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Review

Insulin, PKC signaling pathways and synaptic remodeling during memory storage and neuronal repair

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Abstract

Protein kinase C (PKC) is involved in synaptic remodeling, induction of protein synthesis, and many other processes important in learning and memory. Activation of neuronal protein kinase C correlates with, and may be essential for, all phases of learning, including acquisition, consolidation, and reconsolidation. Protein kinase C activation is closely tied to hydrolysis of membrane lipids. Phospholipases C and A2 produce 1,2-diacylglycerol and arachidonic acid, which are direct activators of protein kinase C. Phospholipase C also produces inositol triphosphate, which releases calcium from internal stores. Protein kinase C interacts with many of the same pathways as insulin; therefore, it should not be surprising that insulin signaling and protein kinase C activation can both have powerful effects on memory storage and synaptic remodeling. However, investigating the possible roles of insulin in memory storage can be challenging, due to the powerful peripheral effects of insulin on glucose and the low concentration of insulin in the brain. Although peripheral for insulin, synthesized in the beta-cells of the pancreas, is primarily involved in regulating glucose, small amounts of insulin are also present in the brain. The functions of this brain insulin are inadequately understood. Protein kinase C may also contribute to insulin resistance by phosphorylating the insulin receptor substrates required for insulin signaling. Insulin is also responsible insulin-long term depression, a type of synaptic plasticity that is also dependent on protein kinase C. However, insulin can also activate PKC signaling pathways via PLC γ, Erk 1/2 MAP kinase, and src stimulation. Taken together, the available evidence suggests that the major impact of protein kinase C and its interaction with insulin in the mature, fully differentiated nervous system appears to be to induce synaptogenesis, enhance memory, reduce Alzheimer's pathophysiology, and stimulate neurorepair.

Keywords: Protein kinase C; Insulin; Synaptogenesis; Neurorepair; Alzheimer's disease

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1. Introduction

Protein kinase C is a serine/threonine kinase that is part of numerous pathways critical for memory storage, synaptic remodeling, and neuronal repair. Protein kinase C exists in at least 10 isoforms, which are divided into three categories: the "conventional" isoforms (α, βI, βII, γ), which are activated by calcium and 1,2-diacylglycerol (DAG); the "novel" isoforms (δ, ε, η, and θ), which are activated by diacylglycerol but insensitive to calcium, and the "atypical" isoforms of protein kinase C (ζ and λ), which are independent of both calcium and diacylglycerol. The requirement for diacylglycerol and calcium means that the activity of conventional protein kinase C isoforms is intimately dependent on two phospholipases: phospholipase C, which hydrolyzes phosphatidylinositol to produce 1,2-diacylglycerol, and phospholipase A2, which produces arachidonic acid, a potent activator of protein kinase C (Fig. 1). Another phospholipase, phospholipase D, produces 1,2-diacylglycerol via phosphatidic acid; although this pool of diacylglycerol is usually considered to be separate from the protein kinase C pathway, there is considerable recent interest in the possibility that this diacylglycerol may also reach protein kinase C and activate it (Becker and Hannun, 2005).

After binding to diacylglycerol, protein kinase C becomes translocated to various intracellular membranes. The ratio of membrane-bound to cytosolic protein kinase C is often used as an index of protein kinase C activation. A variety of natural plant and animal metabolites, including phorbol esters, bryostatins, and some terpenes, are potent activators of protein kinase C. These molecules bind to the diacylglycerol site in conventional and novel protein kinase C and act as a "glue" that fixes the protein kinase C protein to the membrane (Zhang et al., 1995). Protein kinase C is phosphorylated by 3-phosphoinositide-dependent kinase 1 (PDK1) at a threonine on its activation loop. This phosphorylation is essential for maximal activation (Seki et al., 2005). Protein kinase C also undergoes autophosphorylation on threonine residues in its turn motif and hydrophobic motif on the carboxy terminal of protein kinase C, which stabilizes the active conformation. Activated protein kinase C is gradually dephosphorylated by protein phosphatases, becomes ubiquitinated, and is then degraded by the proteasome, a process referred to as 'down-regulation'. Dephosphorylation predisposes membrane-bound protein kinase C to proteolysis (Lee et al., 1997); thus, activation of protein kinase C by diacylglycerol, phorbol esters, or bryostatin produce a brief pulse of protein kinase C activity, which is automatically shut off in a time-dependent manner. This prevents continuous activation of protein kinase C that would otherwise occur following a stimulus.

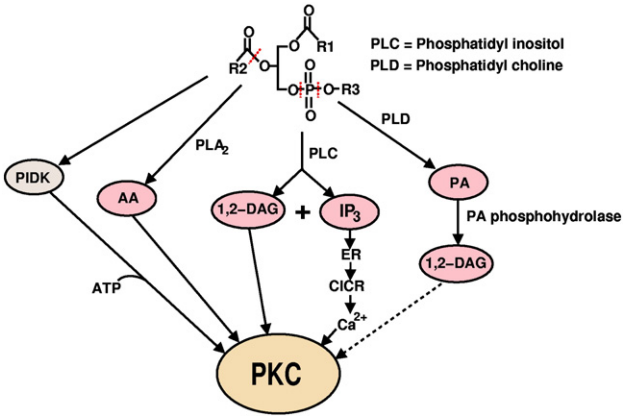

Fig. 1. Activation pathways of protein kinase C. Phosphatidyl inositol is hydrolyzed by phospholipase C (PLC) to produce 1,2-diacylglycerol (1,2-DAG) and inositol 1,4,5-triphosphate (IP_3). The IP_3 binds to IP_3 receptors in the endoplasmic reticulum, which undergoes calcium-induced calcium release (CICR). Phospholipase A2 produces arachidonic acid (AA) and 1,3-diacylglycerol, while phospholipase D (PLD) hydrolyzes phosphatidylcholine to produce choline and phosphatidic acid (PA), which is then rapidly hydrolyzed by PA phosphohydrolase to 1,2-diacylglycerol. 1,2-diacylglycerol, AA, and calcium activate protein kinase C (PKC). The diacylglycerol produced from PA was originally believed to be in a separate pool and unavailable to protein kinase C; however, recent evidence suggests that this is not always the case. Binding of 1,2-diacylglycerol to protein kinase C induces translocation to membranes. Phosphatidyl inositol-dependent kinase (PIDK) is responsible for phosphorylating protein kinase C, which is necessary for full activation.

