Atypical protein kinase C in insulin action and insulin resistance

R.V. Farese1, M.P. Sajan and M.L. Standaert
Research Service, James A. Haley Veterans Administration Medical Center, and Department of Internal Medicine, University of South Florida College of Medicine, Tampa, Fl 33612, U.S.A.

Abstract
It now seems clear that aPKC (atypical protein kinase C) isoforms are required for insulin-stimulated glucose transport in muscle and adipocytes. Moreover, there are marked defects in the activation of aPKCs under a variety of insulin-resistant conditions in humans, monkeys and rodents. In humans, defects in aPKC in muscle are seen in Type II diabetes and its precursors, obesity, the obesity-associated polycystic ovary syndrome and impaired glucose tolerance. These defects in muscle aPKC activation are due to both impaired activation of insulin receptor substrate-1-dependent PI3K (phosphoinositide 3-kinase) and the direct activation of aPKCs by the lipid product of PI3K, PI-3,4,5-(PO4)3. Although it is still uncertain which underlying defect comes first, the resultant defect in aPKC activation in muscle most certainly contributes significantly to the development of skeletal muscle insulin resistance. Of further note, unlike the seemingly ubiquitous presence of defective aPKC activation in skeletal muscle in insulin-resistant states, the activation of aPKC is normal or increased in livers of Type II diabetic and obese rodents. The maintenance of aPKC activation in the liver may explain how insulin-dependent lipid synthesis is maintained in these states, as aPKCs function mainly in the activation of enzymes important for lipid synthesis. Thus increased activation of liver aPKC in hyperinsulinaemic states may contribute significantly to the development of hyperlipidaemia in insulin-resistant states.

Introduction
Type II diabetes mellitus, and its precursors, obesity and the metabolic syndrome, are responsible for much of the cardiovascular disease seen in most populations. The major pathogenetic factor that seems to underlie the hyperlipidaemia, hypertension and subsequent cardiovascular disease under these conditions is systemic insulin resistance. In this regard, defects in insulin signalling mechanisms have been consistently found in muscles of humans and laboratory animals afflicted with Type II diabetes or obesity, and in rodents consuming a high fat diet. On the other hand, the situation is less defined and to a certain extent perplexing in the liver, another major target tissue for insulin. Some defects in insulin signalling have been observed in livers of Type II diabetic rodents, but these defects in signalling do not appear to be present in high-fat fed, insulin-resistant rodents. On the other hand, defective ability of insulin to regulate hepatic carbohydrate metabolism may be seen with high-fat feeding, but in the absence of defects in insulin signalling, observed metabolic defects may largely reflect lipid-induced increases in gluconeogenesis and glucose release.

As alluded to, a perplexing aspect of Type II diabetes, obesity and the metabolic syndrome is that lipid synthesis is increased rather than decreased, as might be expected a priori if the normal stimulatory effects of insulin on hepatic lipid synthesis were inhibited. As we shall see, however, unexpected alterations in insulin signalling mechanisms seem to offer a reasonable explanation for this seeming paradox.

Insulin signalling mechanisms for metabolic regulation
During the past few years, it has become increasingly clear that many of the metabolic effects of insulin are mediated through downstream effectors of PI3K (phosphoinositide 3-kinase), namely aPKC (atypical protein kinase C) and PKB (protein kinase B or Akt). In particular, both aPKC [1–5] and PKB [6–9] appear to be important for mediating glucose transport effects of insulin in muscle and adipose tissue. On the other hand, PKB, rather than aPKC, appears to be important for (i) stimulating glycogen synthesis and promoting glucose storage in muscle, adipose tissue and liver [10,11]; and (ii) diminishing gluconeogenesis [12] and glucose release [13] by the liver.

In contrast with hepatic glucose regulation, lipid synthesis in the liver has been postulated to be controlled largely by the activation of aPKC, which appears to mediate insulin effects on the expression of SREBP-1c (sterol-regulatory-element-binding protein-1c) [14], an important transactivation factor that regulates a battery of genes that promote lipid synthesis [15]. Whether or not PKB participates in the activation of SREBP-1c is controversial (see [14]).

