

Orally active insulin mimics: where do we stand now?

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The war against diabetes through the development of new drugs is an ongoing continuous process to counter the alarming global increase in the prevalence of diabetes and its complications, particularly in developing countries like India. Unfortunately, the speed with which our knowledge of diabetes and its effects is expanding is not matched by the availability of new drugs. Following the identification of the insulin receptor (IR), its intrinsic kinase activity and molecular cloning, many studies have looked at IR as an ideal drug target. This review summarizes in brief the latest advancements in this field with particular reference to the current situation in respect of the development of orally active insulin mimetics in the treatment of type 2 diabetes.

1. Introduction

Ever since the discovery of insulin in 1921, the development of an orally administered insulin or insulin mimetic has been the 'global wish' for the treatment of diabetes mellitus. Unfortunately, insulin has to be taken as an injection simply because it is a protein which is broken up and digested by the enzymes of the gut if taken orally. Therefore, an oral insulin would both obviate the need for injections and more importantly, would closely mimic the physiological action of insulin by initial entry into the portal circulation. Oral therapies for diabetes mellitus have emerged out of this interest and are widely used still today. But rather than acting by directly mimicking insulin signalling, these drugs act by stimulating insulin release (sulphonylureas), potentiating insulin action (thiazolidinediones), lowering hepatic glucose production (biguanides) or by interacting with other intracellular events. The present day diabetic treatment pose the following problems: (i) insulin therapy does not accurately emulate normal *b*-cell function, (ii) oral agents only partially correct the multiple cellular disturbances that characterize type 2 diabetes, (iii) the present range of drugs do not completely normalize or reinstate glucose

homeostasis, (iv) insulin resistance is still relatively impervious to treatment and (v) diabetic patients are still at risk of chronic morbidity and premature mortality, despite the assiduous use of the available drugs. Therefore, it has become quite clear that elucidation of the molecular mechanisms of insulin signalling in diabetes would be necessary both to identify cellular targets and to aid in development of a 'lead' drug, most probably an orally active insulin mimetic.

This is what probably provided the motivation for Zhang *et al* (1999) who for the first time materialized the goal of an oral insulin almost within reach (up to the preclinical stage). The team of researchers (from USA, Sweden and Spain) laboured through some 50,000 potential compounds and finally zeroed in on one promising small non-peptidyl molecule that activated more specifically the human insulin receptor tyrosine kinase (IRTK) and acted as an insulin mimetic in several biochemical and cellular assays. The research was supported by Merck Research Laboratories and the molecule designated as L783,281 (compound 1) was identified as a demethyl-asterriquinone B-1, after isolation by high throughput screening of a fungal extract (*Pseudomassaria* sp.) which originated in the African jungles. This elegant work has

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Abbreviations used: EGFR, Epidermal growth factor receptor; GPI, glycosyl-phosphoinositol; IGF1R, insulin growth factor-1 receptor; IR, insulin receptor; IRS, insulin receptor substrate; IRTK, insulin receptor tyrosine kinase; OBA, 2-(oxalylamino)-benzoic acid; pVs, peroxovanadiums; PI 3-kinase, phosphatidylinositol 3-kinase; PIG, phosphoinositolglycan; PTPases, phosphotyrosine phosphatase; PDGFR, platelet-derived growth factor receptor.

implications for future therapies for diabetes, and the drug, if successful in clinical trials, could in future be popped as a pill!. Could this make painful daily pricks of insulin injections redundant?

2. Activation of insulin receptor kinase – a key step in insulin signalling

In the past, there have been many failed attempts at trying to find an oral substitute for insulin. The cellular response to insulin is mediated by its binding to the insulin receptor (IR). The IR is a heterotetrameric structure consisting of two **a**-subunits (135 kDa) on the outside of the plasma membrane connected by disulphide bonds to **b**-subunits (95 kDa) which are transmembrane proteins. Insulin binding to the **a**-subunits induces conformational changes that lead to autophosphorylation of the membrane-spanning **b**-subunits. This in turn, leads to the activation of a tyrosine kinase activity which is intrinsic to the cytoplasmic domains of the **b**-subunit. Extensive studies have indicated that the ability of the receptor to autophosphorylate and phosphorylate intracellular substrates is essential for its mediation of the complex cellular responses of insulin (Kasuga *et al* 1982; Rosen *et al* 1983; Yu and Czech 1984; Ellis *et al* 1987). Insulin receptors *trans*-phosphorylate several immediate substrates (on Tyr residues), including IR substrate (IRS) proteins 1–4, Shc, and Gab 1, each of which provide specific docking sites for other signalling proteins containing Src homology 2 domains (White 1998; White and Yenush 1998). These events result in a spectrum of biological responses by activating downstream signalling molecules including phosphatidylinositol 3-kinase (PI 3-kinase). PI 3-kinase has recently assumed great significance as the metabolic effects of insulin are primarily activated by PI 3-kinase dependent steps. Although the discrete pathways that couple PI 3-kinase to glucose regulation remain poorly defined, Akt (or protein kinase B), a Ser/Thr kinase known to be PI 3-kinase-dependent, appears to be involved in insulin-mediated activation of glucose transport (Kohn *et al* 1996) and glycogen synthesis (Alessi and Cohen 1998).

