HYPOTHESIS

Diacylglycerol/protein kinase C signalling: a mechanism for insulin resistance?

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Abstract. Shmueli E, Alberti KGMM, Record CO. An increase could occur in three different ways. Chronic hyperinsulinaemia could increase DAG levels by de-novo synthesis from phosphatidic acid, by hydrolysis of phosphatidylcholine, or by hydrolysis of glycosyl-phosphatidylinositol: DAG is also formed by hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP₂). This reaction, known as the 'PI response,' may be the connection between hypertension and insulin resistance. A third mechanism for an increase in DAG involves neural abnormalities. Thus, muscle denervation in the rat is characterized both by a profound insulin resistance and a large increase in DAG. It is possible that a similar increase occurs in humans and may explain the association between denervation, inactivity, and insulin resistance.

Keywords: denervation, diacylglycerol, insulin resistance; protein kinase C.

Insulin resistance, and particularly impaired muscle glycogen synthesis in response to insulin, is found in a number of different conditions including diabetes [1], cirrhosis [2], and hypertension [3, 4]. Insulin resistance has also been found in patients with various neuromuscular disorders including motor neurone disease [5], dystrophica myotonica [6], and Friedrich's ataxia. Although frequently clinically insignificant, the impaired glucose tolerance and insulin resistance of these conditions is not explained by decreased muscle bulk [7]. However, it is well known that exercise increases insulin sensitivity in both normal and diabetic subjects [8], yet the mechanism underlying changes in insulin sensitivity remains obscure.

There is considerable evidence from in-vitro and in-vivo work in rats that insulin increases synthesis of 1,2-diacylglycerol (DAG) which then activates protein kinase C (PKC). In BC3H-1 myocytes DAG increased within 30 s of stimulation by insulin. Membrane PKC activity increased two-fold within 60 s, and cytosolic PKC activity increased by 80% over 20 min [9]. An increase in rat skeletal muscle DAG in response to insulin has been demonstrated in vitro [10, 12] and in vivo [11–13] and was dose-related [12]. Furthermore, insulin also increased DAG synthesis in hepatocytes [14, 15]. Pre-labelling of DAG precursors suggests that the DAG is synthesized mainly from glycerol through phosphatidic acid [9–11, 14, 15] and by hydrolysis of phosphatidylcholine by an insulin-dependent phospholipase D [15].

Increases in DAG were associated with both membrane- and cytosol-associated increases in PKC activity [9], or with translocation of PKC to the membrane, and an increase in membrane-associated
Phosphorylates serine residues on insulin activity [15-121. In one study however, the increase in PKC activity in response to insulin was minor [16], whilst in another insulin had no effect on PKC activity [17].

The role of PKC in glucose metabolism remains to be clearly defined. It has been suggested that PKC is intimately involved in negative feedback control of intracellular signals [18]. Indeed, PKC activated by DAG inactivates glycogen synthase in hepatocytes [19, 20] and in skeletal muscle [21]. Moreover, there is evidence that PKC diminishes tyrosine kinase activity in the insulin receptor by phosphorylation of serine/threonine residues [22-24], and phorbol esters (DAG analogues) also inactivate insulin receptor tyrosine kinase through PKC activation [25]. The issue is confused by evidence that PKC activation has 'insulin-like' effects in increasing glucose transport [17, 26], pyruvate dehydrogenase activity [26], and lipogenesis [20]. However, these increases only occur in the basal state and are not additive to the effect of insulin [12, 17]. Indeed there is more evidence that phorbol esters (PKC activators) antagonize the action of insulin [20, 25, 27]. The evidence suggests two pathways for glucose transport—one being insulin dependent and inhibited by PKC, and the other being insulin independent and enhanced by PKC. It is therefore of interest that basal glucose utilization is increased in both type 1 and type 2 diabetes [28], while in non-insulin-dependent diabetes insulin-independent glucose uptake increased [29].

The sectioning of motor nerves to rat skeletal muscle 1–3 days before incubation of the muscle in vitro reduces the ability of insulin to induce glucose transport and glycogen synthesis by 70–100% [30, 31]. As insulin binding and receptor numbers are not affected, it is probable that denervation induces a post-receptor defect [30, 32]. Indeed, when the denervated muscle was preincubated in buffers for 2–4 h before analysis, glucose transport returned to normal but glycogen synthesis remained profoundly insulin resistant [33], suggesting that denervation may affect the insulin receptor signal transduction at more than one site. In this context glucose transporters 1 and 4 (GLUT-1 and GLUT-4) mRNA were unchanged 1 day after denervation when insulin resistance was already well established, whilst at days 3 and 7 post denervation GLUT-4 was decreased by 50% whilst GLUT-1 was increased by 60–100% [34]. Glycogen synthase activity was diminished by about 80% after 3 days of denervation, and studies with 32P-labelled glycogen synthase...
demonstrated that the ability of insulin to stimulate dephosphorylation was impaired [35].

Denervation of rat skeletal muscle has also now been associated with increases in DAG levels. Turinsky et al. [36] found that rat skeletal muscle DAG increased by 21.51 and 117%, 3.16, and 32 days after denervation: whilst insulin resistance was maximal at about 3 days. Heydrick et al. [37] showed a 40% increase in muscle DAG 24 h after denervation, associated with an increase in PKC activity. The effect of denervation on DAG in human muscle is unknown, although it is interesting that diseases involving denervation have been associated with insulin resistance [5–7].

Hypertension is associated with an increase in the ‘PI response’ and an increase in DAG containing arachidonate in the C2 position [38]. Inhibition of glycogen synthase by this DAG, through PKC activation, may be the link between hypertension and insulin resistance. Indeed, treatment of hypertension with captopril or prazosin—drugs that diminish the PI response—increases insulin sensitivity [39].

Whether DAG is increased in insulin resistant states in humans is not known. However, DAG levels are increased by 40–136% in the skeletal muscles of insulin-resistant genetically obese Zucker rats [40], whilst streptazotocin-induced diabetes increases DAG levels and PKC activity in a number of rat tissues so far studied including adipose tissue [11], aorta and heart [41], and mesangial cells [42–43]. Furthermore, a two-fold increase in PKC activity was found in both the cytosol and membrane extracts from starved rats [24]. It is thus conceivable that mild hyperinsulinaemia leads to increased DAG levels and PKC activity, which then clinically inhibits insulin action and starts a vicious cycle whereby insulin levels increase further: the consequent increased insulin resistance eventually leading to glucose intolerance and diabetes (Fig. 1).

References

14 Cooper DR, Hernandez H, Kuo YJ, Farese RV. Insulin increases the synthesis of phospholipid and diacylglycerol and protein kinase C activity in rat hepatocytes Arch Biochem Biophys 1990; 276: 486–94.

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