Activation of aPKC and PKB in various tissues
Although both aPKC and PKB operate downstream of IRS (insulin receptor substrate) family members and PI3K,
there are, perhaps surprisingly, considerable tissue-specific differences in how aPKC and PKB are activated. Thus, in muscle, both aPKC and PKB appear to operate largely downstream of IRS-1-dependent PI3K, as judged from the finding that insulin effects on both kinases are markedly diminished in IRS-1 knockout mice [16]. However, as we shall discuss, this does not imply that the activation of aPKC and PKB in muscle fluctuate in parallel in various disease states in which IRS-1-dependent PI3K is impaired.

In contrast with muscle, in the liver of IRS-1 knockout mice, insulin activation of PKB is markedly diminished, but, most surprisingly, aPKC activation is uncompromised [16]. On the other hand, activation of aPKC, as well as PKB, is diminished in immortalized murine hepatocytes deficient in IRS-2 [17]. Accordingly, it may be surmised that, in mouse liver: (i) aPKC activation is largely dependent on IRS-2, rather than IRS-1; and (ii) PKB activation is dependent on both IRS-2 and IRS-1.

To add further to the tissue-specific complexity, in studies of white [16] and immortalized brown [18] adipocytes, aPKC activation is compromised by the absence of either IRS-1 or IRS-2, whereas PKB activation is not inhibited by loss of either IRS-2 or IRS-1. Thus, in adipocytes, it appears that aPKC activation is dependent on both IRS-2 and IRS-1, and, in contrast, PKB activation can be satisfied, at least quantitatively, by activation of either IRS-1, IRS-2 or other factors that activate PI3K. Interestingly, insulin-stimulated glucose transport follows the same pattern as aPKC activation in white and brown adipocytes deficient in IRS-1 or IRS-2 [18,19].

Finally, in white and brown adipocytes, insulin-induced activation, and plasma membrane localization of aPKC within specific lipid raft microdomains, is dependent, not only on IRS-1 and IRS-2, but also on Cbl [20] and, we believe, Cbl-dependent PI3K [21]. Also note that Cbl is required for activation of Crk, C3G and TC10, independent of PI3K [20]. Thus, Cbl appears to co-ordinate two pathways, one PI3K-dependent and one PI3K-independent, needed for insulin-stimulated GLUT4 translocation and glucose transport in adipocytes. On the other hand, these actions of Cbl and Cbl-dependent factors do not appear to be operative in rat skeletal muscles (see [21]). Nevertheless, it seems probable that an analogous pathway serves the purpose of localizing aPKC to sites important for GLUT4 translocation. Of further interest, Cbl activation is compromised in adipocytes of Type II diabetic rats and restored by thiazolidinedione treatment (M.L. Standaert, M.P. Sajan, A. Miura and R.V. Farese, unpublished work).

Defects in insulin signalling in muscle in diabetes and obesity

Defective activation of aPKC has been observed in muscles of Type II diabetic rats [22], monkeys [23] and humans [24–26]. This defect in muscle aPKC activation in these diabetic states is at least partly due to impaired activation of IRS-1-dependent PI3K. However, the activation of both PKBα and PKBβ has been found to be intact under these conditions [22–27]. The surprisingly normal activation of PKBα/β in diabetic muscle is enigmatic. One possibility is that a partial defect in IRS-1-dependent PI3K activation may inhibit aPKC, but not PKB activation, if PKB is more effectively activated at lower levels of insulin and IRS-1-dependent PI3K activity. However, insulin dose–response studies do not support this idea (Y. Kanoh, M.P. Sajan, A. Miura, M.L. Standaert and R.V. Farese, unpublished work). Another possibility is that diabetes may up-regulate alternative factors that control overall PKB activity. In the latter scenario, PKB activation may occur in intracellular compartments that are not involved in glucose transport.