The mechanism for activation of IRTK has been a subject of intensive investigation. High resolution structural information has been obtained through crystallographic studies of IR kinase domain (Hubbard *et al* 1994; Hubbard 1997; Till *et al* 2001). Based on crystal structures of the unphosphorylated low activity form, as well as the phosphorylated active form of the IR, a model of *cis*-inhibition and *trans*-activation of the receptor was proposed. The unliganded receptors exist in the auto-inhibitory conformation that prevents access of ATP and substrate to the active site. Upon autophosphorylation of Tyr¹¹⁵⁸, Tyr¹¹⁶², and Tyr¹¹⁶³ in the activation loop, the IR

kinase undergoes a major conformational change resulting in unrestricted access of ATP and substrate to the active site and full activation of kinase. More recently, the three-dimensional structure of insulin receptor bound to insulin was determined by electron cryomicroscopy (Luo *et al* 1999). The three-dimensional reconstruction of the quaternary structure reveals that the both **a**-subunits are involved in insulin binding and that the two **b**-subunits are poised for *trans*-autophosphorylation. These structural studies have thus thrown new light on the molecular basis of activation of IR. In a nutshell, activation of the tyrosine kinase activity of the insulin receptor represents an essential and foremost step in the transduction of an insulin signal across the plasma membrane of target cells (figure 1).

The central role of the insulin receptor in metabolic and growth control has been validated in insulin receptor-null mouse models (Accili *et al* 1996; Joshi *et al* 1996). Mutations in insulin receptor gene that lead to alterations of receptor synthesis, degradation, and function have been described in patients with several uncommon syndromes associated with severe insulin resistance (Taylor 1992). The molecular basis for insulin resistance that precedes, or is associated with, common forms of type 2 diabetes remains poorly understood. However, several studies have shown modest decreases in insulin receptor number attributed to down-regulation in response to hyperinsulinemia in tissues or cells from type 2 diabetic patients (Olefsky 1976; Caro *et al* 1986). Specific decreases in insulin-stimulated receptor tyrosine kinase activity and an even more substantial defect in receptor-mediated IRS phosphorylation or PI 3-kinase and/or Akt activation have been described using cell types from rodents or human subjects with type 2 diabetes (Caro *et al* 1987; Freidenberg *et al* 1987; Haring *et al* 1987; Bulangu *et al* 1990; Goodyear *et al* 1995; Kerouz *et al* 1997; Krook *et al* 1998; Kim *et al* 1999). Thus, in humans with type 2 diabetes there are clear defects involving the insulin receptor and proximal steps in insulin signalling. The message from the above studies is that pharmacological intervention aimed at augmenting insulin receptor function may ultimately prove beneficial in patients with diabetes.

The studies of Zhang *et al* (1999) demonstrate that L783,281 directly modifies the receptor intracellular domain, apparently changing its conformation to one favouring tyrosine kinase activity. By enhancing the insulin-stimulated IRTK activation, L783,281 was also stated to function as an insulin sensitizer. The selectivity of L783,281 towards the insulin receptor, instead of the very closely related and more mitogenically active IGF-1 receptor (84% identical in the kinase domain), is truly remarkable, as the catalytic activities of these two proteins are extremely similar. After looking at the effects of L783,281 on insulin growth factor-1 receptor (IGF1R),

epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR), the compound was claimed to be more specifically selective for insulin receptor activation. The *in vivo* efficacy of L783,281 was also tested in db/db and ob/ob mice in which blood glucose was lowered by 50% over 6 h and about 40% over 7 days of oral therapy with the drug. The rodent models used in this study exhibit insulin resistance due to deficient production or signalling by leptin, the fat-derived hormone. At present, it is not clearly understood how the activity of L783,281 in these rodent models could be extrapolated to human type 2 diabetes, in which neither leptin deficiency nor resistance are the fundamental defects (Flier 1992). Nevertheless, the fact that exogenous insulin or insulin secretagogues improve the metabolic defect in many type 2 diabetics suggests that L783,281

would elicit a similar corrective action. In addition to its effect on IRTK, L783,281 was also shown to activate other components of the insulin signalling pathway such as stimulation of PI 3-kinase activity and phosphorylation of Akt kinase. As expected, L783,281 did stimulate glucose uptake in rat primary adipocytes and mouse soleus muscle.

Merck Research Laboratories speculated that their work represents a lead from which the directed synthesis of new compounds with improved features can be derived. Indeed, the follow-up work proved this is so and provides a role model for the systematic high-throughput natural product drug discovery. Zhang and his colleagues (Liu *et al* 2000; Qureshi *et al* 2000) have recently synthesized a number of derivatives of L783,281 (compound 1) and tested in a cell-based assay that monitors activation of

