (other than ATP). Malonyl-CoA has been proposed in this role (Prentki et al., 1992) but the disruption of malonyl-CoA accumulation during glucose metabolism did not reduce insulin secretion (Antinollo et al., 1998). Therefore, the function of long-chain acyl-CoA derivatives as coupling factors remains in dispute. Glutamate is produced from the TCA cycle intermediate α-ketoglutarate by glutamate dehydrogenase (Fisher, 1985) and has been shown to be involved in priming secretory vesicles for exocytosis with positive correlations between intracellular β-cell glutamate concentration and insulin secretion (Maechler and Wollhein, 1999).

The mitochondrial permeability transition pore facilitates the passage of calcium across the mitochondrial membrane in the absence of external adenine nucleotides (reviewed in Duchen, 2000 and Crompton, 1999). The main circumstances in which the channel appears to be active are when the intramitochondrial calcium concentrations are high, the ATP/ADP ratio is low, and there is a rise in inorganic phosphate (Pi) or when the cell is in a state of oxidative stress (Szewczyk and Wojtczak, 2002). This pore has various points of contact on the inner and outer membranes of the mitochondrion and consists of the outer membrane voltage-dependent anion channel (VDAC), the inner membrane adenine nucleotide translocase (ANT), and cyclophilin D.

B. Components of the Insulin Secretory Pathway

1. Ion Channels.

a. The Potassium Channels. The K\textsubscript{ATP} channel on the β-cell consists of a hetero-octomeric complex of four pore subunits, which are known as K\textsubscript{ir6.2}, and four regulatory, or SUR, subunits (Aguilar-Bryan and Bryan, 1999). The K\textsubscript{ir6.2} does not form functional channels in the absence of the SUR protein. Thus, the SUR protein may serve as a chaperon protein for K\textsubscript{ir6.2} (Zerangue et al., 1999) and there is evidence that the reverse may also be true (Clement et al., 1997). Both subunits contain binding sites for ligands that initiate conformational changes in the channel and can effect the channel’s sensitivity to other ligands and/or open or close the channel.

The K\textsubscript{ir6.2} subunit forms the pore of the channel through which the potassium ions permeate, and the “gate” consists of four M2 helices that are thought to form an inverted tepee shape that converges on the cytoplasmic face (Doyle et al., 1998). Both adenosine nucleotides ATP and ADP bind effectively to the K\textsubscript{ir6.2} subunit. The ATP binding site on K\textsubscript{ir6.2} is not defined, although an -F\textsubscript{333}GNTIK338 motif found in ATPases present in the intracellular region of K\textsubscript{ir6.2} is a candidate site (Drain et al., 1998). ATP binds K\textsubscript{ir6.2} in an Mg\textsuperscript{2+}-independent manner and changes the conformation of the tepee orientation to close the gate. This effect of ATP on K\textsubscript{ir6.2} is modulated by the configuration of the SUR1 subunit. There is also evidence that ADP inhibits the K\textsubscript{ATP} channel by binding in an Mg\textsuperscript{2+}-independent manner to this subunit (Gribble et al., 1997a). In the same manuscript, Gribble and colleagues also show evidence for a low-affinity sulfonylurea site on this subunit.

The SUR1 subunit contains the high-affinity sulfonylurea binding site and two well-defined nucleotide binding domains 1 and 2 (NBD1 and NBD2). Ueda and colleagues have suggested a model for the interaction between the binding of nucleotides to these sites and their effect on channel activity (Ueda et al., 1999). When the ATP/ADP ratio is low, ATP is bound to NBD1 and ADP is bound to NBD2. The relationship between SUR1 and K\textsubscript{ir6.2} in this configuration is such that the channel is open and ATP is not bound to the K\textsubscript{ir6.2} subunit. When the ATP/ADP ratio is increased, the decrease in MgADP leads to the release of bound MgADP from NBD2 and a consequential release of ATP from NBD1. The change in conformation increases the affinity of K\textsubscript{ir6.2} for ATP, and the K\textsubscript{ATP} channel closes.

Although it is known that the nucleotide binding site is located on the cytoplasmic side, the exact position of the sulfonylurea binding site on the SUR1 is not known. There is much indirect experimental evidence to suggest that it is located on the inner surface of the membrane (reviewed in Ashcroft and Gribble, 1999).

Other cytosolic agents that can affect the ATP sensitivity of the K\textsubscript{ATP} channel are the membrane phosphatidylinositol phosphates (PIPs), which increase open probability and reduce ATP sensitivity. The effect probably involves an electrostatic component, as the negatively charged phosphate groups at the inositol ring are critical. The highly negatively charged phosphatidylinositol-3,4,5-triphosphate and phosphatidylinositol 4,5-bisphosphate (PIP\textsubscript{2}) are more effective than phosphatidylinositol-4-phosphate (PIP), and phosphatidylinositol (PI) has no effect (Baukrowitz et al., 1998; Shyng and Nichols, 1998). The relative contributions of these effects remain to be determined but are important when considering K\textsubscript{ATP} sensitivity in excised patches. These become more sensitive with the washing out of the membrane phospholipids (Ashcroft and Gribble, 1999). A recent report indicates that the PIPs act by displacing ATP from its nucleotide binding site (Krauter et al., 2001).

Other potassium channels found on the β-cell include the delayed rectifier channels, the Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels, α-adrenoreceptor-activated K\textsuperscript{+} channels and G-protein-gated K\textsuperscript{+} channels. For a comprehensive review of these we refer the reader to a review by Dukes and Philipson (Dukes and Philipson, 1996).

b. The Voltage-Dependent Ca\textsuperscript{2+} Channels. With the exception of mouse β-cells, two types of voltage-dependent calcium channels have been identified on the β-cell. These can be distinguished from each other by their
generates cAMP and PPi from ATP, and the cyclic nu-
activity of two enzyme systems, AC, activation of which
lular cAMP is regulated by the balance between the
activity of the L-type Ca$^{2+}$ channel is potentiated by the
protein kinases A and C (Åmmälä et al., 1994). It is
important to note that when comparing results from
experiments across species that mouse islets contain
only the L-type channel (Rorsman et al., 1988).

2. Second Messengers.

a. G-Protein-Coupled Receptor Systems. In terms of
insulin secretion the most important transducer of li-
gand activation is the guanylnucleotide-binding (GTP)
protein system or G-protein-coupled system. This con-
ists of a seven-transmembrane receptor that is coupled
to a heterotrimeric G-protein; i.e., it consists of three
subunits: the $\alpha$-subunit, which contains the guanine
nucleotide binding site, and the tightly associated $\beta$- and
$\gamma$-subunits. The activated receptor induces a conformation-
tional change in the G-protein $\alpha$-subunit-releasing
guanosine diphosphate (GDP) followed by binding of
GTP. The GTP-bound form of the $\alpha$-subunit dissociates
from the receptor and from the stable $\beta\gamma$-dimer. Both the
GTP-bound $\alpha$-subunit and the released $\beta\gamma$-dimer can
either stimulate or inhibit an effector enzyme on the
inner surface of the membrane that converts precursor
molecules into second messengers. In the case of the
$\beta$-cell there are two membrane-bound enzyme systems:
adenylyl cyclase (AC), which converts adenosine triphos-
phate (ATP) into cyclic AMP (cAMP); and phospholipase
C (PLC), which cleaves phosphoinositides into two sec-
ond messengers, inositol 1,4,5-trisphosphate (IP$_3$) and
diacylglycerol (DAG), which are involved in Ca$^{2+}$ release
and the activation of PKC (serine/threonine kinases),
respectively.

Three G-protein subfamilies are found in $\beta$-cells: $G_a$,
which stimulates AC and so increases cAMP production;
$G_i/G_o$, which inhibits adenylyl cyclase; and the $G_q$ sub-
family, which is associated with the phosphatidylinosi-
tol system alone. The difference between the different
G-protein-coupled receptors is determined by the struc-
ture of the $\alpha$-subunit that is unique to each subfamily.
Table 1 outlines the types of G-protein-coupled receptors
present on the $\beta$-cell and indicates the second messenger
pathways to which they are coupled. (For a comprehen-
sive review of G-protein-coupled receptors see Haga et
al., 1999.)

i. Adenylyl Cyclase System. The amount of intracellu-
lar cAMP is regulated by the balance between the
activity of two enzyme systems, AC, activation of which
generates cAMP and PPi from ATP, and the cyclic nu-
cleotide phosphodiesterases (PDEs), which metabolize
cAMP. Activation of AC and the consequent rise in
cAMP results in a significant up-regulation of the activity
of the cAMP-dependent protein kinase PKA family,
comprising ubiquitous serine/threonine phosphorylating
enzymes. This leads to a cascade in which phosphoryla-
tion of vesicular and plasma membrane proteins, volt-
age-dependent calcium channels, and potentially other
ion channels (possibly even the GLUT2 transporter) re-
sults in augmentation of glucose-induced exocytosis of
insulin, as well as phosphorylation of transcription fac-
tors, which increases insulin gene promoter activity and
therefore will influence insulin secretion long-term (re-
viewed in Jones and Persaud, 1998). Within the $\beta$-cell,
stimulation of AC through $G_\alpha$ under normal physiolog-
ical circumstances occurs mainly via the gut hormone
receptors for glucose-dependent insulinotropic factor
(GIP) and glucagon-like peptide 1 (GLP-1), whose levels
increase dramatically after eating (see Section
II.B.1.b.i.). Somatostatin (SST), released from the en-
tero-endocrine cells and from $\delta$-cells of the islets, inhib-
its AC by activating $G_\delta$ (see Section II.B.1.b.i.). cAMP
synthesis plays a major role in augmenting glucose-
induced insulin release. The importance of this system is
clearly demonstrated in type 2 DM, where $\beta$-cells of the
pancreas have become resistant to the insulinotropic
effects of GIP (Elahi et al., 1994), probably because of a
maladaptation within the AC/PKA system because of the
high glucose (Livak and Egan, 2002).

Under experimental conditions cAMP analogs, activa-
tors of AC and PDE inhibitors, most notably isobutyl
methylxanthine (IBMX), all increase glucose-mediated
insulin secretion from islets (Prentki and Matschinsky,
1987). Basal levels of cAMP (most likely by controlling
basal, or tonic, PKA activity, as we will see below) must
be required for full expression of glucose-induced insulin
secretion because the addition of IBMX not only to islets
but to insulinoma cell lines (where no hormones, etc.,
which might activate AC are present) greatly potentiate
so glucose-induced insulin secretion (Montrose-Rafiza-
deh et al., 1994).

At least 11 families of PDE inhibitors have been iden-
tified (for a review see Conti, 2000). PDEs present in
$\beta$-cells have been determined as total PDE activities
from crude islet preparations and the presence of PDEs
3 and 4, and calcium-sensitive PDEs, in $\beta$-cells have

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>G-Protein</th>
<th>Effector Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretin, GLP-1, GIP</td>
<td>$G_\alpha$</td>
<td>Adenylyl cyclase</td>
</tr>
<tr>
<td>Purinergic $P_1$</td>
<td>$G_i/G_o$</td>
<td>Adenylyl cyclase</td>
</tr>
<tr>
<td>Muscarinic/cholinergic</td>
<td>$G_q$</td>
<td>Phospholipase C</td>
</tr>
<tr>
<td>Adrenergic</td>
<td>$G_\sigma$</td>
<td>Adenylyl cyclase</td>
</tr>
</tbody>
</table>

Table 1

Outline of the G-protein-coupled receptors present on the $\beta$-cell

Adrenergic Muscarinic/cholinergic
Purinergic P1
Purinergic $P_{xy}$
Muscarinic/cholinergic
Adrenergic

At least 11 families of PDE inhibitors have been identified (for a review see Conti, 2000). PDEs present in $\beta$-cells have been determined as total PDE activities from crude islet preparations and the presence of PDEs 3 and 4, and calcium-sensitive PDEs, in $\beta$-cells have
been inferred (Sugden and Ashcroft 1981; Parker et al., 1995; Shafiee-Nick et al., 1995). One, in particular, PDE 3B, has been shown to be activated by IGF-1 (Zhao et al., 1997) and leptin (Zhao et al., 1998). Leptin, by activating PDE 3B (activation of which is PI3 kinase-dependent), inhibits GLP-1-mediated increases in glucose-induced insulin secretion. The direct involvement of PDE 3B in this inhibition was confirmed by the ability of N6-monobutyl-cAMP, a form of cAMP resistant to hydrolysis by this isozyme, to increase insulin secretion in the presence of glucose (Zhao et al., 1998). In a similar paper from the same laboratory Zhao and colleagues demonstrated the same PDE 3B-dependent inhibition of insulin secretion (Zhao et al., 1997). Han and colleagues (Han et al., 1999) have shown in isolated islets that PDE 1C and PDE 4 inhibit glucose-mediated insulin secretion. Inhibition of PDE 1C activation stimulated glucose-induced insulin secretion in a dose-dependent manner. The combined inhibition of PDE 1C, 3, and 4 had as potent an effect on augmentation of insulin secretion by glucose as nonspecific inhibition by IBMX. Interestingly, PDE 1C activity was elevated upon stimulation of β-cells with glucose, pointing to a feedback control of glucose-induced insulin secretion via degradation of cAMP. The authors speculate that it is the increased intracellular calcium from glucose treatment of the islets that is causing activation of the calcium/calmodulin-dependent PDE 1C.