In addition to the canonical life cycle of protein kinase C described above, there are other factors that are important in understanding how protein kinase C contributes to synaptic remodeling. For example, in cell extracts, cytosolic protein kinase C can also be rapidly degraded by calcium-activated proteases, producing protein kinase M, a calcium-independent form of protein kinase C. Also, phorbol esters, diacylglycerol and bryostatin bind to a number of other proteins in addition to protein kinase C, including Munc13, a protein involved in synaptic vesicle release; RasGRP, a Ras GTP exchange factor; and chimaerins, a family of Ras GTPase-activating proteins (Kazanietz et al., 2000). In many cases, these proteins complement the effects of activated protein kinase C.

2. Protein kinase C, insulin and memory

Activation of neuronal protein kinase C correlates with, and may be essential for, all phases of learning, including acquisition, consolidation, and reconsolidation (Alkon, et al., 1998; Olds et al., 1989; Bank et al., 1988; Bonini et al., 2007). Consolidation ordinarily requires protein synthesis contemporaneous with the conditioned/unconditioned stimulus pair. However, when animals are exposed to bryostatin for several days prior to the training, long-term learning still occurs even when protein synthesis is blocked with anisomycin (Alkon et al., 2005). The effect is completely blocked by protein kinase C inhibitors. The most likely explanation for this is that protein kinase C induces the synthesis of the specific set of proteins that are required for consolidation.

Many protein kinase C substrates are involved in the biochemical pathways that are important in learning and memory. Early work with *Hermissenda* associative learning (Alkon, 1984; Nelson et al., 1990) and rabbit nictitating membrane conditioning (Bank et al., 1988; Olds et al., 1989, 1990; Scharenberg et al., 1991) demonstrated that long-term translocation of protein kinase C in specific brain regions is a critical event in memory storage. Numerous studies have confirmed the importance of protein kinase C and identified other protein kinase C substrates important for learning and memory. Zhang

et al. (2005) demonstrated that overexpressing constitutively active protein kinase C enhanced learning that correlated with increasing phosphorylation of protein kinase C substrates. Downstream protein kinase C targets such as Erk 1/2, src non-receptor tryrosine kinase, type II ryanodine receptors, mRNA stabilizing proteins (Quattrone et al., 2001), and molecules upstream of protein kinase C such as insulin growth factor and fibroblast growth factor-18 (Cavallaro et al. 2002), are all engaged in the memory storage process. Protein kinase C phosphorylation also increases the opening rate of *N*-methyl-D-aspartic acid (NMDA) receptors and the number of functional channels (Lin et al., 2006). Growth-associated protein kinase C substrate (GAP-43) is another protein kinase C substrate essential for hippocampal-dependent memory (Rekart et al., 2005).

Protein kinase C interacts with many of the same pathways as insulin (Zhao and Alkon, 2001); therefore, it should not be surprising that insulin signaling and protein kinase C activation can both have powerful effects on memory storage and synaptic remodeling. However, investigating the possible roles of insulin in memory storage can be challenging, due to the powerful peripheral effects of insulin on glucose and the low concentration of insulin in the brain. Peripherally administered insulin has profound effects on behavior, making studies of any central role for insulin signaling in memory difficult. Like insulin-like growth factors, insulin is imported into the brain through the cerebrospinal fluid and across the blood–brain barrier by a saturable specific receptor-mediated transporter. Most of insulin's actions in the brain complement its peripheral role in regulation of glucose. For example, intracerebroventricular infusion of insulin results in the decrease of the hypothalamic expression of neuropeptide Y (Schwartz et al., 1992), and increases the expression of corticotrophin-releasing hormone (Schwartz et al., 1996) and α-melanocyte stimulating hormone (Benoit et al., 2002), which enhances the glucose-depleting effects of peripheral insulin by inhibiting food intake.

However, there is also evidence that insulin can have direct effects on learning and memory, instead of acting through glucose. Intranasally-administered insulin, which bypasses the blood–brain barrier and has no effect on circulating blood glucose levels, has been reported in several studies to enhance hippocampal-dependent learning and memory in human patients (Benedict et al., 2007, 2004). Since peripheral glucose levels are not affected, this effect must be due to stimulation of insulin receptors in the brain. Several reports have demonstrated low levels of insulin receptor mRNA (Havrankova et al., 1979; Zahniser et al., 1984; Zhao et al., 1999) as well as insulin mRNA (Devaskar et al., 1994) in brain, especially the hippocampus (Steen et al., 2005). However, the question of whether the brain synthesizes insulin has been controversial (Woods et al., 2003). Insulin receptor expression and mRNA levels are also elevated after water-maze training (Dou et al., 2005). Training increases the levels of downstream molecules such as insulin receptor substrate-1, Shc and Akt, and decreases Akt phosphorylation (Dou et al., 2005).

Although insulin clearly facilitates learning, the extent to which peripheral-derived insulin and central nervous system insulin receptors are essential for learning, as opposed to merely facilitating learning, is still unclear. Inducing artificial diabetes with intraperitoneal injections of streptozotocin has little or no effect on learning behavior or retention (Dou et al., 2005). However, while mice heterogeneous for a targeted gene deletion of olfactory bulb insulin receptor, and homozygous null knockouts are both able to learn to a limited extent, both groups demonstrate reduced performance in short-term and long-term memory tests (Das et al., 2005). Insulin receptor heterozygous knockout mice also demonstrate an impairment in recognition of familiar objects. Corresponding electrophysiological changes were also found, including decreased peak currents (Das et al., 2005) and changes in the $Kv1.3K^+$ channel. On the other hand, a knockout of the brain/neuron-specific insulin receptor produced no change in neuronal survival, memory, or brain glucose metabolism, even though activation by insulin of pathways downstream of the insulin receptor, including Akt phosphorylation and GSK-3β, was impaired (Schubert et al., 2004). Thus, it appears that insulin's role is principally that of a modulator of learning.

