Introduction to the Kinases in Diabetes
Biochemical Society focused meeting: are protein kinases good targets for antidiabetic drugs?

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Abstract
Insulin regulates whole-body glucose homeostasis by modulating the activities of protein kinases in its target tissues: muscle, liver and fat. Defects in insulin’s ability to modulate protein kinase activity lead to ‘insulin resistance’ or impaired insulin action. Insulin resistance in combination with defective insulin secretion from the pancreas results in the elevated blood glucose levels that are characteristic of diabetes mellitus. Pharmacological agents that selectively modulate protein kinase activities in insulin-resistant tissues may act either as insulin-sensitizing or insulin-mimetic drugs. Consistent with this, small molecule modulators of a number of protein kinases have demonstrated efficacy in animal models of insulin resistance and diabetes. Moreover, emerging data in humans suggest that marketed anti-diabetic agents may also act in part through modulating protein kinase activities. This meeting was convened to consider the potential to treat insulin resistance and Type II diabetes by modulating protein kinase activity.

Protein kinases as drug targets
Protein kinases play a key role in regulating cellular processes, and their dysregulation is associated with disease states including cancer, inflammatory disorders, and diabetes [1–4]. Over the last decade the feasibility of identifying cell-permeable, small molecule inhibitors of protein kinases has been established within the pharmaceutical industry [1]. More recently, it has also been demonstrated that it is possible to activate protein kinases with small molecules [5]. Importantly, the first protein kinase inhibitors are now launched or in late-stage clinical development for the treatment of a variety of cancers and chronic inflammatory diseases [6–10]. This meeting was convened to consider the potential to treat Type II diabetes by modulating protein kinase activity.

Protein kinases in insulin signalling
Insulin exerts a dominant role in whole-body glucose homeostasis through stimulating glucose disposal into peripheral tissues (primarily muscle) and suppressing HGO (hepatic glucose output) [10]. Insulin activates the IRTK (insulin receptor tyrosine kinase) that in turn, phosphorylates IRS (insulin receptor substrate) proteins (Figure 1). Tyrosine-phosphorylated IRS proteins then recruit and activate the lipid kinase PI3K (phosphoinositide 3-kinase). PI3K, in concert with the protein serine/threonine kinases PDK-1 and -2 (phosphoinositide-dependent kinases 1 and 2), triggers the activation of PKB (protein kinase B) and aPKC (atypical protein kinase C). The protein serine/threonine kinases PKB and aPKC are believed to mediate the critical metabolic responses to insulin, including the stimulation of peripheral glucose utilization by increased glucose transport and glycogen synthesis in muscle [11]. Additionally, insulin stimulation of PKB appears important for the hormone’s suppression of HGO [12]. In Type II diabetes, insulin fails to maintain normoglycaemia owing to defective insulin action at its target tissues. This ‘insulin resistance’ is characteristic of Type II diabetes, and reflects post-receptor defects in the insulin signalling pathway, as typified by defective activation of the PKB kinase cascade [13–16]. Elevated insulin secretion from the pancreas initially compensates for peripheral insulin resistance at the muscle and fat, with the resulting hyperinsulinaemia maintaining normoglycaemia [17]. The pathologically elevated blood glucose (hyperglycaemia) that
Figure 1 | The signalling pathway by which insulin regulates whole-body glucose homeostasis

Insulin binding to the extracellular domain of the insulin receptor results in the activation of the IRTK on the intracellular domain of the receptor. Activated IRTK then tyrosine-phosphorylates and activates IRS. IRS recruits and activates PISK, which generates the membrane phospholipid second messenger PIP3. PIP3 in turn is required for the PDK1/2-dependent activation of PKB and aPKC. These two protein kinases are believed to mediate the critical metabolic responses to insulin, as described in the text. The protein kinases IRTK, PDK1/2, PKB and aPKC, the lipid kinase PISK and IRS are shaded green as these signalling molecules mediate insulin action. IRTK and IRS in their inactive state are shaded blue. In the interests of clarity, none of the other signalling molecules are shown in their inactive state. Signalling molecules that oppose insulin action are shaded red. Solid arrows and hammerheads denote direct activation and inhibition respectively. Broken lines denote undefined pathways. The question mark represents unknown signalling molecules that are shown in their inactive state. Signalling molecules that oppose insulin action are shaded red. Solid arrows and hammerheads denote direct activation and inhibition respectively.

Characterizes frank diabetes presents itself when pancreatic β-cell exhaustion leads to an absolute deficiency of insulin [18]. At this stage, hepatic insulin resistance and increased HGO also contribute to the observed hyperglycaemia.