Clearly, therefore, an agent that could be used in vivo to inhibit PDE 1C in β-cells would be a powerful tool to increase intracellular cAMP (and therefore insulin secretion), especially if it was used in conjunction with cAMP elevating agents such as GLP-1 or PACAP (pituitary adenyl cyclase activating peptide), and could have potential in the treatment of type 2 diabetes. No such therapeutic agent is yet available. The methylxanthines used in the treatment of asthma stimulate insulin secretion and are known to antagonize phosphodiesterases, but at pharmacological concentrations this is not the primary mode by which they induce insulin secretion (see Section IV.E.).

PKA is a key component in the regulation of insulin secretion by cAMP. It mediates many of the phosphorylation reactions required for secretion by β-cells. Inhibition of PKA in isolated islets and most insulinoma cell lines abolishes GLP-1-mediated insulin secretion and diminishes glucose-mediated insulin secretion (Wang et al., 2001). Thus basal (nonstimulated) levels of PKA activity are required for optimal glucose-mediated insulin secretion. PKA enzymes are composed of a regulatory and catalytic subunit. There are at least four different types of regulatory unit (RIα, RIβ, RIλα, RIλβ) and three catalytic subunits (Cα, Cβ, Cγ). It is not clear which isoforms of PKA are present in the β-cell, as very little work has been done to examine this. RIα, RIλα, and Cα have been found in all tissues examined so far, so it is probable that they are expressed in the β-cell (Jones and Persaud, 1998). When cAMP binds to the R subunit in the β-cell, a shift in confirmation causes the release of the monomeric C from the holoenzyme. The catalytic subunit is inactivated when it again rearranges with the R subunit. Spacial regulation is through compartmentalization via A-kinase anchor proteins (AKAPs). These AKAPs tether the R subunits to various subcellular structures, therefore placing PKA in proximity to upstream effector molecules (AC, for example) and downstream targets (secretory vesicles and L-type calcium channels, for example). To choreograph insulin secretion PKA must be associated with the plasma membrane, and this localization is mediated through AKAPs. When an inhibitor of RII-AKAP (Ht31 peptide) association was introduced into cultured islets, GLP-1-mediated insulin secretion was blocked (Lester et al., 1997), demonstrating that PKA must be tethered for cAMP generation by GLP-1 to modulate insulin secretion. AKAP18, in RINm5F cells (an insulinoma cell line), couples PKA to L-type calcium channels (Lester et al., 2001).

To add to this complexity there is now evidence that PDEs are also tethered in the AKAP/PKA complex (Dodge et al., 2001). This has been shown for heart muscle, where PDE4, mAKAP, and PKA form part of a complex. Under basal conditions it appears that tethered PDE4 metabolizes cAMP diffusing into the local environment. Upon hormone stimulation, the increased flow of cAMP overrides the PDE activity and active C subunits are released. Two important regulatory factors built into the complex favor signal termination. The anchored PDE is active and PKA, in turn, phosphorylates PDE, which increases its V max (Oki et al., 2000). As PDE4 is also expressed in β-cells, such a system is likely to be operative in the regulation of insulin secretion (Han et al., 1999).

Recently, very elegant work by Takahashi and colleagues (Takahashi et al., 1999) also emphasized the importance of PKA for ATP-mediated insulin secretion by demonstrating that PKA is important for the action of ATP on exocytosis. Using mouse islets, they showed that ATP, after controlled and uniform elevations in intracellular calcium, hastened and augmented exocytosis of insulin granules. This agrees with previous data that showed ATP increased insulin secretion even when the KATP channel was open and intracellular calcium was clamped at a high level (Eliasson et al., 1997). Takahashi and colleagues then proceeded to demonstrate that ATP was not inducing exocytosis by simply being hydrolyzed, and thus serving as a source of energy for motor protein to transport vesicles, because ATP hydrolysis did not appear to be required for ATP-induced insulin secretion, as the introduction of a hydrolysis-resistant ATP analog (ATP3S) worked even better at inducing insulin secretion than ATP itself. They further dissected the adenyl cyclase/cAMP/PKA pathway to determine whether it was involved. The action of ATP on insulin secretion was abolished by 1) an antagonist of
cAMP (Rp-cAMP), 2) an irreversible inhibitor of adenylyl cyclase (MDL-12,330A), and 3) inhibitors of PKA (H89 and H7). AMP-PPN, another hydrolysis-resistant analog, could not substitute for ATP. These data suggest that the action of ATP is mediated by phosphorylation because ATP(γS), but not AMP-PPN, can serve as a phosphate donor for kinase reactions. Both analogs can be converted to cAMP by adenylyl cyclase. These findings are significant for a number of reasons. They again show that even without exogenous stimulation of PKA by activators of cAMP (ATP-induced insulin secretion without addition of cAMP-generating compounds, for example), PKA is still needed for full expression of insulin secretion. Indeed, the addition of forskolin did not further induce insulin secretion in the presence of high levels of ATP, but did so at low levels, indicating that basal activities of AC (and presumably PKA activation) are sufficient to mediate the maximal effects of ATP, which under usual physiologic situations would be generated from glucose metabolism in the TCA cycle. Almost certainly there are cyclical phosphorylation/dephosphorylation reactions ongoing at any one time within the β-cell that maintain the β-cell in a state of readiness for minute-to-minute requirement of insulin release or inhibition. Takahashi et al. also unify the thinking behind the actions of ATP and PKA in β-cells. Besides ATP simply enhancing Ca²⁺ entry into the cells, it serves as the source for cAMP and donates the phosphate for PKA-mediated phosphorylations (Takahashi et al., 1999).

To reiterate, it should be noted that no therapeutic agent has yet been demonstrated to directly stimulate or inhibit PKA activity. If PKA down-regulation were a feature of type 2 DM, in which the β-cell no longer releases insulin in response to a glucose load, then an agent that could restore PKA activity would be useful. However, it has not been shown that such is the case. It also important to note that cAMP also regulates insulin secretion via mechanisms that do not require activation of PKA (Renstrom et al., 1997).

It is evident from the above review of the literature that much is left to be explored in the phosphorylation and dephosphorylation steps involved in insulin secretion in normal physiological conditions. The possibility of the existence of more than one of the isomeric forms for the G-proteins (Emami et al., 1998), AC (Fimia and Sassone-Corsi, 2001), AKAPs, and PKA subunits in the β-cell, together with the compartmentalization of PKA with phosphatases (Coughlan et al., 1995) allows for multiple and differential levels of control of insulin secretion by glucose, hormones, and various drugs.

ii. Calcium/Phosphatidylinositol System. Of the four main phosphoinositide-specific PLCs (PLC-β, -γ, -δ, and -ε) only the PLC-β isoforms are known to be activated by G-protein-coupled receptors (Williams, 1999). The collective evidence weighs in favor of the presence of all four PLC-β isoforms in the pancreas (PLC-β 1, 2, 3, and 4), based on data from rat pancreas (Kim et al., 2001; Wang et al., 2000). However, it is not yet known which of these isoforms are coupled to the muscarinic receptor in the β-cell. As with the AC second messenger system, the presence of the various isoforms raises the possibility that neurohumoral agonists such as acetylcholine activate different PLC isoforms from nutrients such as glucose or fatty acids, thus allowing for differential regulation. There is evidence that PKC, which is activated by DAG, is instrumental in second-phase insulin secretion, as the general PKC inhibitors staurosporin and Gö 6976 are known to inhibit this phase (Zawalich and Zawalich, 2001).

b. G-Protein-Coupled Receptors on the β-Cell
i. Gut Hormone Receptors. The G-protein-coupled receptors present on the plasma membrane of the β-cell in this category belong to the class B family of G-protein-coupled receptors. Included in this family are GLP-1, glucagon, GIP, secretin, pituitary adenylyl cyclase activating peptide, calcitonin, latrophilin, and parathyroid hormone and calcitonin gene-related peptide receptors. These peptide hormones are ligands for hormone-specific seven-transmembrane receptors that are coupled to the Gs protein and stimulate cAMP production in the β-cell. In general, there is very little cross-reactivity among these receptors, e.g., glucagon binds with 100-1000-fold less affinity to the GLP-1 receptor than does GLP-1 itself (Fehmann et al., 1994). Of these peptide hormones only GLP-1 has so far been used clinically to stimulate insulin secretion (see Section III.D.1.) and glucagon is used to treat hypoglycemia secondary to exogenous insulin and as a test of β-cell reserve.

Five types of SST receptor genes have been cloned and have been shown to produce six somatostatin receptor types (sstr) of protein, five of which (sstr1, sstr2b, sstr3, sstr4, and sstr5) have been found on human cells (reviewed in Viollet et al., 1995). All of the ss receptors are of the seven-transmembrane G-coupled type and are coupled to the Gs protein (Viollet et al., 1995). In mammals there are two biologically active forms of ss that are 14 and 28 amino acids long (ss 14 and ss 28, respectively). While all subtypes of the ss receptor have been detected by immunohistochemistry in β-cells (Portela-Gomes et al., 2000; Kumar et al., 1999), rodent experiments indicate that inhibition of insulin release is mediated through sstr5 only (Strowski et al., 2000; Zambre et al., 1999); sstr5 displays a higher specificity for ss 28 (Thermos et al., 1990). Ss receptor ligands are used in the treatment of diseases of hyperinsulinemia and tumors (reviewed in Lamberts et al., 1996; see Section IV.C.).

ii. Muscarinic Receptors. Acetylcholine (ACh), the major parasympathetic neurotransmitter, is released from intrapancreatic nerve endings. The arrival of an action potential at the nerve terminal triggers ACh release due to the influx of calcium. The effects of ACh at the effector cell are mediated by muscarinic cholinergic receptors, of which five subtypes are known to exist
(M₁–M₅). The M₂ and M₄ subtypes are known to be coupled to Gᵢ and are pertussis toxin-sensitive, while the remaining subtypes (M₁, M₃, and M₅) are coupled to Gₛ. Activation of the muscarinic receptors on the β-cell of the pancreas are not pertussis toxin-sensitive, and thus their activation is mediated by the Gₛ-coupled M₁/M₃/M₅ category and not the M₂/M₄ adenylyl cyclase-linked pertussis toxin-sensitive pathway (reviewed in Gilon and Henquin, 2001). Reverse transcriptase-polymerase chain analysis of extracts of rat islets indicates the presence of all three Gₛ-coupled subtypes with a predominance of M₁ and M₃, which are expressed approximately to the same extent (Iismaa et al., 2000). Presently we do not know of any muscarinic receptor ligands that are used specifically to modulate insulin secretion; neither have we encountered any reports of the use of agonists the muscarinic receptors as potential agents to enhance insulin secretion and hence treat diabetes. It is known that the drug tacrine, an acetylcholinesterase inhibitor, affects insulin secretion (see Section IV.H.).