Insulin also produces an insulin-specific form of synaptic plasticity known as insulin-long term depression (van der Heide et al., 2005; Huang et al., 2004, 2003). This form of learning is also dependent on protein kinase C and phosphatidylinositol 3-kinase (PI3K)-dependent insulin signaling, and is characterized by changes in the 3-hydroxy-5-methylisoxazole-4-propionic acid receptor (Huang et al., 2004).

3. Protein kinase C, insulin and Alzheimer's disease

An interesting side-product of these studies has been the discovery of a possible role for insulin and insulin receptor in Alzheimer's disease, which most commonly presents at its earliest stages as a disease of memory (Alkon et al., 2007). Patients with diabetes have long been known to be at increased risk for Alzheimer's disease (Freude et al., 2005). Alzheimer's disease patients have higher levels of plasma insulin, but lower levels of insulin in the cerebrospinal fluid (Craft et al., 1998). Several reports have demonstrated that intracerebroventricularly-administered streptozotocin produces large (five-fold) increases in hyperphosphorylated tau, a hallmark indicator of the neurofibrillary tangles that occur in Alzheimer's disease (Grünblatt et al., 2007; Clodfelder-Miller et al., 2006), in mouse hippocampus. These changes can be rapidly reversed by peripheral administration of insulin. The increase in phosphorylation was accompanied by a 44–55% decrease in the activity of PP2A (Clodfelder-Miller et al., 2006), a protein phosphatase that dephosphorylates tau. Paradoxically, peripheral hyperinsulinemia (Freude et al., 2005) and starvation (Yanagisawa et al., 1999) also promote tau hyperphosphorylation, presumably by activating phosphatidylinositol 3-kinase and mitogen-activated protein kinase (MAP kinase), which are components of the signaling pathways downstream of the insulin receptor. Because of insulin's ability to improve memory, there is also considerable interest in using intranasal sprays of insulin as a treatment for Alzheimer's disease.

Insulin also has other beneficial effects that would benefit Alzheimer's disease patients. A ten-minute portal vein infusion

of 230 pM insulin was found to increase the hepatic clearance of the 40-amino acid β-amyloid fragment (Aβ(1-40)) by about 2.2-fold (Tamaki et al., 2007). This increase was abolished by low-density lipoprotein receptor-associated protein, indicating that the effect was mediated by translocation of low-density lipoprotein receptor-related protein from the cytosol to the hepatic plasma membrane. Insulin promotes the release of intracellular β-amyloid (Gasparini et al., 2001) and also regulates the expression of proteases that aid in the clearance of β-amyloid (Zhao et al., 2004). Intra-CA1 injections of insulin also protect against stress-induced memory deficits (Moosavi et al., 2007). Thus, insulin may play an important role in disposing of the toxic β-amyloid peptide. Consistent with this is a finding that blockade of insulin-like growth factor-I receptors in the choroid plexus in mice produces amyloidosis and hyperphosphorylated tau deposits similar to those seen in Alzheimer's disease (Carro et al., 2006).

There are many parallels between Alzheimer's disease and insulin resistance syndrome. Insulin resistance syndrome, which is characterized by inflammation, reduced brain insulin levels, down-regulation of insulin transport into the brain, and chronic elevations in peripheral insulin levels, is associated with Alzheimer's disease and age-related memory impairment (Craft, 2005). It has been suggested that the inflammation produced in insulin resistance, which is mediated by tumor necrosis factor-α and other cytokines, reduces uptake and clearance of β-amyloid by the liver (Craft, 2005; Griffin et al., 1998). Insulin resistance and Alzheimer's disease have many similarities, including deficiencies in insulin growth factor signaling and expression and mitochondrial dysfunction. Intracerebral streptozotocin produces neurodegeneration that shares many features of Alzheimer's disease (Lester-Coll et al., 2006). Some researchers have therefore suggested that Alzheimer's disease is a form of brain-specific diabetes-like syndrome, dubbed "Type 3 diabetes" (de la Monte et al., 2006).

Protein kinase C is also important in Alzheimer's disease (Alkon et al., 2007). Numerous studies have shown that protein kinase C activates α-secretases, enzymes which produce soluble amyloid precursor protein fragment α (sAPPα) from amyloid precursor protein (APP) (Gandy and Greengard, 1994). This is called the 'non-amyloidogenic' amyloid precursor protein cleavage pathway because cleavage by α-secretase prevents the formation of β-amyloid, which is created by β- and γ-secretases. Activation of protein kinase C by bryostatin and other protein kinase C activators prolongs the lifespan of Alzheimer's disease mutant mice, and greatly reduces the rate of production of β-amyloid (Etcheberrigaray et al., 2004). Because formation of β-amyloid and sAPPα are mutually incompatible, it was originally believed that α-secretase might compete with β-secretase for a limited pool of amyloid precursor protein. However, this theory has been largely abandoned as the complexity of amyloid precursor protein metabolism and its regulation have been revealed.

4. Insulin-like growth factor I

Insulin-like growth factor-I affects all tissues, including the brain. Cells in the blood–brain barrier and particularly the blood-cerebrospinal fluid barrier contain receptors for insulin-like growth factor-I (Lee et al., 1993). High levels of insulin-like growth factor-binding proteins such as insulin-like growth factor-binding protein-2, which translocates insulin-like growth factors to their target organs, are also present in brain and in cerebrospinal fluid. Peripheral administration of insulin-like growth factor-I produces many central nervous system effects, such as hippocampal neurogenesis, growth factor expression, and clearance of β-amyloid. Small amounts of insulin-like growth factor-I are also synthesized in the brain (Bondy and Lee, 1993).