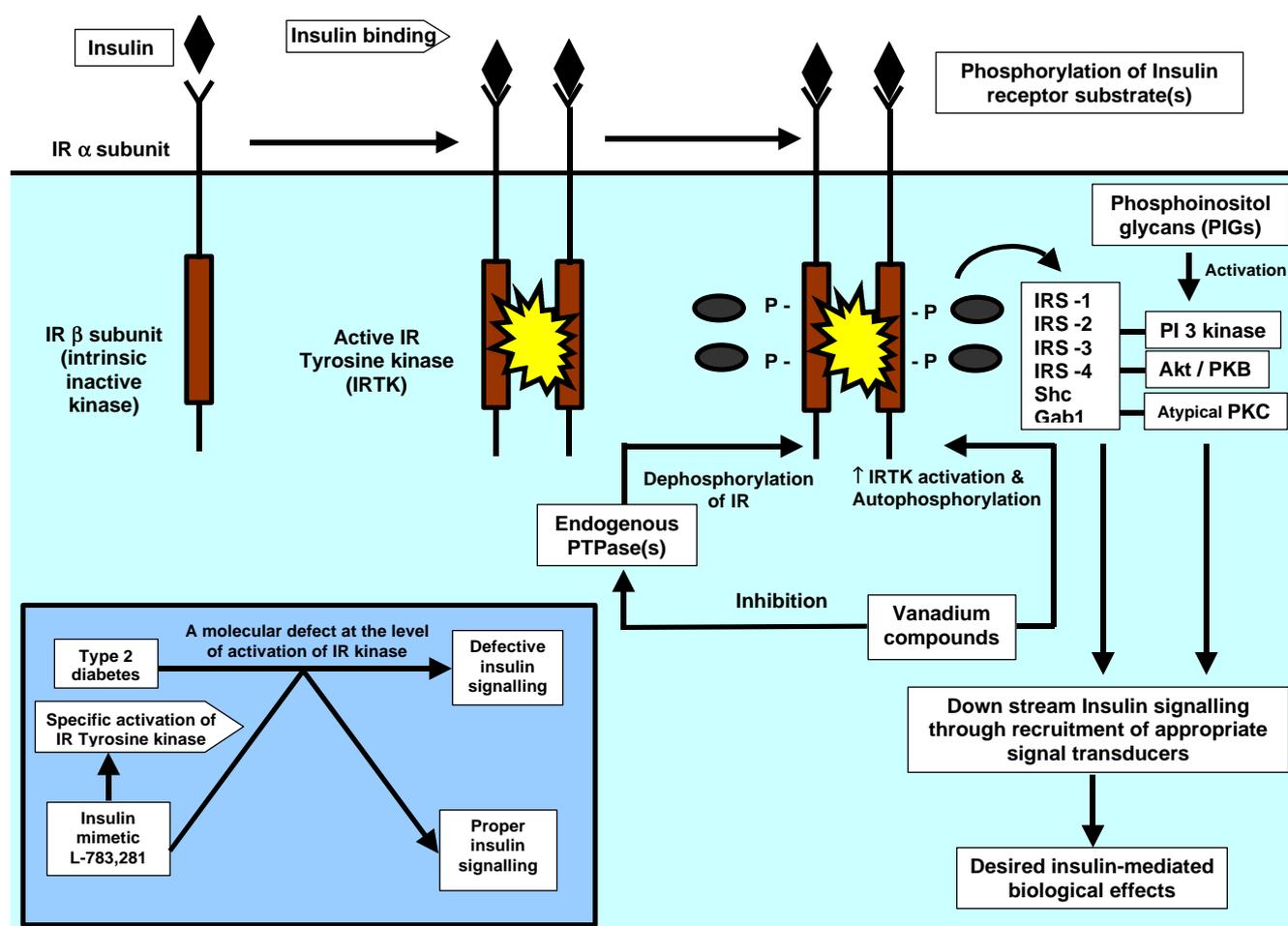


Figure 1. Insulin binds to the extracellular α -subunit of the IR, and induces a conformational change in the kinase domain of the transmembrane β -subunit resulting in activation of IR tyrosine kinase, an essential step for the downstream insulin signalling events. In type 2 diabetes, part of the insulin resistance is due to inability of insulin to activate the receptor kinase activity (see box). The Merck compound L783,281 is claimed to bypass this defect by specifically activating IR tyrosine kinase. Also depicted in the figure are other cellular sites wherein vanadium compounds and synthetic phosphoinositol glycans act.

IRTK activity in CHO cells expressing human insulin receptor (CHO-IR). One of these derivatives (designated as compound 2) found increased the IRTK activity in these cells with an EC_{50} of 300 nM, reflecting a greater than 10-fold improvement in the potency compared with compound 1. In contrast, a closely related analogue, compound 3, was not effective in activating IRTK in the same assay at concentrations up to 100 μ M. From the basic research and pre-clinical point of view, the results with the compound 2 appear very promising. In CHO-IR cells, it stimulated tyrosyl phosphorylation of the IR β -subunit and IRS-1 as well as phosphorylation of Akt in a dose-dependent manner. Moreover, the effect of insulin and compound 2 on activation of Akt can be blocked by pre-treatment with wortmannin (an inhibitor of PI 3-kinase), suggesting the activation of a PI 3-kinase-dependent pathway. Compound 2 stimulated tyrosine kinase activity of partially purified native IR and recombinant IR tyrosine kinase domain. Their administration to mice was associated with increased IR tyrosine kinase activity in liver. While oral administration of compound 2 to db/db mice elicited significant correction of hyperglycemia, in a streptozotocin-induced diabetic mouse model, it potentiated the glucose-lowering effect of insulin. In normal rats, compound 2 improved oral glucose tolerance with significant reduction in insulin release following glucose challenge. Quite interestingly, the additional insulin sensitizing actions other than IRTK activation of the Merck compound imply that the compound is unlikely to pose a great concern for desensitization of the insulin signalling during long term exposure. In fact, Qureshi *et al* (2000) has stressed that the extent of *in vivo* IR activation by the compound 2 was moderate (as against the exaggerated action in CHO-IR artificial system) only in part to mimic that of insulin with the additional benefits of the compound's action as an insulin sensitizer. Therefore, the results of the potent L783,281 endorses their potential insulin mimetic and sensitizing effects and raises our hopes for the development of a novel class of orally active antidiabetic agents.