iii. Adrenergic Receptors. There is physiologic and pharmacologic evidence for the presence of inhibitory and stimulatory adrenoreceptors on the β-cell. The inhibitory α-adrenoreceptor has been characterized as being of the α₂-subtype (Cherksey et al., 1983) and the stimulatory β-adrenoreceptor as the β₂-subtype (Fyles et al., 1986). The α₂-subtype is coupled to Gₛ/G₁₆ and the β₂ is coupled to the Gₛ protein. Stimulation of the α₂-adrenoreceptors in the β-cell is believed to directly activate a G-protein-gated K⁺ channel, thereby inhibiting exocytosis (Rorsman et al., 1991). Epinephrine, which activates both types of adrenoreceptors, inhibits glucose-induced insulin secretion (Cawthorn and Chan, 1991) indicating that α₂ is the predominant adrenergic receptor in β-cells. Such an inhibitory action is suppressed when islets are exposed to pertussis toxin (which causes irreversible ADP-ribosylation of the Gₛ-subunit and prevents it interaction with the receptor). Interestingly, however, selective α₂ blockade with deriglidole, while preventing epinephrine-induced inhibition of insulin secretion, did not potentiate basal or intravenous or oral glucose tolerance-induced insulin release in nondiabetic humans (Natali et al., 1998). This leads to the assumption that in healthy people neither basal nor postabsorptive insulin secretion is under tonic adrenergic tone. This may not be the case in type 2 diabetes, as it was shown that acute α-adrenergic blockade improved glucose-potentiated insulin secretion in that specific group of people (Broadstone et al., 1987). While plasma noradrenaline levels were increased by the blockade in both control and diabetic subject, it was increased much more in the diabetic condition, so it is conceivable that synaptic cleft levels of norepinephrine are higher in diabetic than nondiabetic subjects. There are a number of α₂-adrenoreceptor agonists and antagonists that are used in the treatment of diseases of the circulatory system (see Section IV.D.).

iv. Purinergic Receptors. There are two main classes of purinergic receptors: those stimulated by adenosine are classified as P₁ receptors and those that respond to ATP are known as P₂ receptors. There are four subtypes within the P₁ class: A₁, A₂a, A₂b, and A₃. The A₂b subtypes are coupled to Gₛ and stimulate adenylyl cyclase, whereas A₁ and A₃ are coupled to Gₛ/G₁ and inhibit adenylyl cyclase. Stimulation of the A₁ receptor on the β-cell inhibits insulin secretion (Bertrand et al., 1989). Using two stable P₂ receptor agonists, α,β-methylene ATP and ADPβS, which are more specific for the P₂Y and the P₂Y receptor agonists, respectively, Petit and colleagues have shown that both of these receptors exist on the β-cell (Petit et al., 1998). Their action is to potentiate glucose-stimulated insulin secretion. Of the purinergic receptors only the A₁ has been shown to be important in pharmacological action in the β-cell, as it is antagonized by the group of compounds known as the methylxanthines (see Section IV.E.). There are as yet no known pharmacological agents that stimulate ATP production, nor are there any pharmacological agents in use that are known to be effective at the P₂ receptors on the β-cell. However, the P₂ receptors are the targets of research for future treatments for diabetes (see Section III.D.3.).

C. Insulin Synthesis

1. Transcriptional and translational regulation. The β-cell is the only cell in the adult body that can make proinsulin mRNA. Other cells have been programmed to transcribe proinsulin (Laub and Rutter, 1983) and other neuroendocrine cells are able to process proinsulin to mature insulin secretory vesicles and secrete insulin in a regulated manner (Moore et al., 1983), but the initiation of transcription of the proinsulin gene is unique to the β-cell of the pancreas. PDX-1 is perhaps the most extensively studied insulin transcription factor because it is essential for the maintenance of the β-cell phenotype and pancreatic invagination and development (Jonsdottir et al., 1996). It binds to the A-box motifs of the insulin promoter and is involved in glucose- and GLP-1-mediated up-regulation of the insulin gene (MacFarlane et al., 1999). Glucose induces translocation of PDX-1 to the nucleus within 15 to 30 min (Wang et al., 1999). This mechanism is PKA-independent (Wang et al., 2000) but is known to be sensitive to wortmannin-LY 294002 (PI 3-kinase inhibitors) and has been shown to require PI 3-kinase activation (Rafiq et al., 2000). Previously it was believed that the restriction of insulin transcription to this single cell type was dependent on the expression of a unique compliment of transcription factors (including PDX-1) within the β-cell. However, PDX-1−/− mice do not have a pancreas, but insulin-positive cells are observed in the rudimentary bud of the organ (Offield et al., 1999). Consequently, PDX-1 is not necessary for transcription because there are other transcription factors that can bind to the A-box element of the insulin promoter and cooperate with the coactivator β2/NeuroD
to activate transcription of insulin and thus act in place of PDX-1 when it is not expressed. In this case the Lim-homeodomain proteins Lmx1.1 and Lmx1.2 are capable of forming a more effective transcription factor complex with β2/NeuroD than is PDX-1 (Ohneda et al., 2000). Thus, factors that may be influential in reserving insulin transcription to the β-cell alone could include the presence, in other cell types, of inhibitory proteins that bind to and inactivate key factors and/or the absence of certain transcription factors upstream of those directly involved in insulin transcription. For a comprehensive review of the transcription factors that bind to the insulin promoter we refer the reader to the following reviews: Melloul et al. (2002); Ohneda et al. (2000); and Sander and German (1997).

Elevation of the second messengers cAMP and Ca$^{2+}$ can enhance insulin transcription by the activation of two separate types of sites on the insulin gene promoter. The human insulin promoter contains two cAMP response elements (CRE, TGACGTCA), both of which are responsible for cAMP inducibility (Inagaki et al., 1992). Activation at this site is through a PKA-dependent phosphorylation of the basic region leucine zipper transcription factor, CREB (CRE binding protein), which then binds the coactivator CREB binding protein (CBP), resulting in the activation of transcription of the insulin gene. The calcium, calcineurin/NFAT (nuclear factor of activated T-cells) pathway is important in the regulation of insulin gene transcription and is triggered by the rise in intracellular calcium. Calcineurin is a serine/threonine phosphatase (protein phosphatase 2B; Rusnak and Mertz, 2000) and is unique among other phosphatasases of its family (PPI and PP2) in that Ca$^{2+}$-calmodulin is required for its activation. Calcineurin dephosphorylates (on multiples serines) the transcription complex, NFAT, exposing its nuclear localization signal (Crabtree, 2001; Rao et al., 1997). The dephosphorylated NFAT complex is maintained in the nucleus as long as Ca$^{2+}$ concentrations are elevated, thus keeping calcineurin in the activated state (Timmerman et al., 1996). NFAT has been shown to bind to and activate at least one of the three putative NFAT binding sites on the rat insulin promoter, and calcineurin inhibitors are known to prevent the glucose induced transcription of insulin (Lawrence et al., 2001).

Within the nucleus of the β-cell the preproinsulin mRNA is modified by the addition of a 5′-methylguanine cap, the RNA is cleaved to signal the addition of a poly-A tail, and the noncoding introns are excised. The now mature mRNA is translocated to the cytoplasm where translation to preproinsulin begins on membrane-bound ribosomes. The cytoplasm of the β-cell contains large amounts of preproinsulin mRNA (10–15% of the total mRNA) that are dormant in glucose concentrations of >3.3 mM. It is the initiation of translation ultimately leading to insulin biosynthesis that is acutely regulated by glucose. Translocation to the ribosomes and translation increase within minutes with glucose concentrations of >3.3 mM (Welsh et al., 1986). The rates of transcription and translation are not acutely coupled, demonstrated by the delay of several hours between inhibition of transcription and a dampening of translation (Jahr et al., 1980). Upon the translation of about 50 residues the nascent chain emerging from the ribosomal complex binds to the signal recognition sequence of an 11S ribonucleoprotein complex and the elongation is halted as the translation complex binds to the endoplasmic reticulum. The rate of elongation is also thought to be regulated by glucose at concentrations up to 5 mM, in which it is seen to increase insulin synthesis without any changes in the distribution of preproinsulin mRNA (Welsh et al., 1986). It is in the endoplasmic reticulum that the translation of proinsulin mRNA is completed and the conversion to proinsulin occurs.

2. Endoplasmic Reticulum, Insulin Secretory Vesicles and Transportation, and Exocytosis. Preproinsulin, once synthesized in the endoplasmic reticulum, exists for about 30 to 60 s before the pre-portion is removed enzymatically, and proinsulin is then transported along the microtubule network in transport vesicles to the cis part of the Golgi apparatus. This latter transportation step is GTP- and calcium-dependent (Beckers and Balch, 1989). It is in the trans network of the Golgi apparatus that proinsulin is converted by the prohormone-converting endopeptidases PC3 (also known as PC1) and PC2, and the exoprotease, carboxypeptidase H into insulin and the inactive byproduct C-peptide. Once in the cis-ternae of the Golgi apparatus insulin is packaged into secretory vesicles ready for export to the plasma membrane. The grains of insulin accumulate in the cisterna of the Golgi apparatus, where they initially form immature clathrin-coated vesicles. Most of the insulin molecules are channeled into vesicles and hence are secreted in the regulated pathway with only about 1% being secreted through the constitutive pathway (Rhodes and Halban, 1987). From the trans-Golgi network the secretory vesicles are carried via the microtubules that form part of the cytoskeleton of the β-cell. The cytoskeleton of the β-cell is an important component of insulin secretion, and disruption of this network hinders the post-translation processing and mobilization of insulin to the plasma membrane. This network consists of polymerized structures of actin filaments and microtubules and they form an important bridge between the endoplasmic reticulum and the Golgi apparatus and the plasma membrane. The microtubules consist of polymerized tubulin, and the application of glucose to the cells is known to increase the amount of polymerized tubulin in the β-cell (Montague et al., 1976). The polymerization of tubulin and mobilization of vesicles through the cytoskeletal network is regulated by proteins that bind to tubulin, known as microtubule-associated proteins. The microtubule-associated protein or proteins that promote polymerization of tubulin are believed to be phosphory-
al-associated protein 25 (SNAP-25), the of a core complex linking the syntaxin and synaptosome with the plasma membrane involves the formation of SNARE on the plasma membrane. Docking of the vesicle-associated protein 2 (VAMP-2)/synaptobrevin-2, -SNARE is recognized by the target-SNARE (reviewed in Easom, 2000). The vesicle-SNARE in directing the specificity of the vesicles to the membrane (Balczon et al., 1992; Meng et al., 1997) and on the vesicles themselves (Donelan et al., 2002), and consists of an ATPase portion, a tubulin binding site and a vesicle binding site (Hirokawa, 1998). In the resting β-cell kinesin is phosphorylated by casein kinase 2, but with increasing calcium levels (as with glucose-induced insulin secretion) it is rapidly dephosphorylated by calcineurin (Donelan et al., 2002).

The insulin vesicles must be recruited from the cytosolic pool, translocated to the plasma membrane, and form a physical association with the membrane; i.e., they are docked and fuse with the membrane and the contents spilled into the extracellular space in the process known as exocytosis. Phosphorylation of the microtubulins and filaments facilitates the navigation of the vesicles toward the cell membrane. A small fraction of the insulin vesicles in the β-cell are primed by an ATP-dependent mechanism and form a fusion-competent ready releasable pool (RRP) of insulin vesicles (Eliasson et al., 1997). It is the fusion of these vesicles with the plasmalemma that allows insulin release (Eliasson et al., 1997). Glucose-induced insulin secretion is biphasic, with the first phase representing the release of the vesicles in the RRP, and occurs in humans within 3 to 5 min of nutrient or glucose ingestion or intravenous glucose administration (reviewed in Nesher and Cerasi, 2002). The second phase of insulin secretion is dependent on the priming of the reserve pool of insulin secretory vesicles and the further processing of newly synthesized insulin, and is referred to as the plateau phase, with blood glucose levels returning to poststimulation levels about 120 min after an oral glucose tolerance test (OGTT). It is important to note that the first phase of insulin secretion is virtually nonexistent and the second phase is severely blunted in the diabetic state (Nesher and Cerasi, 2002).

A group of proteins known as SNAP REceptors (SNARES) [for soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptors] are important in directing the specificity of the vesicles to the membrane (reviewed in Easom, 2000). The vesicle-SNARE (v-SNARE) is recognized by the target-SNARE (t-SNARE) on the plasma membrane. Docking of the vesicle with the plasma membrane involves the formation of a core complex linking the syntaxin and synaptosomal-associated protein 25 (SNAP-25), the t-SNARE, with vesicle-associated protein 2 (VAMP-2)/synaptobrevin-2, the v-SNARE (Wheeler et al., 1996; Daniel et al., 1999). Studies in an insulinoma cell line (RIN 1046-38) have shown that glucose phosphorylates SNAP-25 (Zhou and Egan, 1997).