Defective activation of aPKC has also been observed in muscles of, not only Type II diabetic rats, monkeys and humans, as described above, but also in muscles of obese diabetic ob/ob mice [28], obese diabetic db/db mice [29], obese prediabetic monkeys [23], obese glucose-intolerant humans [24,25] and obese glucose-tolerant humans [26,30]. Thus, defects in aPKC activation are seen in obesity, even in the absence of clinical abnormalities of glucose metabolism. Defects in both IRS-1-dependent PI3K and PKB activation have been found under some of these conditions, e.g. ob/ob-diabetic [28] and db/db-diabetic [29] mice, but are not necessarily present or statistically significant in other situations, e.g. obese glucose-intolerant [25] and obese glucose-tolerant humans [26,30].

The failure to observe consistently significant defects in IRS-1-dependent PI3K alluded to activation in the latter situations prompted us to examine the direct activation of aPKC by PIP3, [PI-3,4,5-(PO4)3], the lipid product of PI3K. Interestingly, marked defects in PIP3-dependent activation of aPKCs were found in muscles prepared from muscles of obese glucose-intolerant [25] and glucose-tolerant [30] humans, obese prediabetic monkeys [23] and Type II diabetic monkeys [23] and humans [25]. Accordingly, it may be surmised that defects in muscle aPKC activation in Type II diabetes are due to both impaired activation of IRS-1-dependent PI3K and poor responsiveness of aPKCs to PIP3. It may be further speculated that poor responsiveness of aPKCs to PIP3 can occur independent of, and perhaps be as or more important than, impaired activation on IRS-1-dependent PI3K in causing defective aPKC activation in both prediabetic and overtly diabetic states.

Of further note, defective activation of aPKC by PIP3 has also been found in cultured myocytes and adipocytes of obese humans [32]. In addition to defects in aPKC activation, there are defects in insulin-stimulated glucose transport, but PKB activation is normal, in these cultured myocytes and adipocytes [24,32]. Further studies are needed to determine why defects in aPKC activation persist in cultured cells.

Insulin signalling in muscles of high-fat fed rodents

High fat feeding has been used to induce rapidly an insulin-resistant but, at least initially, glucose-tolerant state in rodents fed rodents.
that may be analogous to obesity. In our experience, aPKC activation by insulin was found to be defective in muscles of both rats [33] and mice [28,34] placed on a moderately high fat diet (40% calories). In mice, the defect in muscle aPKC activation is due to both impaired activation of IRS-1-dependent PI3K and diminished responsiveness of aPKC to PIP3, and PKB activation is similarly impaired [28,34]. Perhaps more interestingly, in rats, this same diet provokes a defect in muscle aPKC activation, with no appreciable change in IRS-1-dependent PI3K, IRS-2-dependent PI3K or PKB activation [33]. In these fat-fed rats, the defect in muscle aPKC activation appears to be solely due to poor responsiveness of aPKC to PIP3.

**Insulin signalling in livers of diabetic rodents**

In the livers of Type II diabetic, non-obese, Goto–Kakizaki rats, insulin activation of IRS-1-dependent PI3K and PKB are markedly diminished, but, in marked contrast, the activation of IRS-2-dependent PI3K and aPKC are intact ([28] and M.P. Sajan, M.L. Standaert, A. Miura and R.V. Farese, unpublished work). Interestingly, this pattern of diminished PKB activation coupled with normal aPKC activation is virtually the same as that observed in livers of IRS-1 knockout mice [16]. Accordingly, compromise of IRS-1 function and maintenance of IRS-2 function, as seen in Goto–Kakizaki-diabetic liver, seems to account for the loss of PKB activation and the retention of aPKC activation in this tissue; the functional importance of this dichotomy in insulin signalling through IRS-1/PKB and IRS-2/aPKC is discussed below.

A remarkably similar pattern of impaired PKB activation and apparently normal activation of aPKC has also been observed in livers of obese-diabetic ob/ob mice [28]. However, the activation of both IRS-1- and IRS-2-dependent PI3K was reported to be impaired in these mice [31]. Further studies are needed to determine what PI3K activity is responsible for maintaining aPKC activation in livers of ob/ob-diabetic mice.

**Insulin signalling in livers of high-fat fed rodents**

In contrast with diabetic rodents, the activation of IRS-1- and IRS-2-dependent PI3K, and both PKB and aPKC, appear to be fully intact in livers of high-fat fed mice ([28,35] and unpublished work). As alluded to above, this does not necessarily imply that glucose handling by the liver is normal, but rather suggests that any such defects in glucose handling are more likely to be reflective of lipid (fatty acids) or other substrate-dependent effects on gluconeogenesis and/or glucose storage or release.