Therapies for Type II diabetes

There is a major unmet medical need for improved pharmacological therapies for Type II diabetes. Current anti-diabetic drugs have limited efficacy in normalizing hyperglycaemia. These drugs can be classified, albeit somewhat crudely, on the basis of their established or putative mechanism of action [19]. The first class are insulin itself and those drugs that stimulate endogenous insulin secretion from pancreatic β-cells, the latter being the insulin secretagogue drugs, the sulphonylureas and prandial glucose regulators. The second class are those drugs that sensitize cells to the actions of insulin: the TZDs (thiazolidinediones) and metformin. Exogenous insulin and insulin secretagogues can be thought of as ‘insulin mimetics’ as they increase total blood insulin, and therefore ‘force’ insulin signalling in insulin-resistant tissues. In contrast, TZDs and metformin act by improving the sensitivity of insulin-resistant tissues to endogenous insulin: hence these drugs are known as ‘insulin sensitzers.’ The third class of anti-diabetic drugs are the α-glucosidase inhibitors, which inhibit carbohydrate digestion in the gut and hence slow glucose absorption. However, these drugs have more limited efficacy than those described above, and will not be discussed further as they are not so widely prescribed. Having introduced the insulin signalling pathway and the concept of ‘insulin mimetic’ and ‘insulin sensitizer’ drugs, it is interesting to consider whether drugs with similar or improved efficacy could be developed from inhibitors or activators of protein kinases. These candidate target kinases may be mediators of insulin action or putative negative regulators of the insulin signalling pathway that may contribute to the genesis and/or progression of insulin resistance. Alternatively, candidate target kinases may act independently of the insulin signalling pathway, but their modulation would still be predicted to impact metabolism in a manner that reduces hyperglycaemia.

Kinases addressed in the meeting

Protein kinases and insulin resistance

The speakers at this meeting addressed the potential of many kinases as targets for the development of novel anti-diabetic agents. Jeremy Tavare and others (this issue, see pp. 346–349) describe the evidence that PKB plays a critical role in insulin-stimulated glucose uptake in adipose tissue and muscle. Jeremy proceeded to highlight the technical challenges inherent in defining the precise mechanism by which PKB mediates increased insulin-stimulated GLUT4 translocation from intracellular vesicles to the plasma membrane. Bob Farese and others (see pp. 350–353) remind us that, in addition to PKB, there is a second insulin-activated protein kinase downstream of PDK1/2, namely aPKC. Evidence from IRS1 knockout mice indicates that intact IRS1/PISK signalling is required for insulin stimulation of muscle aPKC and PKB. In contrast, only insulin activation of aPKC was impaired in adipocytes, and only insulin activation of PKB in liver. Similar observations were made in the tissues of a number of rodent diabetic models. Assuming a selective defect in insulin activation of PKB exists in the diabetic liver, then this might explain why insulin fails to suppress HGO (via PKB activation) while retaining the ability to stimulate hepatic lipogenesis [via activation of aPKC, which is known to induce SREBP-1c (sterol-regulatory-element-binding protein-1c)]. Bob also reports that defects in insulin activation of aPKC in diabetic muscle can be attributed to both defects in PISK activation and to reduced sensitivity of the PKC itself to PIP3 (phosphatidylinositol 3,4,5-trisphosphate). Juleen Zierath and M. Bjornholm (see pp. 354–357) describe the defect in insulin’s ability to promote tyrosine phosphorylation of IRS1, and hence the activation of PISK, in diabetic muscle. Juleen raises the possibility that
increased serine/threonine phosphorylation of IRS1 was the mechanism for reduced insulin-receptor-mediated tyrosine phosphorylation of this protein. Bei Zhang and G. Jiang (see pp. 358–361) extend this line of thought by reviewing the evidence in cells and animal models that phosphorylation of serine-307 on IRS1 parallels the development of insulin resistance. Furthermore, Bei reports that PPAR (peroxisome-proliferator-activated receptor) agonists reduce IRS1 serine phosphorylation at this and other sites concomitantly with improved insulin sensitivity. The critical question is: ‘which kinase(s) are responsible for IRS1 serine phosphorylation in insulin-resistant or diabetic individuals?’ Bei describes the evidence implicating p38 MAPK (mitogen-activated protein kinase) and JNK (c-Jun N-terminal kinase) in the inhibitory serine phosphorylation of IRS1. IKKβ (IkB kinase β) has also been suggested as a ‘culprit’ since IKK heterozygous knockout mice have improved insulin sensitivity and are refractory to high-fat-diet-induced insulin resistance. Additionally, salicylates, including aspirin, which are IKKβ inhibitors, improve insulin sensitivity in diabetic humans and mice. Steve Shoelson proposed in his talk a model by which IKK contributes to the development of insulin resistance in obese diabetics. In this model, direct phosphorylation of IRS1 by IKK is not believed to play a role in insulin resistance in obese diabetics. Overnutrition results in obesity, and this is associated with hepatic steatosis and the activation of IKKβ in both adipose tissue and the liver. Activated IKKβ then triggers NFκB (nuclear factor κB) activation of transcription. It is this low-grade inflammatory response that induces insulin resistance, not only locally in adipose tissue and the liver, but also in the muscle, although the mechanism for this referred effect is not yet known [19a]. Further evidence for this model is provided by the observation that increased expression of IKKβ in the fat or liver of mice mimics the effects of a high fat diet to induce insulin resistance. On the other hand, expression of the IKK super-repressor in fat or liver protects mice from obesity-associated insulin resistance. Intriguingly, IKKβ overexpression in muscle does not induce insulin resistance, but causes profound muscle wasting akin to clinical cachexia [20]. This is interesting in view of a recent report describing that IkκB protein is reduced in insulin-resistant muscle, suggesting that IKKβ activity is in fact elevated [21]. Emmanuel Van Obbergen offered in his talk another mechanism for insulin resistance related to cytokine signalling and inflammation. He reported that SOCS (suppressors of cytokine signalling) proteins can interfere with insulin signalling through blocking IRS association with, and its tyrosine phosphorylation by, the insulin receptor. Additionally, SOCS can bind to IRS proteins and target these for proteosomal degradation [22]. The potential relevance of this mechanism to insulin resistance in animals is supported by the observation that SOCS3 expression is increased in obese mice, probably through increased insulin and/or TNFα (tissue necrosis factor α), both of which induce SOCS3 expression in adipocytes. Furthermore, SOCS3 knockout mice exhibit increased insulin sensitivity.