III. Pharmaceutical Agents Active in the Treatment of Disorders of Glucose Homeostasis

A. Insulinotropic Agents

Of all compounds known to directly positively modulate insulin release, the sulfonylureas are the most studied. Their insulinotropic properties have been much exploited by the drug industry in the treatment of type 2 DM. As sulfonylureas require intact β-cells, they have no value in treating type 1 DM. They are named for their common core configuration, which consists of a sulfonylurea group attached via the sulfur to a benzene ring (Fig. 2). They are derivatized by varying the substituents on the nitrogen of the urea group (R1) and on the para position of the benzene ring (R2). In the case of first-generation sulfonylureas (chlorpropamide, tolbutamide, tolazamide, and acetohexamide) the R1 substituents are small and polar, and therefore render the aryl-sulfonylurea more water-soluble. In the second-generation sulfonylureas (glyburide, glipizide, gliclazide, and gliimepiride) the substituents are large, non-polar, lipophilic groups that more readily penetrate cell membranes and are thus more potent.

Sulfonylureas release insulin by binding to the SUR subunit of the KATP channel and reducing its probability of opening (see Section II.B.1.i.). They are exogenous ligands of the SUR subunit, closing the channel and eliciting insulin release regardless of plasma glucose concentrations. In the presence of sulfonylureas KATP channel activity is disconnected from glucose sensing, so hypoglycemia resulting from hyperinsulinemia may occur in the fasting state (Ferner and Neil, 1988; Seltzer, 1989). The longer the half-life of a particular analog the greater will be its probability of inducing hypoglycemia. There is evidence that some sulfonylureas actually enhance the usual inhibitory action that MgADP has on channel activity so that the nucleotide binding site of the Kir6.2 subunit and reducing its probability of opening (see Section II.B.1.i.). They are exogenous ligands of the SUR subunit, closing the channel and eliciting insulin release regardless of plasma glucose concentrations. In the presence of sulfonylureas KATP channel activity is disconnected from glucose sensing, so hypoglycemia resulting from hyperinsulinemia may occur in the fasting state (Ferner and Neil, 1988; Seltzer, 1989). The longer the half-life of a particular analog the greater will be its probability of inducing hypoglycemia. There is evidence that some sulfonylureas actually enhance the usual inhibitory action that MgADP has on channel activity so that the nucleotide binding site of the Kir6.2 subunit
occurs via uptake of H$	extsuperscript{+}$ and is a necessary priming step for release of insulin. This increase in positive charge within the vesicle must be balanced by an influx of Cl$^{-}$ to prevent an excessive build-up of positive charge and thus permit vesicle acidification. Sulfonylureas are known to bind to a 65-kDa protein found on the vesicle fraction of the β-cell (Kramer et al., 1994) and are thought to modulate the activity of the granular CIC-3Cl$^{-}$ channel, which is instrumental in the acidification of the insulin vesicle (Renstrom et al., 2002).

Much has been written on the pharmacology and treatment regimens of the various generations of sulfonylureas used in the maintenance of euglycemia in the diabetic state and the combination of these drugs with other oral agents and insulin in the treatment of diabetes and its complications (DeFronzo, 1999; Inzucchi, 2002; Holmboe, 2002). In particular, many of the publications continuing to emanate from the United Kingdom Prospective Study on Diabetes (UKPDS) provide recent information on the efficacy of intensive combination therapy; these publications are listed on the homepage of the UKPDS website (http://www.dtu.ox.ac.uk/index.html).

A new class of drugs that also close the K$\textsubscript{ATP}$ channels has been discovered, and arose from the observation that the second-generation sulfonylurea, glimepiride, binds to a low-affinity binding site on the SUR1 subunit. These benzoic acid derivatives (also referred to as the meglitines) are structurally distinct from the sulfonylureas but show some chemical resemblance to the non-sulfonylurea part of glimepiride. Two benzoic acid derivatives are currently in use to treat type 2 DM. They are repaglinide and nateglinide with a third, mitiglinide, still in clinical trials (at the time of writing). Many studies have been performed comparing these drugs with each other and with glibenclamide both in vitro and in vivo. Both repaglinide and nateglinide are potent K$\textsubscript{ATP}$ channel blockers, with repaglinide being 10-fold more potent than glibenclamide (Fuhlendorff et al., 1998) and the rank order of potency being repaglinide > glibenclamide > nateglinide (Hu et al., 2000). Binding studies on repaglinide and glibenclamide in an insulinoma cell line (βTC-3 cells) indicate that there are probably three distinct binding sites for these two compounds: a high-affinity repaglinide site and two lower-affinity sites for glibenclamide (Fuhlendorff et al., 1998). Repaglinide, in contrast to glibenclamide, did not stimulate insulin secretion in islets in the absence of glucose and is more effective than glibenclamide at higher glucose concentrations (16.7 mM; Fuhlendorff et al., 1998). However, nateglinide (30 μM) shows a glucose-induced insulin secretion profile similar to that of glibenclamide (0.3 μM) (Ikenoue et al., 1997). Nateglinide has the advantage in that it rapidly dissociates from the SUR1 and displays a more rapid onset of channel inhibition and faster reversal of same than rapaglinide (Hu et al., 2000).

Because the effects of both drugs are rapid and short-lived they are used to curtail postprandial excursions in glucose (de Souza et al., 2001; Kalbag et al., 2001). The plasma half-life of both compounds in healthy human volunteers is between 1 and 1.5 h, with nateglinide producing a much more rapid rise in insulin postprandially than does rapaglinide, when the agents are administered 30 min before eating (Kalbag et al., 2001). Thus the risk of hypoglycemia when treating with these drugs is lower than with traditional sulfonylureas. When nateglinide (120 mg) was administered to fasted type 2 DM patients 15 min before receiving an i.v. bolus of glucose (300 mg/kg body weight), it produced an incremental response in the first 10 min of 788 pM/min versus 303 pM/min for glyburide (10 mg; Kahn et al., 2001). For nateglinide the incremental response was greatest at 60–120 min, whereas for glyburide it was greatest at 120–300 min after the intravenous glucose tolerance test (IVGTT).

At the end of the 300-min observation period plasma insulin levels in the patients who received nateglinide had returned to prevailing basal levels, whereas those for glyburide were still 2-fold basal values. Thus, due to its rapid action on the β-cell, administration of nateglinide to diabetic individuals preprandially produces a more physiologically normal insulin response than is seen with the sulfonylureas. There is a rapid 5-fold increase of insulin above basal values within 30 min of eating, which represents the prompt release of the insulin in the vesicles of the RRP followed by a decline within 120 min to values 2-fold above basal. It must be noted, however, that this does not necessarily reflect a
B. Thiazolidinediones

Drugs in the class of insulin sensitizers known as thiazolidinediones (Berger and Moller, 2002) act primarily on the proxosome proliferator activated receptor-\(\gamma\). They are used to treat insulin resistance, therefore we will not address them here but refer only to two reports where there is an indication of improvement of \(\beta\)-cell function and consequently insulin secretion following treatment with this class of drugs. Two groups have measured the ratio of proinsulin/insulin, sometimes used as an indication of \(\beta\)-cell function and insulin secretion following treatment with this class of drugs. In one study it was found that after 52 weeks of rosiglitazone therapy (Prigeon et al., 1998), the proinsulin/insulin ratio was significantly decreased (Porter et al., 2000). The second study showed that a decrease in the ratio was associated with troglitazone therapy (Prigeon et al., 1998).

C. Agents Used in the Treatment of Hyperinsulinemia

Glucagon is a polypeptide hormone that consists of 29 amino acids. In its endogenous form it is naturally secreted by the \(\alpha\)-cells of the islets and stimulates glycogenolysis in the liver resulting in increased blood glucose levels. At physiological concentrations it augments glucose-induced insulin secretion by stimulating the glucagon G-protein-coupled receptor on the \(\beta\)-cell (Huypens et al., 2000) resulting in increased intracellular cAMP levels (Henquin, 1985). When used as an emergency treatment of hypoglycemia due to hyperinsulinemia it is administered as a 1-mg dose usually intramuscularly or subcutaneously, but may also be given intravenously (Hall-Boyer et al., 1984). At these concentrations it counteracts the anabolic effect of insulin on the hepatocytes and stimulates glycogenolysis. This latter effect counteracts the increased endogenous insulin induced by glucagon in non-type 1 DM states. In routine clinical practice glucagon is used to test \(\beta\)-cell reserve. A fasting C-peptide level is obtained followed by a second level 6 min after i.v. glucagon (1 mg) administration. For normal \(\beta\)-cell reserve function the C-peptide levels should be at least double the fasting value.

Diazoxide is an antihypertensive, antidiuretic, benzothiadiazine derivative that is also used to treat hyperinsulinemia as a consequence of inoperable insulinoma or persistent hypoglycemic hyperinsulinemia of infancy. The most potent \(\beta\)-cell \(K_{\text{ATP}}\) channel opener known, diazoxide, hyperpolarizes the \(\beta\)-cell, thereby inhibiting insulin secretion (Panten et al., 1989). It is only effective in opening the \(K_{\text{ATP}}\) channel in the presence of Mg\(^{2+}\) and ATP (reviewed in Ashcroft and Rorsman, 1989). Diazoxide does not competitively displace glibenclamide at the \(K_{\text{ATP}}\) channel, suggesting that it has a different binding site on the channel than the sulfonylureas (Panten et al., 1989).

D. The Potential Agents and Targets for Future Treatment of Diabetes

1. Agonists at the Glucagon-Like Peptide-1 Receptor

The risk of hypoglycemia is a major and recurring drawback of the sulfonylureas (see Section III.A.). Research has intensified over the last 10 years on endogenous mammalian peptide or incretin that is insulinotropic only in the presence of glucose (3 mM glucose threshold; Fehmann et al., 1992). GLP-1 is a 30-amino acid peptide synthesized in the intestine and secreted upon nutrient ingestion (Mojsov et al., 1986). It is effective in augmenting glucose-mediated insulin secretion in the diabetic state and in suppressing glucagon secretion. GLP-1 acts to potentiate glucose-induced insulin secretion and has pleiotropic effects on the \(\beta\)-cell (reviewed in Doyle and Egan, 2001) as well as inhibiting glucagon secretion. Due to its clinical potential the mechanisms of these effects have been explored extensively (Drucker, 2002). It acts through a specific G-protein-coupled receptor on the \(\beta\)-cell and ultimately increases both the number of cells secreting insulin and the amount secreted per cell by increasing insulin biosynthesis and recruiting more vesicles into the RRP (Drucker, 2002). It synergizes with the glucose-induced insulin secretion pathway at a molecular level and increases intracellular cAMP (Drucker et al., 1987); regulates insulin gene promoter activity by CREB (Skoglund et al., 2000) and NFAT (Lawrence et al., 2002) sites on the insulin promoter; stimulates PDX-1 translocation, transcription, and translation (Wang et al., 1999, 2001); and phosphorylates SNAP-25 (Zhou and Egan, 1997).

GLP-1 has a very short half-life in vivo, and thus to be effective in maintaining euglycemia it must be given as a continuous subcutaneous infusion or injections at frequent intervals (Parkes et al., 2001). Many modifications have been made to the GLP-1 molecule to rectify this deficiency, and thus exploit the activation of the GLP-1 receptor (GLP-1R) so that it can be applied to treat diabetes in a clinical setting. Most of these modifications have been made to the N-terminal end (HisAlaGlu), which is subject to cleavage by the enzyme dipeptidyl-peptidase IV (DPP IV). These modifications include substitution of the alanine with threonine, serine, \(\gamma\)-aminoisobutyric acid (Deacon et al., 1998) or glycine (GLP-1 Gly\(^8\); Deacon et al., 1998; Burcelin et al., 1999), as well as glycation of the alanine (O’Harte et al., 2000) and insertion of an aliphatic six-carbon chain between the histidine and the alanine (Doyle et al., 2001). In general, these modifications only marginally increase the half-life of the
compound. However, Novo Nordisk A/S ( Bagsvaerd, Denmark) currently has a long-acting analog of GLP-1 in clinical trials (Agerso et al., 2002). This is an acetylated albumin-bound analog that has a considerably longer half-life of $12.6 \pm 1.1$ h in humans.