**Phenotypic consequences of altered insulin signalling patterns in muscle and liver**

From the above considerations, it would appear that defects in aPKC activation, with or without associated defects in PKB activation, play a key role in the development of skeletal muscle insulin resistance. Such defects in muscle aPKC activation are present both in early and later stages of insulin-resistant disorders, i.e. from high fat or high caloric feeding, through simple obesity and the metabolic syndrome, and thence to impaired glucose tolerance and overt Type II diabetes mellitus. Since hepatic signalling defects are not evident in livers of high fat-fed rodents, it is tempting to postulate that systemic insulin resistance is initially largely due to defective aPKC activation in muscle. As a corollary, it may be inferred that poor responsiveness to PIP3, is a major cause of poor aPKC activation in early as well as later phases of insulin resistance.

On the other hand, in Type II, obese and non-obese, overtly diabetic rodents, defects in insulin signalling to IRS-1-dependent PI3K and PKB are readily apparent in the liver. Unfortunately, there is only limited information on insulin signalling in the livers of high fat-fed or obese non-diabetic rodents or other laboratory animals, and we therefore do not know the point at which insulin signalling to IRS-1-dependent PI3K and PKB becomes defective in the liver, as rodents or other animals progress from a hyperlipidaemic/hypercaloric state to recognizable obesity and thence to overt diabetes. At this point, it is tempting to suggest that insulin signalling to IRS-1 and PKB becomes defective only with the development of glucose-intolerance or overt Type II diabetes, and, indeed, such defects in hepatic insulin signalling undoubtedly make an important contribution to hyperglycaemia in Type II diabetes mellitus.

Apart from IRS-1 and PKB and their effects on glucose homeostasis, it is particularly interesting that aPKC activation in the liver appears to be intact during all stages of high fat feeding, obesity and Type II diabetes mellitus, at least in rodents so far examined. This maintenance of aPKC activation would provide an explanation for maintenance of certain effects of insulin in the liver, most notably increased activation of SREBP-1c and thus hepatic lipid synthesis, as has been observed in insulin-resistant lipodystrophic and ob/ob-diabetic mice [36]. Moreover, if indeed insulin effects on lipid synthesis are largely mediated through aPKC, the maintenance of aPKC activation would explain how lipid synthesis and secretion of very-low-density lipoproteins are increased in each of these insulin-resistant hyperinsulinaemic states. Thus a major cardiovascular risk factor present in diabetes, obesity and the metabolic syndrome, namely hyperlipidaemia, may be at least partly due to increased aPKC activity in the liver.

**Conclusions**

It now seems clear that a defect in muscle aPKC activation by insulin is present in all, of as yet examined, insulin-resistant states in rodents, monkeys and humans. Accordingly, defective activation of aPKC may be an important proximate cause of systemic insulin resistance in states of obesity, the metabolic syndrome and Type II diabetes. This defect in muscle aPKC activation appears to be due to diminished activation of IRS-1/PI3K and/or poor responsiveness to PIP3.
It seems most probable that these defects are acquired, as they can be largely prevented [23] or diminished [26] by caloric restriction and weight control or by thiazolidinedione treatment [25]. On the other hand, insulin signalling defects to aPKC are maintained in cultured adipocytes and myocytes, and the reason for this memory needs to be elucidated.

Presently, we have no information on insulin signalling in livers of diabetic or obese humans. If, however, defects in insulin signalling in livers of humans are similar to those seen in rodents, it may be surmised that IRS-1 and PKB activation are compromised in liver and contribute to hyperglycaemia, whereas aPKC activation remains intact or in fact may be increased in response to hyperinsulinaemia, and thereby contributes to hyperlipidaemia, in diabetic humans. Accordingly, therapeutic efforts in Type II diabetes should be focused on (i) improving aPKC activation in muscle, (ii) inhibiting aPKC activation in liver, and (iii) improving PKB activation in muscle and liver.

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References

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