5. Molecular pathways for Insulin and insulin-like growth factor signaling

When insulin binds to the insulin receptor, the receptor phosphorylates a number of intracellular proteins, including the insulin receptor substrates insulin receptor substrate-1 to insulin-receptor-6, Grb2-associated binder-1 (Gab1), Cas-Br-M-ectopic retroviral transforming sequence homologue (Cbl) and the Src-homology-2-containing protein (Shc) (Fig. 2). The receptor for insulin growth factor I is structurally similar to the insulin receptor, and there is some cross-talk between the insulin receptor and the insulin growth factor-I receptor (Denley et al., 2007), both of which are tyrosine kinases that phosphorylate similar insulin receptor substrate proteins. Insulin receptor substrate proteins possess numerous phosphorylation sites, and can also be phosphorylated by other kinases, a phenomenon that contributes to insulin resistance (Draznin, 2006). One such kinase is protein kinase C. Protein kinase C has been shown to phosphorylate insulin receptor substrate-1 protein at the plekstrin-homology domain (Nawaratne et al., 2006). Inhibition of protein kinase Cε has been shown to block hepatic insulin resistance in a rat model for fatty liver disease (Samuel et al., 2007). Phosphorylation of insulin receptor substrates by the insulin receptor or the insulin-like growth factor-I receptor links the receptor to intracellular signaling cascades. Insulin receptor substrates bind to the insulin receptor through pleckstrin-homology domains and phosphotyrosine binding domains on the insulin receptor substrate protein. After phosphorylation by the insulin receptor, insulin receptor substrate proteins bind to proteins containing Src-homology-2 (SH2) domains. A complex series of protein interactions (reviewed in Taniguchi et al., 2006)) results in a stimulation of glucose uptake, glycogen synthesis, and protein synthesis, and an inhibition of gluconeogenesis.

Although insulin and insulin-like growth factor-I exert their primary effects on glucose metabolism and uptake, there are a number of points in the insulin signaling pathway that could account for insulin's effects on learning and memory, which are observable *in vivo* even under glucose clamp conditions. Recent research has pointed to dendritic spines and spine proteins as among the earliest and most robust indicators of memory formation. For example, a large percentage of the protein interactions that are induced by spatial maze learning is among proteins found primarily or exclusively in dendritic spines (Nelson et al., 2004). These protein interactions may underlie the dramatic changes in dendritic spine morphology observed after learning (Hongpaisan and Alkon, 2007). Three insulin

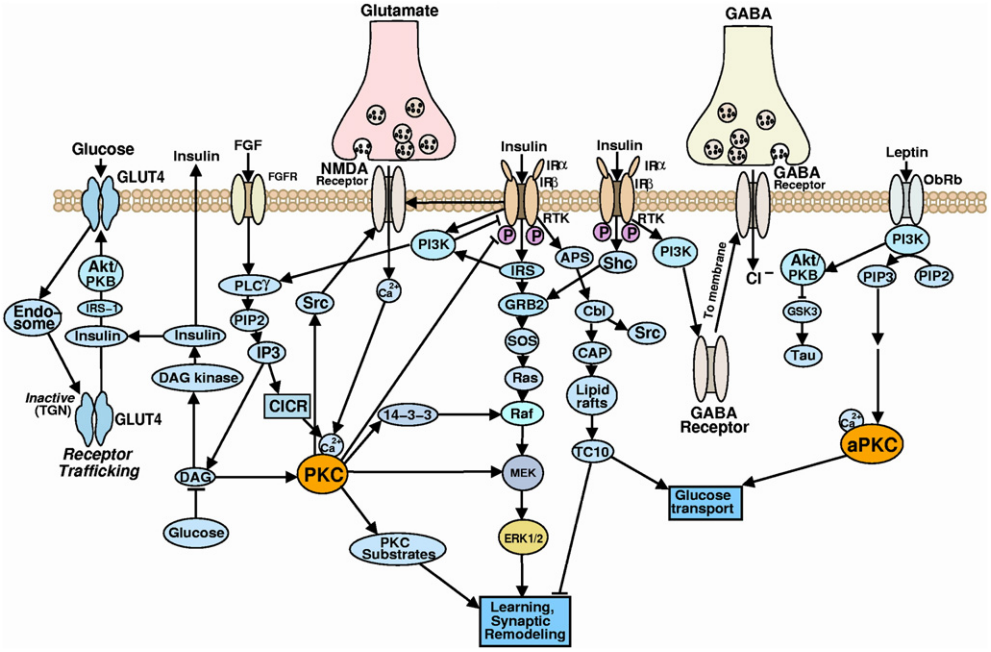

Fig. 2. Schematic diagram of insulin signaling pathways that affect synaptic remodeling and glucose transport. The insulin receptor tyrosine kinase (RTK) phosphorylates insulin receptor substrates (IRS), activating the Ras/Raf pathway through a series of protein interactions involving Grb2, son of sevenless (SOS), Ras, Raf, MAP kinase kinase (MEK), and mitogen-activated kinase (extracellular signal-regulated kinases, Erk 1 and 2). The insulin receptor also phosphorylates APS, activating the Cbl pathway, which is involved in glucose transport, which is also activated by atypical protein kinase C isoforms (aPKC). The aPKC isoforms are activated by phosphatidylinositol 3-kinase (PI3K), which produces phospatidylinositol 3,4,5-triphosphate (PIP_3) from PIP_2 at the plasma membrane. PI3K is activated by the hypothalamic obese receptor b (ObRb), which is a signal for satiety. Leptins, which are structurally related to cytokines, bind to the ObRb and stimulate the JAK-STAT pathway (not shown), eventually leading to activation of the PI3K pathway. Fibroblast growth factors (FGF) activate protein kinase C indirectly, by activating phospholipase C (PLC) gamma, which hydrolyzes phosphatidylinositol to release diacylglycerol (DAG), an activator of the conventional and novel forms of protein kinase C. Protein kinase C then phosphorylates a number of substrates, including MEK and 14-3-3, leading to synaptic remodeling. In pancreatic islet cells, diacylglycerol kinase converts diacylglycerol to phosphatidic acid, activating insulin release. Protein kinase C phosphorylates the beta subunit of the insulin receptor, inhibiting its tyrosine kinase activity. Activators of protein kinase C therefore act to desensitize cells to insulin. Insulin receptor substrates, such as insulin receptor substrate p53, are enriched in postsynaptic densities and participate in synaptic remodeling and plasticity. The insulin receptor also activates PI3K, which recruits GABA receptors to the membrane. Insulin also activates receptor trafficking of GLUT4, the insulin transporter. Insulin binding to the insulin receptor phosphorylates the insulin receptor substrate-1, which acts through Akt/PKB to translocate GLUT4 to the membrane.