A natural agonist of the GLP-1R was found by Eng and coworkers in the saliva of the Gila monster lizard and is known as exendin-4 (Eng et al., 1992). This peptide has a 52% amino acid homology with GLP-1 and binds with greater avidity to the GLP-1R than does GLP-1 (Goke et al., 1993). It is also resistant to many of the circulating enzymes that degrade GLP-1 (Hupe-Sodmann et al., 1995) and thus has a longer half-life (Parkes et al., 2001). A long-term study completed in our laboratory has shown that it decreases the deposition of subcutaneous and visceral fat in diabetic Zucker rats (Szayna et al., 2000). When administered acutely it reduced food intake, inhibited gastric emptying, and stimulated insulin secretion in healthy volunteers (Egan et al., 2002).

Interestingly, there are many observations of increased $\beta$-cell mass after acute and chronic treatment with GLP-1 receptor agonists (Perfetti et al., 2000; Stoffers et al., 2000); thus activation of the GLP-1R produces endocrinotrophic effects and stimulates neogenesis of $\beta$-cells in rodent pancreata. One must caution that there is as yet no evidence for this increase in $\beta$-cell mass in humans treated with GLP-1R agonists. Treatment with these drugs does, however, lead to a restoration of first-phase insulin response (Meneilly et al., 2001) and an increase in pulse amplitude of insulin secretion (Porksen et al., 1998) in type 2 DM.

2. Agonists at the Purinergic 2 Receptor. One group in Israel has done some work on producing insulinotropic agonists to the purinergic P2Y receptor on the $\beta$-cell (Fischer et al., 1999, 2000). These are a series of 2-thioether 5’-O-(1-thiotriphosphate)-adenine nucleotides that are stable to degradation by porcine ATP-Dase. In perfusions of whole rat pancreata the compounds were found to be 100-fold more potent at stimulating insulin secretion than ATP. However, the effects of these derivatives are not specific to the purinergic receptors on the $\beta$-cell and they also induce vascular effects, thus limiting their potential for clinical applications as they stand.

3. Imidazolines. Imidazolines such as phenolamine, yohimbine, and efaroxan, which are known to be $\alpha_2$-adrenoreceptor blockers, can enhance insulin release (Chan et al., 1991). This effect occurs via the inhibition of the $K_{ATP}$ channel of the $\beta$-cell (Plant and Henquin, 1990; Proks and Ashcroft, 1997) and not through the $\alpha_2$-adrenoreceptors or the conventional imidazole binding sites. Imidazolines also exert a direct effect on exocytosis, an action that is distal to closure of the $K_{ATP}$ channel (Zaitsev et al., 1996). This $K_{ATP}$-independent effect of imidazolines on insulin secretion differs from the $K_{ATP}$ channel-independent sulfonylurea effect, as the latter is not sensitive to PKA inhibition but is PKC-dependent (Eliasson et al., 1996), but that of the imidazolines is dependent on both kinases (Zaitsev et al., 1996). There have been successful efforts to exploit this $K_{ATP}$ channel-independent action of the imidazolines to synthesize a glucose-dependent insulinitrope. At least two laboratories have synthesized derivatives that do not interact with the $K_{ATP}$ channel and only stimulate insulin secretion in a glucose-dependent manner (Mest et al., 2001; Efano et al., 2001).

IV. Drugs Administered in the Treatment of Disorders Other Than Diabetes That Have Effects on Pancreatic Insulin Secretion and $\beta$-Cell Function

A. Drugs Implicated in Post-Transplant Diabetes Mellitus

1. Calcineurin Inhibitors. Impaired glucose tolerance and post-transplant diabetes mellitus (PTDM) are common complications following solid organ transplantation (Dubernard and Frei, 2001). The immunosuppressive regimens that include corticosteroids, cyclosporin, and tacrolimus (fungal macrolides that are calcineurin inhibitors) have been directly implicated (Weir, 2001). Prednisolone causes significant insulin resistance in skeletal muscle by impairing activation of glycogen synthase (Ekstrand et al., 1996). This in turn puts increased demand on the pancreas to increase insulin secretion. If the pancreas cannot do so, DM occurs. In contrast, cyclosporin and tacrolimus probably induce PTDM by directly compromising $\beta$-cell function, with tacrolimus being the most commonly implicated (reviewed in Weir and Fink, 1999). Studies have quoted incidences of PTDM due to use of tacrolimus as anywhere from 15 to 29% (Weir and Fink, 1999). A higher incidence of PTDM is reported in tacrolimus-treated (36.6%) African Americans than in cyclosporin-treated (12.2%) (Neylan, 1998). In the European FK506 study 15.1% of tacrolimus-treated patients developed DM compared with 8.7% of those treated with cyclosporin (European FK506 Multicentre Liver Study Group, 1994). In follow-up studies on kidney transplant patients it was found that 41.2% of tacrolimus-treated patients who developed PTDM over the first year ceased with insulin treatment and successfully remained on tacrolimus treatment (Vincenti et al., 2002). The incidence of tacrolimus-related PTDM is considered to be dose-dependent, and First and colleagues suggest that a lowering of the tacrolimus dose and/or combination with maintenance doses of prednisone and mycophenolate mofetil is the preferred option to transferring to cyclosporin treatment (First et al., 2002). This is because the risk of graft rejection is lower with tacrolimus treatment.

Insulin secretion in pediatric patients treated long-term with either tacrolimus (15 patients) or cyclosporin...
(14 patients) post renal transplantation was examined using an IVGTT with frequent plasma sampling (Filler et al., 2000). The 1- to 3-min insulin secretion response to the IVGTT in tacrolimus-treated children was severely blunted, and insulin secretion followed to 60 min was significantly less than that of children treated with cyclosporin. There was a dose relationship between the trough tacrolimus levels and the inhibition of insulin secretion. Thirteen percent of the tacrolimus-treated patients and none of the cyclosporin-treated patients developed PTDM. Some patients had their tacrolimus dose decreased and subsequently showed improvement in insulin secretion during a repeat IVGTT. This would indicate that the effects of tacrolimus are reversible and the drug, at concentrations used in clinical practice, is not causing permanent β-cell damage.

A recent study of adults (Duijnoven et al., 2001) confirmed and expanded on the findings in pediatric patients. An IVGTT, again with frequent plasma sampling for 60 min, was performed on 18 patients (mean age 49 years; 10M, 8F; mean BMI 23.3 kg/m²) before renal transplantation and 5 days after starting tacrolimus (0.15 mg/kg b.wt. b.i.d., p.o.) post-transplantation. Insulin secretion decreased significantly (p < 0.0001) from 864.5 to 600 mU/l · min. Insulin sensitivity was not altered using the homeostasis model assessment of glucose (HOMA: fasting glucose [mmol/l] × fasting insulin [mU/l]/22.5; Matthews et al., 1985). Fourteen of the patients were followed for a median of 34 months. All three patients on monotherapy with tacrolimus acquired diabetes: two were treated with sulfonylurea, and one controlled with diet alone. The other 11 patients remained normoglycemic after transplantation. Boots and coworkers attempted to separate the individual effects of glucocorticoids and tacrolimus on glucose tolerance (Boots et al., 2002). Glucose metabolism was evaluated by IVGTT before and after reduction in tacrolimus trough levels. After prednisone (10 mg) withdrawal insulin resistance decreased, as demonstrated by an improved insulin/glucose ratio. In the second part of the study tacrolimus trough levels were reduced. There were approximately 10 months between the first and second IVGTT after reduction of trough levels. C-peptide secretion increased significantly, by 36% (from 49 to 67 nM · min). Of clinical significance, HgbA₁c decreased from 5.9 to 5.3% between the two IVGTTs. This study produced similar results to that of Filler and colleagues in that the effects of tacrolimus on insulin secretion are reversible.

Both tacrolimus and cyclosporin act by blocking the antigen-activated transcription of early T-cell activation genes such as interleukin 2 (II-2) and by inhibiting the phosphatase calcineurin (Fruman et al., 1992). Tacrolimus and cyclosporin require their respective cognate intracellular immunophilins (for a comprehensive review of immunophilins see Marks, 1996), FKBP12 (FK506 binding protein 12), and cyclophilin A, to which they bind before complexing with the calcineurin B binding site, thus preventing the formation of the NFAT transcription factor complex (Crabtree, 2001; Crabtree and Olson, 2002). This inhibits transcription of genes such as insulin that contain NFAT binding sites in their promoter regions (Lawrence et al., 2001). Treatment of the insulinoma cell line HIT-T15 and isolated islets with these immunosuppressants resulted in a decrease in both insulin mRNA and protein content (Redmon et al., 1996; Paty et al., 2002). A similar observation was made in islets isolated from rats treated with tacrolimus (10 mg/kg/day) for 14 days (Tamura et al., 1995). There was a decrease in insulin mRNA and insulin content of the islets of the treated rats compared with vehicle-treated animals. What is important to note about this experiment is that similar to the effects observed in the IVGTTs, the insulin on the insulin synthesis machinery in islets was reversible upon withdrawal of tacrolimus.

A chronic sustained decline in insulin synthesis, with continuation of calcineurin inhibitor medication, would ultimately lead to a decline in the insulin available for ready release. This would be expected to have long-term consequences for glucose homeostasis. Patients, if placed under stress where increased insulin demand due to insulin resistance is needed, as in infections, use of other medications, or addition of corticosteroids to the immunosuppressant regimen, for example, would have a reduced capacity to respond to the increased demand and glucose intolerance could and does arise. Age would also be expected to have an impact on the incidence of PTDM as mRNA and insulin production in islets declines longitudinally with age (Perfetti et al., 1995). Indeed, the age of the graft recipient is a factor in the etiology of PTDM (Rao et al., 1992). It is possible to make a correlation between the decline in β-cell function with age and an increased risk of developing PTDM, as in one study a comparison has been made of pre- and post-renal transplant levels of β-cell function. Nam and colleagues compared the fasting and 2-h plasma glucose levels and proinsulin/insulin ratios of patients who carry the diagnosis of PTDM with those defined as having impaired glucose tolerance (as determined by World Health Organization criteria) and normal glucose tolerance (Nam et al., 2001). The plasma glucose levels and proinsulin/insulin values were found to be significantly higher in the impaired glucose tolerance and PTDM groups that in the normal subjects. They concluded that patients who had a low insulin secretory capacity before receiving a graft were predisposed to PTDM. As insulin sensitivity did not vary significantly between the groups they also proposed that reduced β-cell capacity weighed more than insulin resistance as a factor in the development of PTDM.

Calcineurin inhibitors have also been demonstrated to reduce insulin secretion by disrupting the dephosphorylation by calcineurin of kinesin on insulin secretory vesicles (Donelan et al., 2002). This prevents the kinesin-dependent forward movement of the vesicles and
lowers the pool of vesicles docked at the plasma membrane. This also explains the reversibility of the effect of tacrolimus on insulin secretion observed upon cessation of treatment with calcineurin inhibitors. Cyclosporin (2–5 μM) was found to diminish insulin release from mouse islets by inhibiting glucose-stimulated oscillations of the cytoplasmic free calcium and disrupting the oscillations in the cell and mitochondrial potential (Dufer et al., 2001). Cyclosporin binds readily to cyclophilin D in the mitochondrial permeability transition pore and blocks the opening of this channel on the mitochondrial (see Section II.A.1.). Tacrolimus was found to have no effect on the glucose-induced oscillations, thus this effect of cyclosporin was not mediated through calcineurin inhibition.

2. Antiproliferative Agents. While the calcineurin inhibitors block the action of T-cells, azathioprine acts as an antiproliferative agent (MICROMEDEX Healthcare Series, 2002). Nucleophiles such as glutathione cleave the prodrug azathioprine to mercaptopurine, and this purine analog is subsequently converted into mercaptopurine-containing nucleotides. Azathioprine is used as a monotherapy for Crohn’s disease and rheumatoid arthritis, and it is occasionally used in the treatment of bullous pemphigoid and pemphigus vulgarus, where it is more usually used as a steroid-sparing agent. There are no reports in which the direct effects of azathioprine on islets have been examined but the drug is toxic to the pancreas, and the incidence of pancreatitis in graft recipients treated with azathioprine is estimated to be between 2 and 12% (reviewed in MICROMEDEX Healthcare Series, 2002). However, the studies are complicated by the fact that the patients were treated with other drugs, so it is difficult to implicate azathioprine alone.

