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Insulin and the insulin receptor in experimental models of learning and memory

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Abstract

Insulin is best known for its action on peripheral insulin target tissues such as the adipocyte, muscle and liver to regulate glucose homeostasis. In the central nervous system (CNS), insulin and the insulin receptor are found in specific brain regions where they show evidence of participation in a variety of region-specific functions through mechanisms that are different from its direct glucose regulation in the periphery. While the insulin/insulin receptor associated with the hypothalamus plays important roles in regulation of the body energy homeostasis, the hippocampus- and cerebral cortex-distributed insulin/insulin receptor has been shown to be involved in brain cognitive functions. Emerging evidence has suggested that insulin signaling plays a role in synaptic plasticity by modulating activities of excitatory and inhibitory receptors such as glutamate and GABA receptors, and by triggering signal transduction cascades leading to alteration of gene expression that is required for long-term memory consolidation. Furthermore, deterioration of insulin receptor signaling appears to be associated with aging-related brain degeneration such as the Alzheimer's dementia and cognitive impairment in aged subjects suffering type 2 diabetes mellitus.

Keywords: Insulin receptor; Learning and memory; Signal transduction; Receptor trafficking; Diabetes mellitus; Alzheimer's disease

1. Introduction

The presence of insulin and insulin receptors in the brain suggests that the brain is a target organ for insulin. However, unlike the classic peripheral insulin target tissues such as adipocyte, muscle and liver, where the primary function of insulin is to regulate glucose homeostasis, insulin in the central nervous system (CNS) exhibits more diverse actions, most of which have not been clearly understood. In addition to its central role in food intake and weight control, a direct role of CNS insulin/insulin receptor signaling in improving cognitive functions, including learning and memory, and the association of insulin receptor deterioration with brain degenerative dementia (e.g., Alzheimer's disease) have attracted increasing interest. Although still at an early stage, efforts in behavioral, electrophysiological and biochemical studies have begun to uncover the cellular and molecular basis for the CNS insulin/insulin receptor action on learning and memory.

2. Memory-improving effects of insulin

The presence of insulin and insulin receptor in the hippocampus and cerebral cortex suggests a functional involvement in brain cognition phenomena such as learning and memory. Insulin has been shown to exert a memory-enhancing action on both humans and experimental animals. Administration of insulin into the third cerebral ventricles of rats shortly after a passive avoidance training experience resulted in higher memory retention levels compared to rats that received saline and a heat-inactivated insulin injection (Park et al., 2000). Because insulin was given after acquisition of the experience, it most likely contributed to processes underlying memory consolidation. Extracranial delivery of insulin has also shown effects on memory. However, the outcomes appear to be complicated by alteration of blood glucose levels. For example, intraperitoneal (i.p.) injection

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of insulin without simultaneous administration of glucose to maintain normal circulating glucose levels caused impaired retention in mice that were trained on either an inhibitory avoidance or a habituation learning task (Kopf and Baratti, 1995, 1996, 1999). Because a peripheral delivery of insulin would certainly lower circulating glucose levels in the absence of insulin resistance, the memory impairment induced by insulin i.p. injection was more likely to be caused by hypoglycemia rather than a direct CNS effect of insulin. Indeed, when these investigators gave concomitant administration of glucose and insulin, the memory impairment was overcome in a glucose dose-dependent manner (Kopf and Baratti, 1995, 1999). On the other hand, systemic infusion of insulin under euglycemic conditions (maintained by glucose clamp) produced significant memory improvement in verbal memory and selective attention (Kern et al., 2001). Recently, an intranasal administration of insulin and other hormonal peptides has been reported to be an advantageous delivery route. Delivery of insulin to human subjects via this method induced a sharp and rapid increase in cerebrospinal fluid insulin concentration without affecting blood insulin and glucose levels (Kern et al., 1999; Born et al., 2002). When tested in a cognitive task responding to an auditory stimulus, those subjects showed a negative shift in auditory-evoked potentials in specific cortical areas (Kern et al., 1999), which are thought to be associated with facilitation of working memory processing (Pelosi and Blumhardt, 1999; Wolach and Pratt, 2001; Shucard et al., 2003). An aged population with impaired memory showed increased latencies and amplitudes in components of auditory-evoked potentials (Pelosi and Blumhardt, 1999). A negative shift of auditory-evoked potentials was also correlated with the insulin-induced memory enhancement tested in a similar task (Kern et al., 2001; Fehm et al., 2000; Born et al., 2002). Consistent with these findings, gene expression of insulin receptor has been upregulated in the CA1 region of the rat brain associated with short-term memory formation after a spatial learning experience (Zhao et al., 1999). Increased insulin receptor at protein levels have also been found in the hippocampal synaptic membrane fractions correlated with short-term memory formation (Zhao et al., 1999). Taken together, these results suggest that insulin/insulin receptor signaling is activated in the early stage of memory formation and may play a role in memory sorting for long-term storage.

