Insulin Action under Arrestin

Jacqueline Stöckli[1] and David E. James[1,*]
[1]Garvan Institute of Medical Research, 384 Victoria St., Darlinghurst, NSW, Australia
*Correspondence: d.james@garvan.org.au
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Insulin signaling is key to the etiology of metabolic syndrome. Recent work (Luan et al., 2009) uncovers a role for β-arrestin, previously known to control GPCR desensitization, in insulin signaling. In mouse models, β-arrestin-2 controls whole-body insulin action by regulating assembly of a complex containing insulin receptor, c-Src, and Akt.

Insulin resistance or impaired insulin action in muscle, fat, and liver is one of the earliest detectable defects associated with a range of metabolic diseases including type 2 diabetes (T2D). Overeating, or a lack of exercise coupled with obesity have been implicated as major factors contributing to insulin resistance. At the cellular level, a range of abnormalities (including ER stress, oxidative stress, mitochondrial defects, intracellular lipids, or inflammation) have been experimentally linked to insulin resistance. However, the molecular basis for insulin resistance remains controversial in that some studies indicate a primary role for a signaling defect at insulin receptor substrate 1 (IRS1) leading to reduced Akt activation (reviewed in Taniguchi et al., 2006), while other studies suggest otherwise (Kim et al., 1999; Hoehn et al., 2008). In a recent issue of Nature, Luan et al. (2009) now provide evidence for yet another insulin action rebel: β-arrestin-2.

β-arrestin-2 was originally identified as a mediator of β-adrenergic receptor endocytosis, leading to attenuation of β-adrenergic signaling. However, subsequent studies have revealed a broad tissue distribution and an array of interacting molecules, suggesting that β-arrestins play a general role in many signaling pathways. For example, they regulate the desensitization and downregulation of many G protein-coupled receptors (GPCRs) (DeWire et al., 2007). They also act as a scaffold for key signaling molecules including the tyrosine kinase c-Src; receptors for insulin, IGF1 and PDGF; and Ser/Thr kinases including Akt, Raf, ERK, and JNK (Hupfeld and Olefsky, 2007).

Luan et al. (2009) begin to integrate some of these disparate attributes of β-arrestin function in the context of insulin action in muscle and liver. They show that β-arrestin-2 expression in muscle and liver, but not fat, is reduced by ~50% in insulin-resistant animal models and in liver from a small cohort of T2D humans; β-arrestin-1 expression was normal. *β-arrestin-2* −/− mice were insulin resistant; results from *β-arrestin-2* haploinsufficient animals were unfortunately not described—these might have provided a direct corollary of the initial physiological data. Regardless, 2-fold overexpression of *β-arrestin-2* in mice led to increased insulin sensitivity, supporting the thesis that *β-arrestin-2* expression is correlated with insulin action.

How might β-arrestin-2 modulate insulin sensitivity? In an extensive series of experiments, Luan et al. first showed that while signaling to PI3 kinase was normal in livers from the *β-arrestin-2* −/− mice, the next step in the cascade, Akt activation, was impaired. Luan et al. showed that β-arrestin-2 acts as a scaffold for the insulin-dependent assembly of an insulin receptor/c-Src/Akt complex leading to c-Src-mediated phosphorylation of Akt at Tyr315/Tyr326. Insulin-dependent Akt tyrosine phosphorylation was defective in *β-arrestin-2* −/− mice, concomitant with reduced phosphorylation of Akt at Thr308 and Ser473, the two sites that are essential for full Akt activation. The converse was observed in *β-arrestin-2* overexpressing mice. Thus, Luan et al. suggest that, in addition to the IRS-1/PI3K pathway, a second pathway downstream of the insulin receptor is required for full Akt activation (see Figure 1B). A disruption in the formation of this complex, due to reduced *β-arrestin-2* expression, leads to impaired Akt activation followed by insulin resistance. This is potentially very exciting in view of the major role that Akt plays in numerous biological processes. However, some aspects of the study by Luan et al. seem to require clarification. The first involves the role of tyrosine phosphorylation in Akt activation and the second involves the link between β-arrestin-2 mediated Akt regulation and whole-body insulin resistance, which are discussed below.

With regard to tyrosine phosphorylation of Akt, the conventional view is that full activation of Akt in response to insulin is mediated via phosphorylation at Thr308 and Ser473 by PDK1 and PDK2 (see Figure 1A). However, several studies have shown that Akt is also phosphorylated at Tyr315, Tyr326, and Tyr474 and that this also plays a key role in kinase activation (Chen et al., 2001; Conus et al., 2002). However, in these studies, there was no evidence that Akt tyrosine phosphorylation regulated PDK-mediated phosphorylation of Akt at Thr308 and Ser473. Rather, inhibition of Akt tyrosine phosphorylation impaired Akt activity independently of PDK function via an ill-defined mechanism (Chen et al., 2001; Conus et al., 2002). In contrast to these studies, Luan et al. show that tyrosine phosphorylation of Akt at Tyr315/326 plays a major role in regulating Thr308/Ser473 phosphorylation (see Figure 1B). In *β-arrestin-2* −/− mice and under other conditions where c-Src function was inhibited, they observed almost complete inhibition of insulin-dependent phosphorylation of Akt at both Thr308 and Ser473, whereas the converse was found in *β-arrestin-2*-overexpressing animals. Thus, while the studies of Luan et al. provide a more plausible mechanism as to how Akt tyrosine phosphorylation might regulate kinase activity, the discrepancy