B. Quinolines

Quinine belongs to the class of drugs known as quinolines, which are used as anti-malarial agents and in nonmalarial countries in the treatment of nocturnal leg cramps. Other drugs in this class are quinidine, chloroquine, mefloquine, and halofantrine. Originally extracted from the bark of the Cinchona officinalis, quinine has been used for over 300 years in the treatment of malaria but its hypoglycemic effect was only observed early in the 20th century (Hughes, 1925). All drugs in this class have been known to lower blood glucose. The degree of reduction in blood glucose associated with each compound is dependent on the lipophilicity, free serum levels, and rate constant of elimination of the compound (Sheiner et al., 1979). In vitro studies performed mainly with quinine have established that quinolines close the $K_{\text{ATP}}$ (Bokvist et al., 1990b; Gribble et al., 2000) and $K_{\text{ir}}$ channels (Bokvist et al., 1990a) of β-cells. Using patch-clamp techniques on Xenopus oocytes injected with a truncated form of Kir$_{6.2}$ (a form that expresses functional channels in the absence of SUR1) Gribble and colleagues (2000) have shown that quinine and the related drug mefloquine act on the Kir component of the $K_{\text{ATP}}$ channel.

Hypoglycemia is a risk factor in malarial patients treated with parenteral quinine (White et al., 1983). This has been seen with hyperinsulinemia but the matter is complicated by the fact that the common infecting organism Plasmodium falciparum consumes glucose (Singh et al., 1998). Hypoglycemia commonly occurs when the drug is administered over an hour or less, probably because of the high free serum levels of the compound during this short period of administration (Molyneux et al., 1989). There is at least one reported case of insulin-mediated hypoglycemia in a non-diabetic individual who was being treated with oral quinine sulfate (325 mg, q.i.d.) for leg cramps (Limburg et al., 1993). The standard oral dose used for leg cramps (primarily in the elderly patient) is 300 to 600 mg quinine sulfate taken at night before retiring. An Australian group studied the effects of 600 mg administered at night on serum glucose and insulin levels (Dyer et al., 1994). Twelve type 2 DM and 10 non-DM subjects (51–79 years of age) were studied on two separate occasions, with or without oral quinine sulfate, which was given at 10 PM, while the last meal before dosing was at 6 PM. Venous blood samples for glucose, insulin, and quinine levels were drawn periodically for the next 38 h. In the non-DM group there was a significant fall in serum glucose of 1.2 mM, 2 h after quinine was administered. It took 10 h for glucose levels to return to nontreated values.

Insulin levels remained the same on both occasions. In the type 2 DM subjects there was also a significant lowering in serum glucose (by 1 mM) 2 h after dosing, which took 8 h to return to nonquinine-treated values. Again, insulin levels were not altered. The nadir in serum glucose corresponded with peak serum quinine levels. This study can be interpreted to show that quinine increased tissue utilization of glucose while maintaining insulin secretion. One would have expected, especially in the non-DM subjects, that serum insulin would fall as serum glucose levels fell. As this did not occur, quinine probably has dual effects on glucose homeostasis.

Although the glucose-lowering effects are similar to those of sulfonylureas, quinine cannot be considered as being of any practical use to treat hyperglycemia clinically, given the number of toxic effects associated with the drug (Bateman and Dyson, 1986) such as chinoism, cardiac conduction abnormalities, and neuropsychiatric disturbances.

C. Somatostatin Receptor Agonists

Octreotide acetate is the first somatostatin (SST) analog used clinically to treat acromegaly, Cushing’s syndrome, carcinoid tumors, vipomas, pancreatic pseudocysts, and gut fistulas. Octreotide is one of the many octapeptide and hexapeptide SST analogs that, unlike
somatostatin, show a high degree of affinity for sstr2 and sstr5, a moderate affinity to sstr3, and little or no binding to sstr1 (reviewed in Patel, 1999). SST and its analogs can inhibit insulin secretion by activation of sstr5, which is mediated by stimulation of the G\(_i\)/G\(_o\) protein (see Section II.B.2.b.i.). Octreotide is known to suppress the secretion of numerous hormones, including pituitary and gut hormones, and activation of SST receptors has pleiotropic effects on hormone-secreting cells. Subcutaneous or intravenous octreotide suppresses first-phase insulin secretion and attenuates insulin responses to activated G\(_\alpha\)-protein-coupled receptors (such as the GLP-1R). Most importantly, as a consequence of these effects, blood glucose levels are significantly increased after eating or after an oral glucose tolerance test. In \(\beta\)-cells, activation of sstr5 inhibits calcium mobilization and AC activity and decreases insulin gene promoter activity, resulting in reduced insulin biosynthesis (reviewed in Benali et al., 2000). SST also exhibits an effect on insulin secretion distal from the inhibition of Ca\(^{2+}\) mobilization and adenylyl cyclase inhibition (Renstrom et al., 1996). By using pancreatic islets under controlled conditions, these authors evoked an increase in intracellular Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_i\)) to 1.5 \(\mu\)M in mouse \(\beta\)-cells (usual resting [Ca\(^{2+}\)]\(_i\) is 0.2 \(\mu\)M) and they then added SST. Insulin secretion was almost completely abolished. Therefore, SST still inhibits insulin secretion, even in the presence of high cystolic Ca\(^{2+}\). In the same manuscript the authors demonstrated that inhibition of calcineurin using calcineurin autoinhibitory peptide and deltamethrin prevented the action of SST on insulin secretion. Thus, the working hypothesis is that sstr5 activation ultimately results in dephosphorylation of specific proteins, which in turn impedes the movement and/or docking of vesicles. This effect is abolished by the inhibition of calcineurin, presumably by permitting the specific rephosphorylations to occur, because it is the sstr5 activation at the plasma membrane by SST that leads distally to the activation of calcineurin (Patel, 1999). It is also important to note that although sstr5 is coupled to G\(_i\)/G\(_o\), the consequent inhibition of AC is not responsible for inhibition of insulin secretion. When Renstrom and colleagues clamped the cytoplasmic cAMP concentration at 100 \(\mu\)M by inclusion of the nucleotide in the solution that was dialyzed into the cell, SST still inhibited insulin secretion (Renstrom et al., 1996).

It has been suggested that the \(\beta\)-cell sstr is coupled to the K\(_{ATP}\) channel (Ribalet and Eddlestone, 1995; Smith et al., 2001) but the effect of this is not considered to be relevant physiologically, as SST is still capable of reducing insulin secretion in the presence of sulfonylureas (Abel et al., 1996). There is some evidence from transfection studies in Xenopus oocytes (Kreienkamp et al., 1997) and electrophysiological experiments (Ribalet and Eddlestone, 1995) in an insulinoma cell line of coupling of the sstr to an inwardly rectifying K\(^+\) channel, but again the significance of this in intact islets or in an in vivo model has not been established. In antiproliferative studies performed on Chinese hamster ovary cells expressing sstr5, it was shown that the human sstr5 activates tyrosine phosphatase, while the rodent sstr5 does not (Sharma et al., 1999). It has also been reported that sstr5 modulates mitogen-activated protein MAP kinase activity and PLC activity (reviewed in Patel, 1999). Whether any of these second messengers are involved in the mechanism by which SST inhibits insulin secretion is not yet known. There are some reports in the literature of the use of octreotide to treat sulfonylurea-induced hypoglycemia, and it appears to be more effective in this instance than diazoxide (reviewed in Harrigan et al., 2001).

D. Drugs Used Mainly to Treat Hypertension

Cardiovascular disease and hypertension are common in type 2 DM patients. In the period from 1988 to 1994, 71% of the population diagnosed with diabetes was recognized as having high blood pressure (Geiss et al., 2002), and about 50% of type 2 DM patients die from complications associated with cardiovascular disease (Geiss et al., 1998). Therefore, many type 2 DM patients on treatment regimens to maintain euglycemia are also being treated for secondary effects to the cardiovascular system. Antihypertensives routinely used are \(\beta\)-blockers, calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, and diuretics.

\(\beta\)-Adrenergic blockers, although of more importance for their ability to blunt counterregulatory responses to hypoglycemia by preventing catecholamine-induced glycogenolysis in the diabetic condition (especially when under treatment with exogenous insulin or sulfonylureas), are potential inhibitors of insulin secretion. The endocrine pancreas is clearly innervated by the autonomic nervous system (Kirchgessner and Gershon, 1990) and \(\beta\)-blockade by propranolol, a \(\beta_1\)- and \(\beta_2\)-blocker, definitely blocks insulin secretion in vitro from isolated islets (Harms et al., 1978). Propranolol infusion into humans has been shown to decrease both phases of insulin secretion (Cerasi et al., 1972), and Robertson and Porte (1973) showed that propranolol caused a significant decrease in basal insulin levels after 1-h infusion (5 mg given rapidly initially followed by 0.08 mg/min). When epinephrine was then added to the infusion regimen (6 \(\mu\)g/min) it caused a further decrease in insulin secretion (60% below pre-epinephrine levels), and this was accompanied by a rise in blood glucose from 100 to approximately 175 mg/ml. These data show that \(\alpha_2\) stimulation and \(\beta_2\) blockade have additive inhibitory effects on insulin secretion. The rise in blood glucose was most likely due to epinephrine-induced glycogenolysis in the liver, unopposed by the inability of the \(\beta\)-cells to increase insulin secretion because of the double blockade.

A few clinical studies have suggested that hypertensive type 2 diabetic subjects on \(\beta\)-blockers have ad-
versely effected glucose tolerance. Twenty type 2 diabetic subjects treated with β-blockade for 4 weeks were reported to have increased blood glucose levels of 25 mg/dl compared with placebo-controls (Wright et al., 1979). Importantly, insulin levels were apparently unaltered with treatment, suggesting that the observed effect on blood glucose was not due to decreased insulin secretion. In contrast, a 6-year study by Berglund and Andersson (1981) of nondiabetic subjects treated with propranolol appeared to show an actual improvement in glucose tolerance. A more recent study by Savage and coworkers (1998) also found no increase in hyperglycemia or diabetes in patients on β-blockers (atenolol). Of importance when considering the effect of diuretics on insulin secretion (see below), when this β-blocker was used with a diuretic (chlorothalidone) there was no increased incidence of glucose intolerance or frank diabetes (Savage et al., 1998). In conclusion, the reproducible effect of propranolol infusion on insulin secretion does not appear to be operative in hypertensive patients on oral treatment. If β-blockade should become an issue in glucose tolerance in the treatment of hypertension (or hyperthyroidism, which may be accompanied by glucose intolerance) in a specific type 2 diabetic subject, then cardioselective (β1-blockade only) would have a theoretical advantage.

Clonidine is an α2-agonist used to treat hypertension. It reduces sympathetic outflow from the central nervous system and decreases plasma norepinephrine levels. As insulin release in nondiabetic individuals is probably not under tonic sympathetic control, clonidine in practice appears to have little effect on insulin secretion in that group of patients with hypertension. Webster and McConnaughey (1982) reported on a case of clonidine in type 2 DM in which glucose tolerance worsened and clonidine withdrawal led to improvement again. A recent study by Lattermann and colleagues (2001) showed that low-dose intravenous clonidine premedication accentuated the usual hyperglycemic (blood glucose levels, clonidine: 6.8 versus control: 5.7 mM) response that is normally seen during surgery (due to anesthesia, elevated cortisol and epinephrine levels, etc.). This appeared to be due to lower plasma insulin levels in the clonidine-treated versus control subjects.

The first-generation calcium channel blockers, verapamil, nifedipine, and diltiazem, are still the most commonly used calcium channel blockers used in clinical practice. Presently, a slow-release form of verapamil (120-, 180-, and 240-mg tablets of verapamil hydrochloride) is administered once daily to treat angina, arrhythmias, and hypertension, and there are no reports of it affecting insulin secretion at these doses. Rojdmark and Andersson studied the effect of oral pretreatment for 1 week and i.v. infusion over 3 h of verapamil on glucose tolerance and insulin secretion. They found no effects on insulin release but did observe improved glucose tolerance, suggesting that at pharmacological doses verapamil has an impact on hepatic glucose output but not on insulin secretion (Rojdmark and Andersson, 1986). Verapamil is used extensively in vitro when examining effects on L-type calcium channel electrophysiology in the β-cell. In vitro verapamil is known to block the L-type calcium channel on the β-cells and also inhibits (in the presence of 11.1 mM glucose), in a concentration-dependent manner, the KATP channels. This latter effect was found to be unique to the phenylalkylamines, i.e., verapamil and its methoxy derivative gallopamil, as 1,4-dihydropyridine, nifedipine, and diltiazem did not block the KATP channels (Lebrun et al., 1997). The second-generation calcium channel blockers, e.g., amlodipine, nicardipine, and felodipine, are not known to have any effect on insulin secretion. Observation of the long-term effects of amlodipine on insulin secretion and plasma insulin in humans shows that this drug has no effect on these parameters (de Courten et al., 1993; Harano et al., 1995) and may even improve insulin sensitivity (Harano et al., 1995). Similarly, insulin levels were essentially unchanged when patients were treated with nicardipine (60 or 120 mg/day dose) for an average of 7.8 weeks (Kihara, 1991). There have been several studies on the effect of nicardipine on glucose tolerance and/or insulin sensitivity when given at doses in the range of 20 to 30 mg in normal patients (4 weeks, Collins et al., 1987; 12 weeks, Wang et al., 1993; Kageyama et al., 1994) with no significant difference observed.