signaling pathways are of particular relevance to memory storage and neurorepair: (1) protein kinase C; (2) MAP kinase; and (3) Cbl.

6. Role of protein kinase C in insulin receptor and insulin-like growth factor signaling

There are several ways insulin and glucose can affect protein kinase C. Elevations in glucose can, in some cases, decrease intracellular levels of diacylglycerol, a lipid essential for protein kinase C activation. These decreases of diacylglycerol produce transient activation of diacylglycerol kinase activity and insulin receptor signaling, resulting in a decrease in protein kinase C activity. However, in hyperglycemic states in diabetes, glucose can activate microvascular PKC isoforms, especially beta, by elevating diacylglycerol, which results in vascular complications (Miele et al., 2007). Protein kinase Cε associates with the insulin receptor *in vivo*, and inhibits receptor tyrosine kinase activity directly (Miele et al., 2007). Inhibition of protein kinase Cα also causes a decrease in insulin degradation (Miele et al., 2007). Protein kinase Cα binds to insulin receptor substrate-1 as well as 14-3-3, a well-known protein kinase C substrate that is also involved in insulin signaling (Oriente et al., 2005). Depletion of 14-3-3 with antisense oligonucleotides blocks insulin signaling and causes a 3-fold increase in the activity of protein kinase C bound to insulin receptor substrate-1 (Oriente et al., 2005). These results indicate that the activity of conventional isoforms of protein kinase C and insulin signaling are inversely regulated.

However, the "atypical" isoforms of protein kinase C (ζ and λ), in contrast to ordinary protein kinase C, also seem to be atypical in their relationship to the insulin receptor. The atypical protein kinase C isoforms differ from the "conventional" isoforms (α, βI, βII, γ) and "novel" isoforms (δ, ε, η, and θ) by being insensitive to calcium and diacylglycerol. Atypical protein kinase C isoforms are required for insulin-stimulated glucose transport in muscle and adipocytes (Farese et al., 2005). These enzymes are downstream of phosphatidylinositol 3-kinase and regulate a variety of insulin-dependent processes. Since one of the primary functions of atypical protein kinase Cs is activation of enzymes involved in lipid synthesis, it has been suggested that activation of atypical protein kinase Cs may contribute to hyperlipidemia in insulin-resistant states (Farese et al., 2005).

Neuronal repair induced by fibroblast growth factors such as FGF-1 and FGF-2, which may be involved in the protection of memory against ischemia, injury, stress, or other insults (Li

et al., 1999; Bland et al., 2007), is mediated by phosphorylation of the tyrosine kinase Src and the lipid-hydrolyzing enzyme phospholipase Cγ (Reuss and von Bohlen und Halbach, 2003), which leads to the activation of protein kinase C by producing more diacylglycerol. Fibroblast growth factors can also activate protein kinase C through SNT/FRS2 (Suc1-associated neurotrophic factor-induced tyrosine-phosphorylated target and fibroblast growth factor receptor substrate 2). Fibroblast growth factor-18 has also been shown to be increased in rat hippocampus after water maze conditioning (Cavallaro et al., 2002).

7. Role of mitogen-activated protein kinase (MAP kinase)

MAP kinase is also involved in insulin signaling. Phosphorylation of the insulin receptor substrate proteins Shc and Gab1 induces binding to Grb2 and the guanyl nucleotide-exchange factor SOS. This in turn activates the small GTP-binding protein Ras, which activates Raf, ultimately resulting in the phosphorylation and activation of mitogen-activated protein kinase kinase I (MEK) and MAP kinase (the 44 and 42 kDa extracellular signal-regulated kinases, ERK1 and ERK2). These signaling kinases mediate a variety of growth and differentiation responses to insulin (Taniguchi et al., 2006). ERK1 and 2 are also essential for spatial learning, fear conditioning, conditioned taste aversion memory, spatial memory, step-down inhibitory avoidance and object recognition memory, as well as for full expression of long-term potentiation (Giovannini, 2006) and long-term depression (Ito-Ishida et al., 2006). ERK 1 and 2 also activate transcription factors, producing changes in protein expression. Thus, PKC and insulin can activate the same pathways, including those involving ERK 1/2 and src, and PKC can be activated indirectly by insulin through common pathways involving PI3K.

ERK 1 and 2 are also components of the neurotrophin receptor pathways. Brain-derived neurotrophic factor (BDNF) acts through ERK1/2 to increase dendritic spine density (Alonso et al., 2004). Dendritic growth induced by glial-derived neurotrophic factor (GDNF) is also mediated by the ERK1/2 pathway (Uchida et al., 2006; García-Martínez et al., 2006). Similarly, potentiation of nerve growth factor (NGF)-induced neurite outgrowth by the immunosuppressant FK506 is mediated through the Ras/Raf/ERK1/2 pathway (Price et al., 2003). However, inhibition of MAP kinase kinase (MEK) enhances rather than blocks neurite outgrowth induced by neurotrophin-3 (Wiklund et al., 2002), indicating that synaptic remodeling by neurotrophin-3 uses a different mechanism.