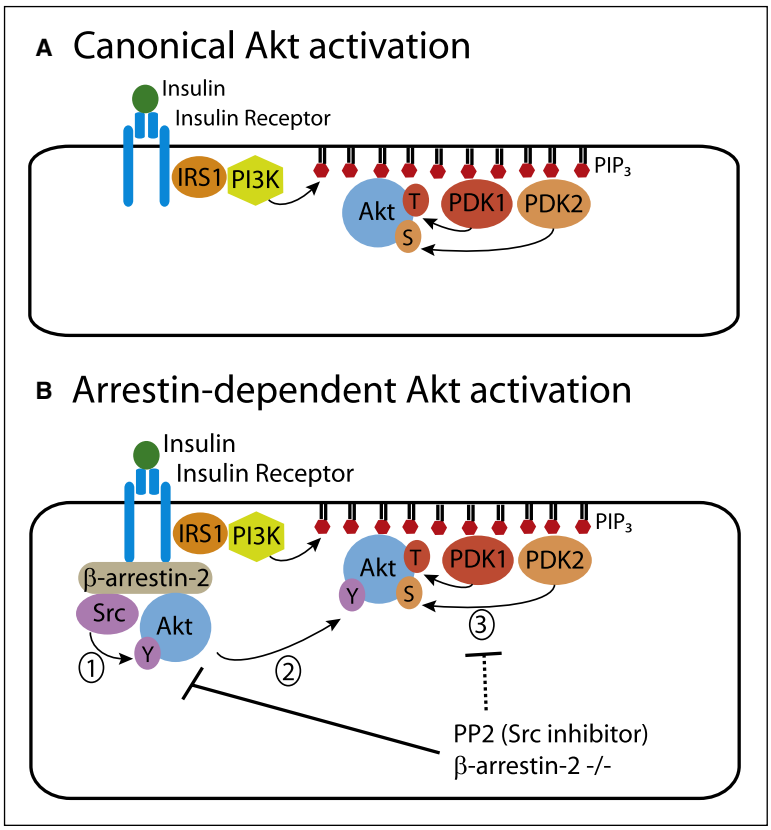

Figure 1. Models of Akt Activation

(A) The conventional model for regulation of Akt by insulin involves tyrosine phosphorylation of IRS1, which recruits PI3K to the plasma membrane, where it generates PIP_3. PIP_3 acts as a docking site for PDKs and Akt leading to the phosphorylation of Akt on Thr308 and Ser473, concomitant with its activation.

(B) Luan et al. propose a two-step model for Akt activation whereby insulin triggers the assembly of a tetrameric complex comprising insulin receptor, β-arrestin-2, c-Src, and Akt. This allows c-Src to phosphorylate Akt on Tyr315 and Tyr326 (step 1), and this step is somehow required (step 2) for phosphorylation of Akt at Thr308 and Ser473 (step 3) and its full activation. Luan et al. showed that impairment of c-Src function using PP2 or deletion of β-arrestin-2 inhibited tyrosine phosphorylation of Akt and its activation.

with previous studies needs to be resolved. These studies also need to be interpreted in light of observations that selective targeting of Akt to the plasma membrane in the absence of any stimulus that would likely activate c-Src kinase, results in robust phosphorylation of Akt at Thr308/Ser473 and kinase activity (Ng et al., 2008).

The demonstration of an insulin-regulated complex involving the insulin receptor, c-Src, Akt, and β-arrestin-2 is extremely exciting and has important implications for our understanding of insulin action. While the physiological data in animal models and the signaling data were compelling in the Luan et al. study, the linkage between these observations is still not completely clear. For example, despite having normal food intake, β-*arrestin-2* −/− mice had increased body weight on a high-fat diet while β-*arrestin-2*-overexpressing animals had reduced body weight. Assuming that these changes were accounted for by adiposity (these data were not reported), one plausible explanation is that the difference in insulin sensitivity in these animals is a simple consequence of altered body weight per se rather than a direct consequence of altered insulin signaling in muscle and liver as a function of modified β-*arrestin-2* expression. Interestingly, previous studies have shown that β-*arrestin-2* −/− mice are hypoactive (Bohn et al., 2003) with defective temperature control (Bohn et al., 1999). Both these traits likely contribute to increased body weight, particularly if they are exacerbated upon high-fat feeding. Because fat accumulation leads to insulin resistance, more work is required to justify the link between β-arrestin-2-mediated tyrosine phosphorylation of Akt and whole-body insulin action. Simultaneous analysis of insulin signaling and insulin action in isolated muscle and primary hepatocytes from wild-type and β-*arrestin-2* −/− mice would clarify matters, as well as more extensive studies of activity and thermogenesis in both chow and fat-fed animals. Despite the lack of firm evidence linking these parameters, the identification of a second insulin-regulated pathway involving Akt tyrosine phosphorylation is potentially very important, but as is often the case with challenging new ideas, more work will be required to both confirm and conquer its mechanistic architecture.

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