The incidence of hypoglycemia associated with patients treated with ACE inhibitors and sulfonylureas is relatively high, and has been documented in several cases (Herings et al., 1995; Shorr et al., 1997). This may be because of improved glucose uptake (Kudoh and Matsuki, 2000) and thus reduce insulin resistance (Vuorinen-Markkola and Yki-Jarvinen, 1995), as there is no documented evidence of these drugs having a direct effect on the β-cell. A new ACE inhibitor, ramipril, has been shown to be effective in reducing the development of diabetes, an effect attributable to its positive impact on insulin resistance and not to any effect on the β-cell (Yusuf et al., 2001).

Of the potassium channel openers, pinacidil and nicorandil, neither is known to affect insulin secretion. These drugs are targeted to interact with the Kir6.2/SUR2A channel on the smooth muscle cells, but not with the Kir6.2/SUR1 channel found on the β-cell. When both channels were expressed in Xenopus oocytes, nicorandil was found to stimulate only the Kir6.2/SUR2A channel and not the Kir6.2/SUR1 channel (Reimann et al., 2001). In a similar set of experiments using Xenopus oocytes, Gribble and colleagues have demonstrated that pinacidil does not activate the SUR1-containing channel found on the β-cell (Gribble et al., 1997b). The only KATP channel opener used to treat hypertension that has an effect on the β-cell Kir6.2/SUR1 channel is diazoxide. The use of diazoxide to treat hypertension is associated with hyperglycemia, which is predictable based on its known ability
to hyperpolarize the β-cell plasma membrane and decrease insulin release (see Section III.C for use in treatment of insulinomas and persistent hypoglycemic hyperinsulinemia of infancy). The dose for treatment of acute hypertension is a 1 to 3 mg/kg (maximum of 150 mg) intravenous bolus over 10 to 15 min (Varon and Marik, 2000). However, the use of diazoxide has been phased out because of its significant side effects, including fluid retention, and that it does not allow for a controlled reduction in blood pressure (Varon and Marik, 2000).

None of the other diuretic agents usually used to treat hypertension, the benzothiadiazines (“thiazides”) and loop diuretics, is known to have direct effects on insulin secretion. The thiazides have a dose-dependent effect on insulin resistance (Brass, 1984; Harper et al., 1995), while the loop diuretic furosemide has no effect on glucose homeostasis (Efendic et al., 1984). Thiazides do not directly affect insulin secretion from islets (Malisse and Malaise-Lagae, 1968) but, because of their induction of glucose intolerance due to insulin resistance, they may induce diabetes in a compromised glucose homeostatic state in which insulin secretion is already maximal. Therefore, they should be used with caution in type 2 DM.

E. Methylxanthines

The two principal therapeutic agents in this class of compounds are caffeine and aminophylline. Aminophylline is administered as a mixture of theophylline and ethylenediamine (2:1). Both caffeine and aminophylline have been used in the treatment of asthma although, of course, aminophylline is by far the more commonly used. The action of methylxanthines is to relax the smooth muscles of the bronchi and therefore produce a definite increase in vital capacity of the lungs. There are three known potential molecular mechanisms of action of this class of drug: they are an ability to 1) translocate intracellular calcium; 2) inhibit phosphodiesterase and thereby increase intracellular concentrations of cAMP and cGMP; and 3) bind to and antagonize the known P1 purinoreceptors, and consequently raise intracellular cAMP levels in β-cells, which contain A1 receptors (Hilaire-Buys et al., 1994), and are negatively coupled to AC and prevent the rise of cAMP in hepatocytes, which contain A2 receptors (Oetjen et al., 1990), and are positively coupled to AC. With aminophylline doses used therapeutically (plasma theophylline levels of the drug should be between 5 and 15 μM) it is only the latter of the three that is considered to be operative. Thus, aminophylline could potentially stimulate insulin secretion by antagonizing the negative regulation of adenosine at the A1 receptor. There is much conflict in the literature of the effect of methylxanthines in vivo on glucose metabolism and insulin secretion, almost certainly reflecting their duality of effects. Cerasi and Luft were the first to study aminophylline and reported that during an IVGTT it had no effect on basal insulin secretion, but did increase glucose-stimulated insulin secretion (Cerasi and Luft, 1969). However, Arias and colleagues recently reported that it increased insulin secretion and consequently reduced plasma glucose (Arias et al., 2001).

Similarly, the reports on caffeine’s effects on insulin secretion contain equally confusing and conflicting data. During an OGTT Pizzoli and coworkers reported an increase in glucose concentration with no effect on insulin levels (Pizzoli et al., 1998), and Graham and colleagues observed a significant increase in insulin but no difference in blood glucose during an OGTT (Graham et al., 2001). However, these investigators used different doses and different preparations of caffeine, which could have had different effects on the liver versus the pancreas. More recently, Greer and coworkers demonstrated, using the hyperinsulinemic-euglycemic clamp, that caffeine decreased glucose disposal without affecting insulin secretion in healthy volunteers (Greer et al., 2001). This adds a new aspect to the effects of caffeine, as it would appear that decreased glucose disposal by skeletal muscles was responsible for the reduction in total glucose disposal. Also in the class is the vasodilator pentoxifylline, which is discussed in the following section.

F. Phosphodiesterase Inhibitors

Realizing that PDE activity within any one cell is due, on the whole, to the action of a subset of the known PDE isozymes, many researchers and pharmaceutical companies have attempted to devise therapeutic interventions based on modulation of individual PDE activities (reviewed in Beavo, 1995). In theory, one should be able inhibit a specific family of PDEs. In practice, several of the second-generation drugs do appear to perform much better in terms of efficacy and have fewer side effects. There are many PDE3 inhibitors now available (cilostazol, enoximone, and milrinone). They are antihypertensives (because of effects on vascular smooth muscle PDE activity), antithrombotics (they affect cGMP-PDE activity in platelets), and they are positive inotropes in congestive cardiac failure (CCF) (again, they affect cGMP-PDE activity in cardiocytes and vascular smooth muscle). Many trials of the use of this class of compounds in CCF (i.e., with vesnarinone; Cohn et al., 1998) have been carried out, but mortality data in long-term studies often show negative data (possibly due to arrhythmias, altered metabolism of the drug in CCF, and/or interactions with the other concomitant drugs; van Veldhuisen and Poole-Wilson, 2001). Cilostazol does not affect blood glucose levels (Okuda et al., 1992; Uchikawa et al., 1992) and we did not find any reports that vesnarinone, enoximone, or milrinone have any effect on glucose metabolism (MICROMEDEX Healthcare Series, 2002).

Dipyridamole (a PDE5 inhibitor) and pentoxifylline (a nonspecific PDE inhibitor of the xanthine class; see Section IV.E.) are two of the most commonly used inhibitors in clinical practice. They are used as antithrombotics.
and vasodilators in peripheral vascular disease and therefore are commonly used in patients with diabetes. Intravenous infusion of pentoxifylline (200–300 mg) did not affect insulin secretion in nondiabetic volunteers (Lenti et al., 1975; Heidrich and Schirop, 1980). In one study a reduction in the amount of insulin required to maintain euglycemia was observed in both type 1 and type 2 DM patients requiring insulin to manage their diabetes during a 2-week observation period (Raptis et al., 1987). As this study showed effects in both types of DM, the decreased insulin requirement must be due to effects on glucose disposal or a decrease in gluconeogenesis. The blood glucose levels and insulin requirements of the patients were measured before and after treatment for a 24-h period and a reduction in blood glucose levels after treatment was noted. Thus the authors concluded that the use of pentoxifylline concurrently with antidiabetic medication is beneficial. Other authors have not observed an improvement (Heidrich and Schirop, 1980).

G. Diamidines

The mesylate and isethionate derivatives of pentamidine are antiprotozoals effective in the treatment of Pneumocystis carinii pneumonia, leishmaniasis, and trypanosomiasis. Pentamidine isethionate is commonly used in prophylaxis against P. carinii pneumonia and administered in aerosolized form (300 mg) every four weeks to patients diagnosed with HIV/AIDS. Aerosolized treatment has a lower incidence of systemic side effects relative to intravenous applications (Stevenson, 1989) and the isethionate form is less toxic than the mesylate (Belehu and Naafs, 1982). Pentamidine, when given intravenously or as an aerosol, has been reported to induce hypoglycemia because of elevated endogenous insulin levels (Fitzgerald and Young, 1984; Karboski and Godley, 1988). There is a dose-response relationship, as in one prospective study all patients with serum pentamidine levels greater than 100 ng/ml developed hypoglycemia (Comtois et al., 1992). In their case study Fitzgerald and Young demonstrated a reversal of this hypoglycemia by treating with oral diazoxide (Fitzgerald and Young, 1984).

Some patients who initially exhibit hypoglycemia may proceed to develop DM (Bouchard et al., 1982; Perronne et al., 1990). This would suggest that pentamidine is initially cytolytic, causing β-cells to release insulin in a nonregulated fashion, and in the final stages of β-cell injury it is cytotoxic. In the study by Bouchard and coworkers the patients had high serum insulin levels in the postabsorptive state and exhibited poor insulin responses to oral glucose, intravenous arginine, or intravenous glucagons, while the α-cell response to arginine was higher than normal.

We have found one report (Hauser et al., 1991) in which the pathology of the pancreas of a patient suffering from AIDS who developed hypoglycemia followed by DM was examined. There was a significant decrease in the number and intensity of insulin-positive cells relative to an age-matched and sex-matched control. There was an increase in the number of cells immunoreactive for glucagon but no change in those positive for SST. The islets displayed increased vascular spaces, but no islet cell necrosis, fibrosis, or lymphatic infiltrate was observed. The accumulative evidence would therefore suggest that pentamidine selectively damages the β-cells of the islets, causing them to release insulin from the secretory vesicles into the cytoplasm and thus increase circulating insulin levels, resulting in hypoglycemia. Persistent toxic effects then cause selective β-cell destruction, which can result in the development of DM, and in some cases this is insulin-requiring DM (Perronne et al., 1990). There is at least one instance in which the hypoglycemic effects of pentamidine were reversed, and clearly not everyone on pentamidine treatment develops diabetes. There is a dose-dependent toxicity with a threshold total dose, which appears to be in the range of 4 to 9 gm, at which irreversible damage to the β-cells may occur (Perronne et al., 1990). This damage is probably similar to that seen with streptozotocin in that the cells having a high energy requirement and highly active mitochondria are more susceptible to the oxidative damage of these compounds (Boillot et al., 1985).

H. Colchicine

Colchicine is an alkaloid derived from the corms and seeds of the plant Colchicum autumnale (meadow saffron, autumn crocus). The drug possesses both anti-inflammatory and antimitotic characteristics. It is commonly used in clinical practice in the treatment of acute gouty arthritis, Behcét’s syndrome, necrotizing vasculitis, and for prophylaxis of familial Mediterranean fever (FMF). Colchicine binds strongly and almost irreversibly to tubulin subunits, inhibiting addition of these units to existing microtubules and disrupting the dynamics of microtubule polymerization (Boyd et al., 1982; Pipeleers et al., 1976); thus the transport of newly synthesized proinsulin from the endoplasmic reticulum to the Golgi complex is retarded, and consequently the proinsulin-to-insulin conversion is hindered (Malaisse-Lagae et al., 1979).