However, ERK 1 and 2 also have a dark side. Activation of ERK1/2 has been reported to mediate neurotoxicity of β-amyloid peptide oligomers by activating the caspase-3 apoptosis pathway (Khan and Alkon, 2006). ERK1/2 signaling is also enhanced in Alzheimer's disease. Increased ERK1 phosphorylation in fibroblasts is reliably enhanced in cells from Alzheimer's patients, and it has been proposed as a diagnostic tool for Alzheimer's disease (Khan and Alkon, 2006; Zhao et al., 2002).

In some cells, insulin produces a decrease rather than an increase in ERK1/2 phosphorylation (van der Heide et al., 2003). This effect is mediated by phosphatidylinositol 3-kinase-dependent phosphorylation of Akt / protein kinase B (PKB). Insulin stimulates both the phosphatidylinositol 3-kinase and the MAPK/ERK pathways (Halevy and Cantley, 2004; Sivaprasad et al., 2004). However, the phosphatidylinositol 3-kinase pathway acts antagonistically on Ras/Raf pathways, since when phosphatidylinositol 3-kinase activity is blocked, insulin produces an increase in ERK1/2 phosphorylation instead of a decrease (van der Heide et al., 2003). Activation of phosphatidylinositol 3-kinase and PKB by insulin also induces long-term depression in hippocampal CA1 neurons in the presence of blockers of GABAergic neurotransmission (van der Heide et al., 2005).

8. Role of Cbl

Cbl is a proto-oncogene that is phosphorylated by the insulin receptor. Cbl proteins are E3 ubiquitin ligases that negatively regulate receptor tyrosine kinases. The three forms of Cbl (c-Cbl, Cbl-b, and Cbl-c/Cbl-3) are all calcium-binding proteins that act as tyrosine kinase substrates. The insulin receptor phosphorylates c-Cbl through the adaptor protein APS. After phosphorylation, Cbl interacts with Cbl-associated protein CAP, which translocates Cbl to lipid rafts, where it activates TC10, a small GTPase similar to Rho. TC10 may then induce membrane translocation of the glucose transporter GLUT4 (Chiang et al., 2000), although the precise details are still being worked out.

Cbl is also a negative regulator of memory. Cbl-b null mice have normal learning, but enhance long-term memory and increased synaptic plasticity. This is associated with markedly enhanced glutamatergic transmission, suggesting enhanced release of neurotransmitter (Tan et al., 2006). Cbl-null mice also exhibit a marked improvement in insulin action, which results in lower body fat and an increase in muscle metabolism (Molero et al., 2004). Thus, like the conventional forms of protein kinase C, Cbl appears to work in opposition to insulin. However, the extent to which the effects of Cbl on learning involve insulin and the insulin receptor is still unclear.

9. Protein kinase C and synaptogenesis

Conventional wisdom is that neurons are distinct from most other cells in that they do not divide, proliferate or recycle. From the perspective of information storage, the lifelong persistence of the brain's integrative neurons is of obvious advantage. The information is stored within the tens of thousands of synapses for each neuron for a lifetime's duration. However, some synaptogenesis does occur during learning even in adults, as recently demonstrated for rat spatial maze learning and memory (Hongpaisan and Alkon, 2007). This synaptic flexibility may also occur in response to brain injury and invasive injury such as can occur during stroke, transient cerebral ischemia, and/or cerebral hypoxia (Sun and Alkon, Soc. Neurosci. Abstract 2007).

After learning, synaptogenesis occurs in the hippocampus (Hongpaisan et al., 2007). This is accompanied by increases in protein kinase C activity and increased interactions among synaptic proteins (Nelson et al., 2004), indicating activation of multiple signaling pathways. Insulin activation of large-

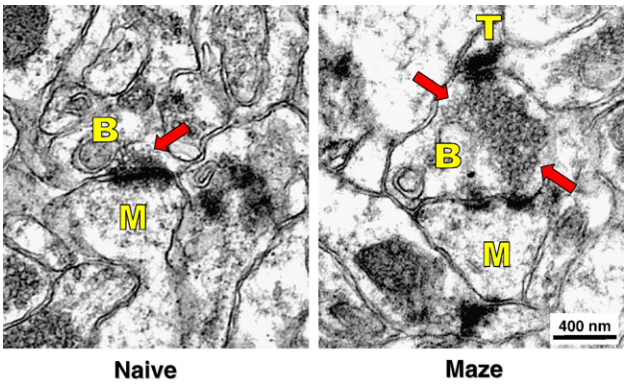

Fig. 3. Electron microscopy of mushroom spines and their presynaptic axonal boutons. Electron micrographs show ultrastructure of axon boutons (B) that synapse with mushroom spines (M) in hippocampal CA1 area at 2 days after 6-day water maze training. Note the increased number of presynaptic vesicles (red arrows) in presynaptic boutons and the presence of two spines (mushroom M, stubby S) that synapse with the bouton for water maze-trained rats.

conductance Ca^{2+}-activated potassium channels is mediated by changes in the actin cytoskeleton (O'Malley and Harvey, 2007), indicating that insulin produces reorganization of dendritic morphology. Similar cytoskeletal changes are seen after leptin stimulation (O'Malley et al., 2005). The dendritic morphology in hippocampal pyramidal cells changes dramatically with learning, with a large increase in mushroom synapses (Fig. 3).