3. Vanadium and protein tyrosine phosphatases

The demonstration that the trace element vanadium has insulin-like properties in isolated cells and tissues and *in vivo* has generated considerable enthusiasm for its potential therapeutic value in human diabetes (Meyero-vitch *et al* 1987; Brichard *et al* 1992; Woo *et al* 1999; Sakurai *et al* 2000). A major advance in the use of vanadium as an insulin-mimetic has been the development of organic vanadium complexes (peroxovanadiums, pVs). In several studies, administration of pV species resulted in activation of IRTK, autophosphorylation at tyrosine resi-

dues and inhibition of phosphotyrosine phosphatases (PTPases) (Posner *et al* 1994; Bevan *et al* 1995; Grillo *et al* 2000). Band *et al* (1997) have established the existence of an IRTK-associated PTPase whose inhibition by pVs correlates closely with IRK tyrosine phosphorylation, activation, and downstream signalling. The insulin-mimetic properties of the pV compounds raises the possibility for their use as insulin replacement in the management of diabetes mellitus. Supporting this view, initial clinical trials have reported a beneficial effect in patients with type 2 diabetes (Cohen *et al* 1995). Another vanadium (IV) compound, KP-102, is also currently under clinical trails in humans (Crans 2000). A few animal studies indicate that oral ammonium dipicolinatooxovanadium (V) is a clinically useful hypoglycemic agent in cats with naturally occurring diabetes mellitus. This compound is particularly interesting since it represents for the first time that a well-characterized organic vanadium compound with the vanadium in oxidation state five can act as an orally effective hypoglycemic agent in animals. A recent report has also claimed that transdermal delivery of pV compounds should be more beneficial in terms of their enhanced insulin mimetic and blood glucose lowering properties in rats (Brand and Hamel 1999). Despite these advances, there is also concern regarding the usage of vanadium in human diabetes, as they lack specificity and augment tyrosyl phosphorylation of a wide variety of cellular proteins and function *in vitro* as an inhibitor of protein tyrosine phosphatases (Denu *et al* 1996). Vanadium compounds could pose toxicity problems as they also target 'P' type ATPases, adenylate cyclases and Ca^{2+} channels. The avoidance of chronic toxicity and potential carcinogenic effects could be critical issues in the further development of vanadium-based insulin mimetics. Furthermore, some of the mechanistic studies indicate that vanadium acts not globally, but selectively and by enhancing rather than by mimicking the effects of insulin *in vivo* (Cam *et al* 2000).

Vanadium studies point out that protein tyrosine phosphatases could also be a suitable target for defining a novel class of insulin mimetics. PTPases play a critical role in regulating insulin action in part through dephosphorylation of the active (autophosphorylated) form of the insulin receptor (IRK) and attenuation of its tyrosine kinase activity. Following insulin binding, the activated IRK is rapidly internalized into the endosomal apparatus, a major site at which the IRK is dephosphorylated *in vivo*. Tyrosine phosphatases such as PTP1B and PTP α are known to dephosphorylate the insulin receptor and may contribute to insulin resistance in diabetes mellitus. In support of this view, overexpression of PTP1B in rat adipose cells significantly impairs insulin-stimulated translocation of GLUT4 (Chen *et al* 1997). Additionally, a phosphotyrosyl mimetic peptide reverses impairment of

insulin-stimulated translocation of GLUT4 caused by overexpression of PTP1B in rat adipose cells (Chen *et al* 1999). These studies suggest that PTP1B is implicated in the attenuation of the insulin signalling. Moreover, mice deficient in the heterotrimeric GTP-binding protein subunit $G_{i\alpha 2}$ exhibit a phenotype of insulin resistance characteristic of type 2 diabetes that correlates with the increased expression of PTP1B (Moxham and Malbon 1996). However, the most convincing results for PTP1B and insulin receptor interaction came from the recent studies of Elchebly *et al* (1999) in which mice lacking *PTP1B* gene showed increased insulin sensitivity and obesity resistance. Additionally, a specific molecular basis for the dephosphorylation of the activation segment of the insulin receptor by PTP1B has been recently reported through crystallographic, kinetic and PTP1B peptide binding studies (Salmeen *et al* 2000). These results make PTP1B a potential therapeutic target for the treatment of type 2 diabetes and obesity. Infact, Novo Nordisk has already on its way to optimizing a lead compound from the recently identified 2-(oxalylamino)-benzoic acid (OBA) which is a relatively weak, nonphosphorus, nonpeptidic inhibitor of several PTPases (Andersen *et al* 2000). This optimization process of structure-based design, guided by PTP mutants and X-ray protein crystallography has been expected to yield a highly selective, low molecular weight PTP1B inhibitor with unique enzyme kinetic features (Iversen *et al* 2000). It is also expected that new inhibitors of PTP1B may arise from the optimization of a series of azolidinediones (Malamas *et al* 2000) and from bioactive compounds extracted from cinnamon (Imparl-Radosevich *et al* 1998). Since PTP1B inhibitors may have the same therapeutic potential as IR kinase activators, there is also much scope in the development of new specific inhibitors of PTP1B-IRK interaction(s).