Burstein and colleagues examined the effect of chronic colchicine treatment on glucose-induced insulin secretion on a group of 31 FMF patients treated with colchicine (1–2 mg daily) continuously for between 2 and 13 years (Burstein et al., 1997). They performed an OGTT on all the patients and an IVGTT on each of nine patients randomly chosen from the FMF cohort. These nine IVGTTs were compared to five age-matched IVGTTs performed on subjects with no history of past or present colchicine use. Essentially, the data from either test did not show any alteration in glucose tolerance or insulin secretory dynamics, which was also the conclu-
sion of the authors, so despite the extensively documented in vitro use of colchicine to disrupt the microtubule network of β-cells we have not found any reports of adverse effects on insulin secretion after long-term treatment with the drug. This is probably due to the different concentrations and exposure times to the drug in both cases. To attain the in vitro effects long pre-exposure periods are required to allow colchicine to permeate the cell membrane (uptake about 30 min) and attach to tubulin. Thus, at the doses commonly used in vivo there is probably not sufficient bathing of the islets in situ in the pancreas in colchicine to allow adequate permeation and attachment of the drug to the microtubulin units.

I. Acetylcholine and Cholinesterase Inhibitors

Acetylcholine is a naturally occurring human neurotransmitter that increases insulin secretion. Carbachol (5 µM), a cholinergic agonist, for example, increases glucose-induced insulin 2- to 3-fold (Zawalich and Zawalich, 2002) from isolated islets. Agonists bind to the M₃ receptor on the β-cell and most likely stimulate insulin secretion by the subsequent generation of DAG and IP₃ (reviewed in Gilon and Henquin, 2001). IP₃ mobilizes Ca²⁺ from the endoplasmic reticulum and DAG is a potent activator of PKC, which increases the efficiency of cytosolic Ca²⁺ in priming the secretory vesicles. ACh and carbamylcholine are used clinically to treat glaucoma. They are applied topically to the anterior chamber of the eye and the commonly used dose is 5 to 20 mg. Although there are some reports of adverse reactions indicative of systemic absorption we have not found any reports of an impact on insulin secretion (MICROMEDEX Healthcare Series, 2002).

Cholinesterase inhibitors have been studied for their ability to cause insulin secretion. Tacrine, used in Alzheimer’s disease, was found to stimulate insulin secretion from rat islets (Karlsson and Ahren, 1992). At tacrine concentrations of 10 to 100 µM the drug increased insulin secretion only in the presence of 8.3 mM glucose, and not at 3.3 mM glucose or in calcium-deficient medium at 8.3 mM glucose. This is consistent with what is known about ACh stimulation of insulin secretion, i.e., that it potentiates glucose-induced insulin secretion via a calcium-dependent mechanism. Other cholinesterase inhibitors, such as pyridostigmine (Del Rio et al., 1997) have been used to study cholinergic stimulation in the pancreas. When pyridostigmine was given (160 mg orally) with an OGTT, it increased total insulin output in obese subjects compared to an OGTT alone. This would seem to indicate that ACh is indeed stimulatory to insulin release.

J. Miscellaneous

1. Anesthetics. Hyperglycemia commonly occurs in the perisurgical period. Some of this is due to the intercurrent illness, but studies with the anesthetics isoflurane, halothane, and enflurane were shown to inhibit insulin secretion in isolated islets (Ewart et al., 1981; Desborough et al., 1993). It is generally agreed that the inhibition observed in these experiments is not due to an inhibition of glucose oxidation, but is associated with a small (but significantly different from controls) inhibition of islet adenylyl cyclase activity (Ewart et al., 1985). In a more recent study (Desborough et al., 1998) IVGTTs (5 g) were performed on 21 patients before and during anesthesia with isoflurane (1 and 2 minimum alveolar concentration) in nitrous oxide or with just nitrous oxide alone. First-phase insulin secretion was affected in that plasma insulin concentration measured at 3 min and area under the curve for plasma insulin levels at 15 min were significantly decreased in all three treatment groups in the tests carried out before, relative those performed subsequent to, anesthesia.

2. Oral Contraceptives. Alterations in carbohydrate metabolism have been reported upon long-term use of oral contraceptives and are known to be due to the progestogen component and not the estrogen content of this treatment (Spellacy, 1969, 1976). Although there is clear evidence for defects in glucose tolerance upon use of oral contraceptives the etiology of it is not clear, as the literature presents conflicting data on this point. For example, 50 women treated with norgestrol (0.075 mg) for 18 months were examined using a 3-h OGTT both before and after the treatment period (Spellacy, 1981). Although all of the glucose tolerance tests were normal initially, after treatment there was a statistically significant increase in blood glucose and plasma insulin levels overall. It is worth noting that the women had a mean increase in body weight of eight pounds, and this would have led to an increase in plasma insulin levels. The glucose levels pre- and post-100 g of oral glucose before and after norgestrel are difficult to interpret. The highest plasma glucose was only 124 mg/dl at 0.5 h after the OGTT. One would have expected much higher levels after this amount of oral glucose. The most recent study that we have found on glucose metabolism in subjects on oral contraceptives compared OGTTs for women on low-and high-dose monophasic norgestrel containing oral contraceptives with women who had never used oral contraceptives or had discontinued use for at least 24 months before the observations (Watanabe et al., 1994). Use of the minimal model to analyze data indicated that the women on the low-dose oral contraceptives (Lo/Ovral, n = 68) exhibited lower insulin sensitivity and glucose tolerance and worse β-cell function relative to the controls (n = 57) or those on the high dose (Ovral, n = 62). This reduced tolerance in only Lo/Ovral users is counterintuitive. It may be that the population in each group was nonrepresentative (i.e., self-selected in some manner), and 18 of the subjects in the Lo/Ovral group were Hispanic versus only 7 in the Orval group. In an earlier study by Luyckx and coworkers in which they observed elevations in blood glucose levels after treat-
Glucosamine and chondroitin sulfate leads to insulin resistance (Hebert et al., 1996; Veerababu et al., 2000). Several studies have suggested that the hexosamine pathway is involved in insulin resistance. Overexpression of GFAT in liver, fat, or muscle, for instance, is linked to hyperglycemia even more so than the older agents (reviewed in Newcomer et al., 2002; Haupt and Newcomer, 2001). Sernyak and colleagues, in a Virginia population of approximately 39,000 people on neuroleptics, showed that patients who received atypical neuroleptics were 9% more likely to have DM than those who received typical neuroleptics; this was after controlling for age (Sernyak et al., 2002). It is not known whether the increased insulin levels reported with treatment of clozapine and olanzapine are secondary to insulin resistance or the drugs have a direct effect on the β-cell. In a study by Melkersson and coworkers (Melkersson et al., 2001) the effects of seven antipsychotic drugs on isolated rat islets over two separate incubation periods of 1 and 4 h were studied. Of those studied, four agents were shown to have an effect on insulin secretion. Clozapine increased insulin secretion during the 4-h incubation period only; haloperidol inhibited glucose-stimulated insulin release, and chlorpromazine inhibited basal insulin secretion (i.e., at 3.3 mM glucose). There have to our knowledge been no investigations of the possible mechanisms by which these drugs or their metabolites may effect insulin secretion. Melkersson and coworkers speculate on the involvement of the dopamine receptors, as these drugs are dopamine receptor antagonists, but there is no clear evidence for this.

### 3. Anti-Psychotic Drugs

There is an increased incidence of hyperglycemia and type 2 DM in schizophrenic patients relative to the general population. Older treatments, such as phenothiazines, cause untoward weight gain, and this could therefore lead to type 2 DM (Thonnard-Neumann, 1968). Recently, reports suggest that the newer agents, clozapine, olanzapine, quetiapine, and risperidone, may also cause hyperglycemia even more so than the older agents (reviewed in Newcomer et al., 2002; Haupt and Newcomer, 2001). Sernyak and colleagues, in a Virginia population of approximately 39,000 people on neuroleptics, showed that patients who received atypical neuroleptics were 9% more likely to have DM than those who received typical neuroleptics; this was after controlling for age (Sernyak et al., 2002). It is not known whether the increased insulin levels reported with treatment of clozapine and olanzapine are secondary to insulin resistance or the drugs have a direct effect on the β-cell. In a study by Melkersson and coworkers (Melkersson et al., 2001) the effects of seven antipsychotic drugs on isolated rat islets over two separate incubation periods of 1 and 4 h were studied. Of those studied, four agents were shown to have an effect on insulin secretion. Clozapine increased insulin secretion during the 4-h incubation period only; haloperidol inhibited glucose-stimulated insulin release, and chlorpromazine inhibited basal insulin secretion (i.e., at 3.3 mM glucose). There have to our knowledge been no investigations of the possible mechanisms by which these drugs or their metabolites may effect insulin secretion. Melkersson and coworkers speculate on the involvement of the dopamine receptors, as these drugs are dopamine receptor antagonists, but there is no clear evidence for this.

### 4. Glucosamine

Glucose metabolism through the hexosamine pathway has been implicated in the many adverse effects of hyperglycemia. In the hexosamine pathway fructose-6-phosphate is converted to N-acetylglucosamine-6-phosphate, which is then converted to N-acetylglucosamine-6-phosphate by glucosamine:fructose-6-phosphate aminotransferase (GFAT). N-acetylglucosamine-6-phosphate is subsequently converted to N-acetylglucosamine-1,6-phosphate and UDP-GlcNAc. UDP-GlcNAc is a substrate for O-linked glycosylation, by O-GlcNAc transferase. It is reported that proteins and even transcription factors are modified and activated by O-GlcNAc (Comer and Hart, 2000; Wells et al., 2001). Several studies have suggested that the hexosamine pathway is involved in insulin resistance. Overexpressing GFAT in liver, fat, or muscle, for instance, leads to insulin resistance (Hebert et al., 1996; Veerababu et al., 2000). Glucosamine and chondroitin sulfate are often used as alternatives to nonsteroidal anti-inflammatory agents for degenerative joint disease and osteoarthritis. They are reputed to serve as building blocks for cartilage and they are exogenous sources of matrix proteins. Of interest to glucose metabolism, glucosamine itself is transported into cells through glucose transporters and phosphorylated by hexosamine to glucosamine-6-phosphate, thus bypassing GFAT and directly entering the hexosamine pathway. Incubation of adipocytes and skeletal muscles with glucosamine has been shown to reduce their insulin-mediated glucose uptake (Traxinger and Marshall, 1991; Robinson et al., 1993; Virkamaki et al., 1997). The importance of the hexosamine pathway is that if it is involved in insulin resistance, then elevations of plasma glucosamine levels would require increased insulin secretion to overcome insulin resistance. However, short-term infusion of glucosamine (300 min, 4 μmol/dl · min) in humans did not have any effect on glucose uptake (Pouwels et al., 2002).

There is also evidence that the hexosamine pathway is directly involved in the β-cells of the pancreas. In 1994, Balkan and Dunning (Balkan and Dunning, 1994) showed that glucosamine (5 mM) reduced glucokinase activity and insulin secretion in response to glucose in isolated islets. Recently, O-GlcNAc transferase has been shown to be highly expressed in β-cells (Akimoto et al., 1999) and incubating islets with glucosamine has been shown to decrease mRNA levels of GLUT2 and glucokinase (Yoshikawa et al., 2002). Kaneto and colleagues have added to the complexity of the effects of glucosamine on β-cells (Kaneto et al., 2001). They demonstrated that islets incubated with glucosamine show a decreased glucose-mediated insulin secretion and increased H2O2 levels in a dose-dependent manner. Insulin secretion was restored to near-normal by the addition of N-acetyl-l-cysteine, and mRNA levels of glucokinase and GLUT2 were also restored. Enhancing O-linked glycosylation in β-cells did not mimic the effects of adding glucosamine alone. The conclusion is that glucosamine does lead to deterioration of β-cell function in isolated islets, but this is because of oxidative stress and is not due to O-linked glycosylation. It should be remembered that the effects seen in isolated islets might not occur in the whole animal, as reactive oxygen species are cleared by defense mechanisms not necessarily present in the isolated islet. So far, we have not seen any reports of deterioration of diabetes control in subjects already diabetic and who are being treated with glucosamine (MICRODEX Healthcare Series, 2002). A recent report on glucosamine metabolism in beagles might explain why we are not likely to see any such reports. It appears that the bioavailability of glucosamine is very low, in that only about 12% of the dose is absorbed even after 2 weeks of treatment, and it is very rapidly metabolized, so very little of an oral glucosamine dose is likely to be available for uptake by islets (Adebowale et al., 2002).
Acknowledgments. We thank Drs. Steve Sollott, Cheryl Fahlin, and Michael Theodorakis for helpful comments on the manuscript and Thomas Wynne for constructing Fig. 1.

References


Diabetes Care 25: S89–94.