**Other candidate kinases that may be targeted in diabetes**

Dave Carling and L. Fryer (see pp. 362–366) review how AMPK (AMP-activated protein kinase) may have a regulatory role in metabolism. This kinase acts as a cellular ‘fuel sensor’ switching off anabolic pathways and switching on catabolic pathways when cellular ATP levels fall. Recently, a role for AMPK in the central regulation of feeding has also been described; its activation promotes feeding. Whereas the evidence for AMPK’s ability to promote fatty acid oxidation in muscle is still persuasive, the evidence for a role of this kinase in contraction-induced muscle glucose uptake has been somewhat eroded by transgenic and knockout mice data. The therapeutic value of activating AMPK in diabetes or metabolic syndrome must now balance the relative merits of any central and peripheral effects.

Rachel Mayers and others (see pp. 367–370) describe the glucose lowering efficacy of small molecule inhibitors of pyruvate dehydrogenase kinase. These compounds promote glucose disposal through the stimulation of glucose oxidation in the muscle, and potentially also act to inhibit hepatic glucose output by suppressing the gluconeogenic precursor supply to the liver. AZD7545 was disclosed as a non-ATP competitive kinase inhibitor, which lowered blood glucose in Zucker diabetic fatty rats.

Brendan Leighton and others (see pp. 371–374) describe the rationale for the sugar kinase glucokinase as a target for anti-diabetic therapy. Glucokinase is expressed primarily in the liver and in pancreatic β-cells, where it catalyses the rate-limiting step in glucose metabolism. Accumulating evidence indicates that activating glucokinase in these tissues promotes glucose utilization. Brendan reported that small molecule activators of glucokinase improve glucose tolerance and lower hyperglycaemia in insulin resistant and diabetic rodents.

**Drug safety issues**

Many of the safety issues surrounding the modulation of protein kinases as a therapeutic approach are common irrespective of the disease state and relate to kinase selectivity. Alex Bridges (see pp. 343–345) reviews kinases as enzymes and targets in general, and then deals with the issues surrounding safety of kinase modulators as drugs with respect to both cancer and diabetes therapy. Here, we present our view of the safety issues. Ideally, the target kinase will have a dispensable role in normal physiology such that inhibition or activation of it is unlikely to elicit unwanted side effects in non-target tissues [23]. This may appear an unrealistic aspiration, as most kinases are components of multiple signalling pathways, regulating pleiotropic responses in a variety of cell types. However, this redundancy of protein kinase function may also mitigate against the induction of undesirable side effects. This assumes, of course, that the target kinase plays an essential regulatory role in the biological effect being targeted, while having a subordinate or redundant role in regulating other potentially adverse biological effects. Side effects may also be minimized by dose titration to limit the extent of inhibition/activation of the kinase by the small molecule. In
and the elucidation of the mechanism of action of existing anti-diabetic drugs. An example of the latter is metformin, which has recently been shown to act at least in part through activation of AMPK. The increased number of candidate kinases will be more effectively validated, including safety assessment, through increasingly refined transgenic animal technology. Finally, a greater structural and functional knowledge of protein kinases will allow the design of highly selective modulators of protein kinases.

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References


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