4. Synthetic phosphoinositolglycans (PIG peptides) mimic insulin by activating PI 3-kinase pathway

PI 3-kinase plays an important role in various insulin-stimulated biological responses including glucose transport, glycogen synthesis, and protein synthesis (Isakoff *et al* 1995; Quon *et al* 1995). Subcellular fractionation analyses indicates that insulin, unlike other growth factors, stimulates PI 3-kinase activity not only in the plasma membrane fraction but also in the low density microsomal compartment and possibly even in GLUT4 containing subfractions of the low density microsomes of adipocytes (Clark *et al* 1998). It appears that insulin-mediated subcompartmentalization of PI 3-kinase may be unique and might be key to the specificity of the effect of insulin on glucose transport. Earlier studies indicate that activation of PI 3-kinase was necessary but not sufficient for stimu-

lation of glucose transporter translocation in insulin-sensitive cells (Jiang *et al* 1998) similar to the actions of other signals such as platelet-derived growth factor (PDGF) and interleukin 4, which stimulate PI 3-kinase without any detectable GLUT translocation (Gould *et al* 1994; Isakoff *et al* 1995). However, it is now clear from the studies of Egawa *et al* (1999) that expression of the membrane-targeted p110 subunit of PI 3-kinase in 3T3-L1 adipocytes was sufficient to induce PI 3-kinase-dependent downstream signalling events, including glucose transport. Recently, synthetic phosphoinositolglycan (PIG) peptides have also been demonstrated to exert insulin-mimetic activity on glucose and lipid metabolism in insulin-sensitive cells and tissues *in vitro* and *in vivo* (Frick *et al* 1998). PIG peptides are designed according to the consensus structure of yeast glycosyl-phosphoinositol (GPI) anchors of GPI proteins and in rat adipocytes their activity depends on the presence of a 115 kDa polypeptide residing in plasma membrane caveolae where they interact with GPI proteins as the natural ligands. In mimicking the metabolic insulin signalling at the cellular level, PIG compounds are more selective for the phosphoinositol 3-kinase (PI 3-K) than mitogen-activated protein kinase (MAPK). These compounds although not activating IR kinase, recruit insulin signalling by invoking tyrosine phosphorylation of IRS-1, as evident from their insulin-mimetic activity in receptor-depleted adipocytes (Kessler *et al* 1998). Therefore, insulin and PIG peptide signalling seems to be divergent upstream of IRS-1 but converge at the level of PI 3-Kinase resulting in activation of further downstream signals and glucose uptake. In response to insulin, a decreased IRS-associated PI 3-kinase activity and a selective resistance to PI 3-kinase pathway were demonstrated in the vascular tissues of obese Zucker rats (Jiang *et al* 1999). Therefore, it is suggested that the early signalling step(s) used by PIG may represent a target for the treatment of insulin-resistant states and/or type 2 diabetes. At present, synthesis of potent and economical insulin-mimic PIG molecules are being carried out along with a strategy for conversion of the peptide and carbohydrate portions into heterocyclic structures which exhibit advantages with regard to oral delivery and bioavailability.

5. Future research

The new millennium holds promise of an explosion of novel anti-diabetic agents. Even in the case of wonder drug insulin, there have been several attempts to create an insulin which will not self aggregate and will exist in monomer form, get absorbed quickly and dissipate rapidly to mimic a physiological release of insulin. Insulin Glargine (Lantus) is the most recent addition to the growing family of insulin analogues and formulations

(‘designer insulins’) which release insulin *in vivo* in a relatively constant and thus peakless profile over 24 h with an additional advantage of restricting hepatic glucose production over prolonged periods (Heinmann *et al* 2000). It is certain that the biotechnological advancements are going to yield more ‘designer’ insulins in the near future. Specific strategies to overcome the barriers to oral insulin administration are also underway with the use of permeation enhancers, protease inhibitors, enteric coatings, polymer microsphere formulations and other drug delivery techniques (Carino and Mathiowitz 1999; Marschutz and Bernkop-Schnurch 2000; Marschutz *et al* 2000). In this context the effort for identifying, optimizing and developing specific insulin mimics has to go a long way to compete with the present day developments in the orally available insulin. However, correction of the exact biochemical defect by the molecular targeting of an insulin mimic will in theory, could be an advantageous therapy in certain subpopulations of type 2 diabetes and/or insulin resistant patients. Therefore, new chemical entities interfering with protein kinase and/or phosphatase interaction with insulin receptor can also be valuable pharmacological agents in the treatment of type 2 diabetes. It would be ideal if the newer drugs also possess insulin-independent blood glucose lowering activities and exhibit extrapancreatic actions that would improve the insulin sensitivity of the peripheral tissues and the reduction in hepatic insulin uptake. In fact, one of the reasons for the renewed interest in new generation sulphonylureas (e.g. glimepiride) in clinical diabetes rests on their beneficial extrapancreatic actions (Muller 2000). The studies on insulin mimics, including the pre-clinical investigations of Merck compounds thus raise much hope for the development of newer anti-diabetic drugs derived from the nature. Global traditional medicine also has great promise of a number of plants and plant products with anti-diabetic activity (Bailey and Day 1989; Balasubramanyam and Mohan 1999). A simple mushroom (*Agaricus campestris*) has been recently documented to have antihyperglycaemic, insulin-releasing and insulin-like activity (Gray and Flatt 1998). *Ipomoea aquatica* that is commonly consumed as a green leafy vegetable in several parts of South Asia is reported to possess an insulin-like activity (Malalavidhane *et al* 2000). It is high time for us to fully exploit our medicinal biodiversity to look for new chemical entities using high throughput biological screening assays. It is possible that many insulin mimetic properties may lie hidden in plants of Indian jungles and tribal areas!