Recently, evidence has become available that protein kinase C signaling pathways are crucially involved in the synaptogenesis underlying learning and memory and in response to ischemic brain injury. These protein kinase C pathways have important convergence with insulin and insulin receptor pathways that have also been implicated in synaptogenesis and the regulation of learning and memory (see Fig. 2, refs.). The potent protein kinase C activator bryostatin, for example, enhances rat spatial maze learning and memory, as well as the accompanying memory-specific synaptogenesis as demonstrated by scanning confocal and electron microscopy. Protein kinase C isozyme-specific blockers such as Ro-08832 block both behavioral and synaptogenesis effects of bryostatin (Hongpaisan and Alkon, 2007). Similarly, the insulin receptor substrate 53 has been shown to regulate dendritic spine morphogenesis (Choi et al., 2005). The protein kinase C and insulin pathways that regulate synaptogenesis include a number of other important molecular steps that involve RAS, MAP kinase, Erk1/2, Cbl and lipid rafts (Fig. 2). Furthermore, both protein kinase C and insulin signaling control the α-secretase-mediated cleavage of the amyloid precursor protein to generate the proteolytic product sAPP (i.e. soluble amyloid precursor protein), which has also been implicated in synaptogenesis (refs., 58). The exact roles of sAPPα and sAPPβ in synaptogenesis, however, have yet to be clearly detailed (cf. Sisodia and Gallagher, 1998). Other data indicate that increased levels of sAPPβ can inhibit protein kinase C function (cf. Favit et al., 1998, Lee et al., 2004; Nelson and Alkon, 2007). Thus, protein kinase C and insulin regulated synaptogenesis may be subject to limiting negative feedback imposed by sAPPβ on protein kinase C and protein kinase C mediated activation of α-secretase.

Insulin may also affect synaptic remodeling at several points. Insulin receptor substrate p53, an SH3 domain-containing protein that is a substrate for insulin receptor tyrosine kinase, is highly enriched in postsynaptic densities. After glutamate or *N*-methyl-D-aspartic acid stimulation, insulin receptor substrate p53 is translocated to postsynaptic sites by a mechanism that requires protein kinase C (Hori et al., 2005). Insulin receptor substrate p53 may be involved in cortical actin dynamics, because it links the small GTPases Rac1 and Cdc42 to downstream effectors for actin regulation (Choi et al., 2005). Overexpression of insulin receptor substrate p53 increases the density of dendritic spines, but has no effect on their shape.

Insulin-like growth factor-I also promotes neurogenesis and synaptogenesis. Transgenic mice overexpressing insulin-like growth factor-I exhibited increases in the number of neurons and a doubling of the number of synapses in the molecular layer of the dentate gyrus (O'Kusky et al., 2000). Although this effect is strongest during postnatal development, insulin-like growth factor-I could also have synaptogenic effects in adult animals. Insulin-like growth factor-I receptors are severely affected in Alzheimer's disease (Dore et al., 1997). Insulin-like growth factor-I acts through a membrane-associated receptor tyrosine kinase, which phosphorylates a number of substrates, including insulin receptor substrate-1 and Shc. These proteins are also phosphorylated by the insulin receptor, and there is considerable cross-talk between the two pathways. The insulin-like growth factor-I receptor activates the MAP kinase pathway (ERK 1/2), as well as the phosphatidylinositol 3-kinase pathway. Phosphatidylinositol 3-kinase activates and phosphorylates Akt, a serine/threonine kinase that regulates a number of survival pathways. For example, active Akt phosphorylates and inhibits the proapoptotic proteins Bad and caspase-9 (del Peso et al., 1997), and Forkhead homolog rhabdomyosarcoma-like1, a transcription factor that is a member of the Forkhead family (Brunet et al., 2001). The end result of these reactions is to increase cell survival.

In mice lacking insulin-like growth factor-I, acquisition of behavioral tasks was impaired, but only in old mice (15–18 months); young mice were unaffected (Svensson et al., 2006), suggesting that insulin-like growth factor-I plays an important role in cell survival even during adulthood. The insulin-like growth factor-I receptor/Akt pathway is inhibited by protein kinase C, and the phorbol ester PMA, which activates protein kinase C, attenuates the insulin-like growth factor-I pathway (Zheng et al., 2000). However, the effects of intracerebroventricular infusion of insulin-like growth factor-I on the number of synapses or neurons in aged rats have been mixed (Poe et al., 2001; Lichtenwalner et al., 2001; Poe et al., 2002; Shi et al., 2005; Aberg et al., 2000), suggesting that the role of insulin-like growth factor-I may be more complex than expected.

Contact with astrocytes can also activate excitatory synaptogenesis by a mechanism involving protein kinase C (Hama et al., 2004). Integrin binding activates the arachidonic acid pathway; thus, arachidonic acid, a strong activator of protein kinase C, may participate in this effect. However, the details of

this pathway are still being uncovered. In PC12 cells, protein kinase C activation with phorbol esters stimulates neurite outgrowth (Burry, 1998). The situation in neuroblastoma cells is murkier, with some groups finding stimulation of neurite outgrowth (Fagerström et al., 1996) and other groups finding inhibition (Tsuneishi, 1992; Shea et al., 1995). In *Hermissenda*, learning produces a dramatic contraction of the overall dendritic volume (Alkon et al., 1990), indicating widespread changes in dendritic morphology. This is more likely to be an active pruning process than an inhibition of outgrowth, because long-term learning is completely blocked by the DNA synthesis inhibitor anisomycin. In the rat, protein kinase C activators (such as bryostatin) also induce morphological changes in synaptic spines that resemble those induced by learning, which activates protein kinase C.

Activation of protein kinase C with bryostatin also produces long-term increases in protein synthesis. These changes are sufficiently similar to the changes in protein synthesis that are required for memory consolidation that, in animals pretreated with bryostatin, long-term memories can be formed even in animals in which protein synthesis is blocked (Alkon et al., 2005).

10. Protein kinase C, insulin and neuronal repair/remodeling after ischemic stroke

Stroke is a leading cause of mortality and long-term morbidity, including cognitive impairment, in the developed world. After the initial ischemic stroke episode, the majority of neurons affected are damaged functionally and structurally, rather than die immediately. Their survival and functional recovery depends on the interplay of many signaling pathways even weeks after the initial insult and can be facilitated by boosting neurotrophic activity, such as insulin/insulin-like growth factor and some protein kinase C isoforms, for neuronal repair and remodeling and blocking delayed cell death.