References

- Accili D, Drago J, Lee E J, Johnson M D, Cool M H, Salvatore P, Asico L D, Jose P A, Taylor S I and Westphal H 1996 Early neonatal death in mice homozygous for a null allele of the insulin receptor gene; *Nat. Genet.* **12** 106–109
- Alessi D R and Cohen P 1998 Mechanism of activation and function of protein kinase B; *Curr. Opin. Genet. Dev.* **8** 55–62
- Andersen H S, Iversen L F, Jeppesen C B, Branner S, Norris K, Rasmussen H B, Moller K B and Moller N P 2000 2-(oxalylamino)-benzoic acid is a general, competitive inhibitor of protein-tyrosine phosphatases; *J. Biol. Chem.* **275** 7101–7108
- Balasubramanyam M and Mohan V 1999 The need for high throughput screening of herbal medicine with special reference to diabetes mellitus; *Brain storming colloquium on Biodiversity* (sponsored by DST), Abstract p 31
- Bailey C J and Day C 1989 Traditional plant medicines as treatments for diabetes; *Diabetes Care* **12** 553–564
- Band C J, Posner B I, Dumas V and Contreres J O 1997 Early signaling events triggered by peroxovanadium [bpV(phen)] are insulin receptor kinase (IRK)-dependent: specificity of inhibition of IRK-associated protein tyrosine phosphatase(s) by bpV(phen); *Mol. Endocrinol.* **11** 1899–1910
- Bevan A P, Drake P G, Yale J F, Shaver A and Posner B I 1995 Peroxovanadium compounds: biological actions and mechanism of insulin-mimesis; *Mol. Cell. Biochem.* **153** 49–58
- Brand R M and Hamel F G 1999 Transdermally delivered peroxovanadium can lower blood glucose levels in diabetic rats; *Int. J. Pharm.* **183** 117–123
- Brichard S M, Ongemba L N and Henquin J C 1992 Oral vanadate decreases muscle insulin resistance in obese fa/fa rats; *Diabetologia* **35** 522–527
- Bulangu L N, Ossowski V M, Bogardus C and Mott D 1990 Insulin-sensitive tyrosine kinase: relationship with *in vivo* insulin action in humans; *Am. J. Physiol.* **258** E964–974
- Cam M C, Brownsey R W and McNeill J H 2000 Mechanisms of vanadium action: insulin-mimetic or insulin-enhancing agent?; *Can. J. Physiol. Pharmacol.* **78** 829–847
- Carino G P and Mathiowitz E 1999 Oral insulin delivery; *Adv. Drug. Deliv. Rev.* **35** 249–257
- Caro J F, Ittoop O, Pories W J, Meelheim D, Flickinger E G, Thomas F, Jenquin M, Silverman J F, Khazanie P G and Sinha M K 1986 Studies on the mechanism of insulin resistance in the liver from humans with non-insulin-dependent diabetes; *J. Clin. Invest.* **78** 249–258
- Caro J F, Sinha M, Raju S M, Itoop O, Pories W J., Flickinger E G, Meelheim D and Dohm G L 1987 Insulin receptor kinase in human skeletal muscle from obese subjects with and without non-insulin dependent diabetes; *J. Clin. Invest.* **79** 1330–1337
- Chen H, Wertheimer S J, Lin C H, Katz S L, Amrein K E, Burn P and Quon M J 1997 Protein-tyrosine phosphatases PTP1B and syp are modulators of insulin-stimulated translocation of GLUT4 in transfected rat adipose cells; *J. Biol. Chem.* **272** 8026–8031
- Chen H, Cong L N, Li Y, Yao Z J, Wu L, Zhang Z Y, Burke T R and Quon M J 1999 A phosphotyrosyl mimetic peptide reverses impairment of insulin-stimulated translocation of GLUT4 caused by overexpression of PTP1B in rat adipose cells; *Biochemistry* **38** 384–389
- Clark S F, Martin S, Carozzi A J, Hill M M and James D E 1998 Intracellular localization of PI 3-kinase and insulin receptor substrate-1 in adipocytes: potential involvement of a membrane skeleton; *J. Cell Biol.* **140** 1211–1225
- Cohen N, Halberstam M, Shlimovich P, Chang C J, Shamooh H and Rossetti L 1995 Oral vanadyl sulfate improves hepatic and peripheral insulin sensitivity in patients with non-insulin-dependent diabetes mellitus; *J. Clin. Invest.* **95** 2501–2509

- Crans D C 2000 Chemistry and insulin-like properties of vanadium (IV) and vanadium (V) compounds; *J. Inorg. Biochem.* **80** 123–131
- Denu J M, Kohse D L, Vijayalakshmi J, Saper M A and Dixon J E 1996 Visualization of intermediate and transition-state structures in protein-tyrosine phosphatase catalysis; *Proc. Natl. Acad. Sci. USA* **93** 2493–2498
- Egawa K, Sharma P M, Nakashima N, Huang Y, Huver E, Boss G R and Olefsky J M 1999 Membrane-targeted phosphatidylinositol 3-kinase mimics insulin actions and induces a state of cellular insulin resistance; *J. Biol. Chem.* **274** 14306–14314
- Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy A L, Normandin D, Cheng A, Himms-Hagen J, Chan C, Ramachandran C, Gresser M J, Tremblay M L and Kennedy B P 1999 Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene; *Science* **283** 1544–1548
- Ellis L, Morgan D O, Clauser E, Roth R A and Rutter W J 1987 A membrane-anchored cytoplasmic domain of the human insulin receptor mediates a constitutively elevated insulin-dependent uptake of 2-deoxyglucose; *Mol. Endocrinol.* **1** 15–24
- Flier J S 1992 Syndromes of insulin resistance. From patient to gene and back again; *Diabetes* **41** 1207–1219
- Freidenberg G R, Henry R R, Klein H H and Olefsky J M 1987 Decreased kinase activity of insulin receptors from adipocytes of non-insulin-dependent diabetes mellitus; *J. Clin. Invest.* **79** 240–250
- Frick W, Bauer A, Bauer J, Wied S and Muller G 1998 Insulin-mimetic signaling of synthetic phosphoinositolglycans in isolated rat adipocytes; *Biochem. J.* **336** 163–181
- Gray A M and Flatt P R 1998 Insulin-releasing and insulin-like activity of *Agaricus campestris* (mushroom); *J. Endocrinol.* **157** 259–266
- Goodyear L J, Giorgino F, Sherman L A, Carey J, Smith R J and Dohm G L 1995 Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects; *J. Clin. Invest.* **95** 2195–2204
- Gould G W, Merrill N W, Martin S, Jess T J, Campbell I W, Calderhead D M, Gibbs E M, Holman G D and Plevin R J 1994 Growth factor-induced stimulation of hexose transport in 3T3-L1 adipocytes: evidence that insulin-induced translocation of GLUT4 is independent of activation of MAP kinase; *Cell Signal.* **6** 313–320
- Grillo S, Gremeaux T, Casamayor A, Alessi D R, Le Marchand-Brustel Y and Tanti J F 2000 Peroxovanadate induces tyrosine phosphorylation of phosphoinositide-dependent protein kinase-1 potential involvement of src kinase; *Eur. J. Biochem.* **267** 6642–6649
- Haring H U, Obermaier B, Ermel B, Su Z, Mushack J, Rattenhuber E, Holz J, Kirsch D, Machicao F and Herberg L 1987 Insulin receptor kinase defects as a possible cause of cellular insulin resistance; *Diabetes Metab.* **13** 284–293
- Heinmann L, Linkeschova R, Rave K, Hompesch B, Sedlak M and Heise T 2000 Time-action profile of the long-acting insulin analog insulin glargine (HOE 901) ion comparison with those of NPH insulin and placebo; *Diabetes Care* **23** 644–649
- Hubbard S R 1997 Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog; *EMBO J.* **16** 5572–5581
- Hubbard S R, Wei L, Ellis L and Hendrickson W A 1994 Crystal structure of the tyrosine kinase domain of the human insulin receptor; *Nature (London)* **372** 746–754
- Imparl-Radosevich J, Deas S, Polansky M M, Baedke D A, Ingebritsen T S, Andersen R A and Graves D J 1998 Regulation of PTP-1 and insulin receptor kinase by fractions from cinnamon: implications for cinnamon regulation of insulin signaling; *Horm. Res.* **50** 177–182
- Isakoff S J, Taha C, Rose E, Marcusohn J, Klip A and Skolnik E Y 1995 The inability of phosphatidylinositol 3-kinase activation to stimulate GLUT4 translocation indicates additional signaling pathways are required for insulin-stimulated glucose uptake; *Proc. Natl. Acad. Sci. USA* **92** 10247–10251
- Iversen L F, Andersen H S, Branner S, Mortensen S B, Peters G H, Norris K, Olsen O H, Jeppesen C B, Lundt B F, Ripka W, Moller K B and Moller N P 2000 Structure-based design of a low molecular weight, nonphosphorus, nonpeptide, and highly selective inhibitor of protein-tyrosine phosphatase 1B; *J. Biol. Chem.* **275** 10300–10307
- Jiang T, Sweeney G, Rudolf M T, Klip A, Traynor-Kaplan A and Tsien R Y 1998 Membrane-permeant esters of phosphatidylinositol 3,4,5-triphosphate; *J. Biol. Chem.* **273** 11017–11024
- Jiang Z Y, Lin Y W, Clemont A, Feener E P, Hein K D, Igarashi M, Yamauchi T, White M F and King G L 1999 Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats; *J. Clin. Invest.* **104** 447–457
- Joshi R L, Lamothe B, Gordonnier N, Mesbah K, Monthieux E, Jami J and Bucchini D 1996 Targeted disruption of the insulin receptor gene in the mouse results in neonatal lethality; *EMBO J.* **15** 1542–1547
- Kasuga M, Karlsson F A and Kahn C R 1982 Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor; *Science* **215** 185–187
- Kerouz N J, Horsh D, Pons D and Kahn C R 1997 Differential regulation of insulin receptor substrate-1 and -2 (IRS-1 and IRS-2) and phosphatidylinositol 3-kinase isoforms in liver and muscle of the obese diabetic (ob/ob) mouse; *J. Clin. Invest.* **100** 3164–3172
- Kessler A, Muller G, Wied S, Crecelius A and Eckel J 1998 Signaling pathways of an insulin mimetic phosphoinositolglycan-peptide in muscle and adipose tissue; *Biochem. J.* **330** 277–286
- Kim Y B, Zhu J S, Zierath J R, Shen H Q, Baron A D and Kahn B B 1999 Glucosamine infusion in rats rapidly impairs insulin stimulation of PI 3-kinase but does not alter activation of Akt/protein kinase B in skeletal muscle; *Diabetes* **47** 310–320
- Kohn A D, Summers S A, Birnbaum M J and Roth R A 1996 Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation; *J. Biol. Chem.* **272** 31372–31378
- Krook A, Roth R A, Jiang X J, Zierath J R and Wallberg-Henriksson H 1998 Insulin-stimulated Akt kinase activity is reduced in skeletal muscle from NIDDM subjects; *Diabetes* **47** 1281–1286
- Liu K, Xu L, Szalkowski D, Li Z, Ding V, Kwei G, Huskey S, Moller D E, Heck J V, Zhang B B and Jones A B 2000 Discovery of a potent, highly selective, and orally efficacious small-molecule activator of the insulin receptor; *J. Med. Chem.* **43** 3487–3494
- Luo R Z, Beniac D R, Fernandes A, Yip C C and Ottensmeyer F P 1999 Quaternary structure of the insulin-insulin receptor complex; *Science* **285** 1077–1080
- Malalavidhane T S, Wickramasinghe S M and Jansz E R 2000 Oral hypoglycaemic activity of *ipomoea aquatica*; *J. Ethnopharmacol.* **72** 293–298