The link between diabetes and stroke is well established. Diabetes increases the frequency of ischemic stroke incidence and ischemic stroke-induced mortality, and adversely affects postischemic stroke outcomes. Infarct volumes are greater in diabetic rats following middle cerebral artery occlusion as compared to non-diabetics (Rizk et al., 2005). Stroke affects metabolic state and probably insulin sensitivity. Impaired insulin sensitivity is associated with stroke independent of glucose level (Bravata et al., 2005). Hyperglycemia, one of the most common abnormalities in acute stroke, occurs in 45% of stroke patients (Capes et al., 2001) and may predict a relatively poor prognosis. Diabetes and stroke impair cognition. The underlying pathology may involve the neurotrophic activity in the brain, including protein kinase C (Sun and Alkon, 2007) and insulin/insulin-like growth factor-1, an important mediator of the anabolic effects of growth hormone in development and adults (Collett-Solberg and Cohen, 2000). Epidemiological studies show that insulin-like growth factor-1 levels in the low normal range may be associated with increased morbidity and mortality from stroke (Schwab et al., 1997; Johnsen et al., 2005). Patients with low insulin-like growth factor-1 levels have been associated with a poor outcome after stroke (Denti et al., 2004; van Rijn et al., 2006).

Insulin and protein kinase C are involved in neurotrophic signaling. Insulin and insulin-like growth factor-1 act on their receptors, such as insulin-like growth factor-1 receptors, promoting neuronal repair and remodeling in the brain. It has been proposed that insulin-like growth factor-1 may mediate much of the ischemia-induced mechanism for neuronal repair and remodeling after stroke, through reorganization of neuronal networks (Sherrard, 1997). Hypoxic ischemia increases the expression of insulin-like growth factor-1 and its receptors in animal models (Guan et al., 2001; Yan et al., 2006), suggesting an endogenous protective role of insulin-like growth factor-1 in limiting injury. Its expression starts to increase 2–3 days after focal ischemia (Beilharz et al., 1998; Zhang et al., 2004) but could not prevent postischemic neuronal damage that occurs in the first 2–3 days after stroke. Ischemia-induced neural progenitor proliferation in the dentate gyrus, for example, occurs with its peak between 2 and 4 days of reperfusion after middle cerebral artery occlusion (Yan et al., 2006). Others, however, reported lower insulin-like growth factor-1 levels after cerebral ischemia, associated with a poor outcome (Schwab et al., 1997; Denti et al., 2004). Insulin-like growth factor-1 administration after ischemia reduces infarct volume and improves neurological function (Guan et al., 2001; Gluckman et al., 1992; Liu et al., 2001; Schabitz et al., 2001; MacKay et al., 2003). Ischemic stroke may decrease or increase protein kinase C activity in the brain (Bright and Mochly-Rosen, 2005), depending on the varying animal models, brain regions, duration and intensities of the ischemic stroke, and isoforms. It increased protein kinase Cγ expression in human ischemic penumbra and protein kinase CβI and βII expression in the infracted core without significant change in protein kinase Cα expression (Krupinski et al., 1998). Neuronal protein kinase Cε, an important component of the signal pathways in ischemic preconditioning-induced neuroprotection (Dirnagl et al., 2003), was reported to decrease moderately in the penumbra but dramatically in the ischemic core (Shimohata et al., 2007). The protein kinase C isoforms may play different roles in ischemic stroke (Bright et al., 2005; Kubo et al., 2007), with protein kinase Cδ being deleterious (Shimohata et al., 2007; Kubo et al., 2007; Savithiry and Kumar, 1994; Dirnagl et al., 1999; Miettinen et al., 1996; Koponen et al., 2000; Bright et al., 2004; Chou et al., 2004) and protein kinase Cγ, potentially beneficial (Aronowski et al., 2000).

Complex processes such as neuronal repair and remodeling after insult are mediated by the synergistic action of many factors. Diabetes and ischemic stroke episode can trigger the endogenous neurotropic activity, but such activation is often insufficient and untimely. Enhancing the neurotropic activity earlier and sufficiently after the initial insult through the use of therapeutic agents may represent one of the best strategies for a better functional recovery after ischemic stroke. These agents include those that act as insulin and insulin-like growth factor-1 enhancers, and those that selectively activate protein kinase C isoforms that are neurotrophic. There is evidence that pretreatment with intracerebroventricular insulin significantly increases

the number of surviving CA1 pyramidal cells at five days of reperfusion in a four-vessel occlusion rat model, probably through inhibiting Jun N-terminal kinase signaling pathway and/or activating phosphatidylinositol 3-kinase/Akt (Hui et al., 2005). In diabetic rats, acute or chronic administration of a high concentration of insulin (12 units/kg) has also been shown to significantly decrease lesion volume and apoptosis subsequent to 2-h middle cerebral artery occlusion followed by 24-h reperfusion (Rizk et al., 2006), whereas a low maintenance dose (2 U/kg) was ineffective. A potential benefit of an acute intervention with insulin in hyperglycemic ischemic stroke patients has thus been proposed (Walters et al., 2006). Activation of the protein kinase C (Sun and Alkon, 2007) and insulin signaling pathways may accelerate neuronal repair and remodeling (Fujiki et al., 2006) and the recovery of the neuronal network involved in cognition. It remains to be studied whether treatment of insulin resistance can facilitate neuronal repair after ischemic stroke.

11. Conclusion

The cellular and molecular processes activated by protein kinase C bear remarkable similarities to those that underlie memory acquisition and consolidation. Protein kinase C activation does not simply produce unlimited growth. As with insulin, nerve growth factor, and many other neurohormones and signaling enzymes, protein kinase C produces different responses in different tissues and even under different conditions in the same tissue. Nevertheless, the major impact of protein kinase C activation and its interactions with insulin signaling in the mature, fully differentiated nervous system appears to be to induce synaptogenesis, enhance memory, reduce Alzheimer's pathophysiology, and stimulate neurorepair. These effects have been demonstrated with animal models of learning and memory (*e.g.*, rat spatial maze learning, *Hermissenda* Pavlovian conditioning, and rabbit nictitating membrane conditioning), transgenic mouse models of Alzheimer's disease, and rodent models of stroke and head trauma. Future studies must test these molecular and pharmacological principles in appropriately controlled clinical trials.

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