- Malamas M S, Sredy, J, Gunawan I, Mihan B, Sawicki D R, Seestaller L, Sullivan D and Flam B R 2000 New azolidinediones as inhibitors of protein tyrosine phosphatase 1B with antihyperglycemic properties; *J. Med. Chem.* **43** 995–1010
- Marschurz M K and Bernkop-Schnurch A 2000 Oral peptide drug delivery: polymer-inhibitor conjugates protecting insulin from enzymatic degradation in vitro; *Biomaterials* **21** 1499–1507
- Marschutz M K, Caliceti P and Bernkop-Schnurch A 2000 Design and *in vivo* evaluation of an oral delivery system for insulin; *Pharm. Res.* **17** 1468–1474
- Meyerovitch J, Farfel Z, Sack Y and Shechter Y 1987 Oral administration of vanadate normalizes blood glucose levels in streptozotocin-treated rats; *J. Biol. Chem.* **263** 6658–6662
- Moxham C M and Malbon C C 1996 Insulin action impaired by deficiency of the G-protein subunit G α 2; *Nature (London)* **379** 840–844
- Muller G, Satoh Y and Geisen K 1995 Extrapancratic effects of sulfonylureas – a comparison between glimepiride and conventional sulfonylureas; *Diab. Res. Clin. Pract.* **28** S115–137
- Muller G 2000 The molecular mechanisms of the insulin-mimetic/sensitizing activity of the antidiabetic sulfonylurea drug Amaryl; *Mol. Med.* **6** 907–933
- Olefsky J M 1976 Decreased insulin binding to adipocytes and circulating monocytes from obese subjects; *J. Clin. Invest.* **57** 1165–1172
- Posner B I, Faure R, Burgess J W, Bevan A P, Lachance D, Zhang-Sun G, Fantus I G, Ng J B, Hall D A, Lum B S 1994 Peroxovanadium compounds. A new class of potent phosphotyrosine phosphatase inhibitors which are insulin mimetics; *J. Biol. Chem.* **269** 4596–4604
- Quon M J, Chen H, Ing B L, Liu M L, Zarnowski M J, Yonezawa K, Kasuga M, Cushman S W and Taylor S I 1995 Roles of 1-phosphatidylinositol 3-kinase and ras in regulating translocation of GLUT4 in transfected rat adipose cells; *Mol. Cell. Biol.* **15** 5403–5411
- Qureshi S A, Ding V, Li Z, Szalkowski D, Biazzo-Ashnault D E, Xie D, Saperstein R, Brady E, Huskey S, Shen X, Liu K, Xu L, Salituro G M, Heck J V, Moller D E, Jones A B and Zhang B B 2000 Activation of insulin signal transduction pathway and anti-diabetic activity of small molecule insulin receptor activators; *J. Biol. Chem.* **275** 36590–36595
- Rosen O M, Herrera R, Olowe Y, Petruzzelli M and Cobb M H 1983 Phosphorylation activates the insulin receptor tyrosine protein kinase; *Proc. Natl. Acad. Sci. USA* **80** 3237–3240
- Sakurai H, Sano H, Takino T and Yasui H 2000 An orally active antidiabetic vanadyl complex, bis(1-oxy-2-pyridinethiolato)oxovanadium(IV), with VO(S₂O₂) coordination mode; *in vitro* and *in vivo* evaluations in rats; *J. Inorg. Biochem.* **80** 99–105
- Salmeen A, Andersen J N, Myers M P, Tonks N K and Barford D 2000 Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B; *Mol. Cell.* **6** 1401–1412
- Taylor S I 1992 Lilly Lecture: Molecular mechanisms of insulin resistance: Lessons from patients with mutations in the insulin receptor gene; *Diabetes* **41** 1473–1490
- Till J H, Ablooglu A J, Frankel M, Bishop S M, Kohanski R A and Hubbard S R 2001 Crystallographic and solution studies of an activation loop mutant of the insulin receptor tyrosine kinase: insights into kinase mechanism; *J. Biol. Chem.* **276** 10049–10055
- White M F 1998 The IRS-signaling system: a network of docking proteins that mediate insulin action; *Mol. Cell. Biochem.* **182** 3–11
- White M F and Yenush L 1998 The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action; *Curr. Top. Microbiol. Immunol.* **228** 179–208
- Woo L C, Yuen V G, Thompson K H, McNeill J H and Orvig C 1999 Vanadyl-biguanide complexes as potential synergistic insulin mimics; *J. Inorg. Biochem.* **76** 251–257
- Yu K T and Czech M P 1984 Tyrosine phosphorylation of the insulin receptor beta subunit activates the receptor-associated tyrosine kinase activity; *J. Biol. Chem.* **259** 5277–5286
- Zhang B, Salituro G, Szalkowski D, Li Z, Zhang Y, Royo I, Vilella D, Diez M T, Pelaez F, Ruby C, Kendall R L, Mao X, Griffin P, Calaycay J, Zierath J R, Heck J V, Smith R G and Moller D E 1999 Discovery of a small molecule insulin mimetic with antidiabetic activity in mice; *Science* **284** 974–977

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