Insulin appears to exert a more significantly beneficial effect on preventing memory loss from brain damage induced by ischaemia, lesions and pharmacological inhibition. When insulin treatment was given with a concomitant glucose supply to rats immediately following ischaemia, it prevented ischaemia-induced learning deficits when those rats received water maze navigation training 1–2 months after experiencing ischaemia. The insulin treatment also significantly reduced the CA1 neuronal necrosis caused by ischaemia, whereas increased glucose supply alone did not have such effects (Voll et al., 1989). Lesions to the hippocampus of rats produced severe loss of learning and memory ability (Horel, 1978; Huppert and Piercy, 1979; Spiers et al., 2001). However, pretreatment with insulin of those rats that received dorsal hippocampal lesions significantly reduced memory deficits for an active avoidance learning experience induced by hippocampal lesions (De Castro and Balagura, 1976). In pharmacological experiments, insulin was shown to overcome scopolamine-induced memory impairment in rats trained in a radial arm maze task (Blanchard and Duncan, 1997), indicating insulin's positive action on adrenergic transmission in the brain.

The significance of understanding roles of insulin receptor in memory has been highlighted by the hypothesis that deterioration of CNS insulin receptor functions is associated with the pathogenesis of aging-related brain degenerative dementias such as sporadic Alzheimer's disease (Hoyer, 1998, 2002; Hoyer and Lannert, 1999; Watson and Craft, 2003). Clinical and experimental evidence supporting this notion have been shown by different investigators. For example, while glucose and insulin improve memory in young animals and humans, only insulin but not glucose exhibits a memory enhancing effect in aged subjects (Long et al., 1992). Brain insulin and insulin receptor are reduced in the brains of sporadic Alzheimer's disease patients (Frolich et al., 1998). Consistent results have also been found in rodents whose brains show aging-reduced insulin receptor numbers (Zaia and Piantanelli, 2000) and insulin receptor mRNA levels (Fig. 1). Systemic administration of insulin under euglycemic or hyperglycemic conditions was shown to improve memory performance of Alzheimer's disease patients whereas hyperglycemia alone did not have such an effect (Craft et al., 1999, 2000). More recent results from those authors have shown that different doses of insulin are required to produce memory improvement in elderly people and Alzheimer's disease patients that were associated with the presence of the apoE-4 allele in tested subjects (Craft et al., 2000, 2003).

Cognitive impairments associated with diabetes mellitus caused by inadequate insulin/insulin receptor functions have also been documented. In this category, memory deficits are not evident in insulin-dependent type 1 diabetes in humans (Ryan and Williams, 1993), and if they occur, are often associated with hypoglycemia (Kaufman et al., 1999; Sommerfield et al., 2003). In diabetic animals most commonly induced by streptozocin, only subtle memory impairment was detected (Biessels et al., 1996, 1998; Popovic et al., 2001), which was accompanied by altered synaptic plasticity (Biessels et al., 1996; Kamal et al., 1999). On the other hand, cognitive deficits have been reported more consistently in non-insulin dependent diabetes (type 2 diabetes) often found concurrent with increased insulin resistance. Cognitive decline in type 2 diabetes depends on duration of

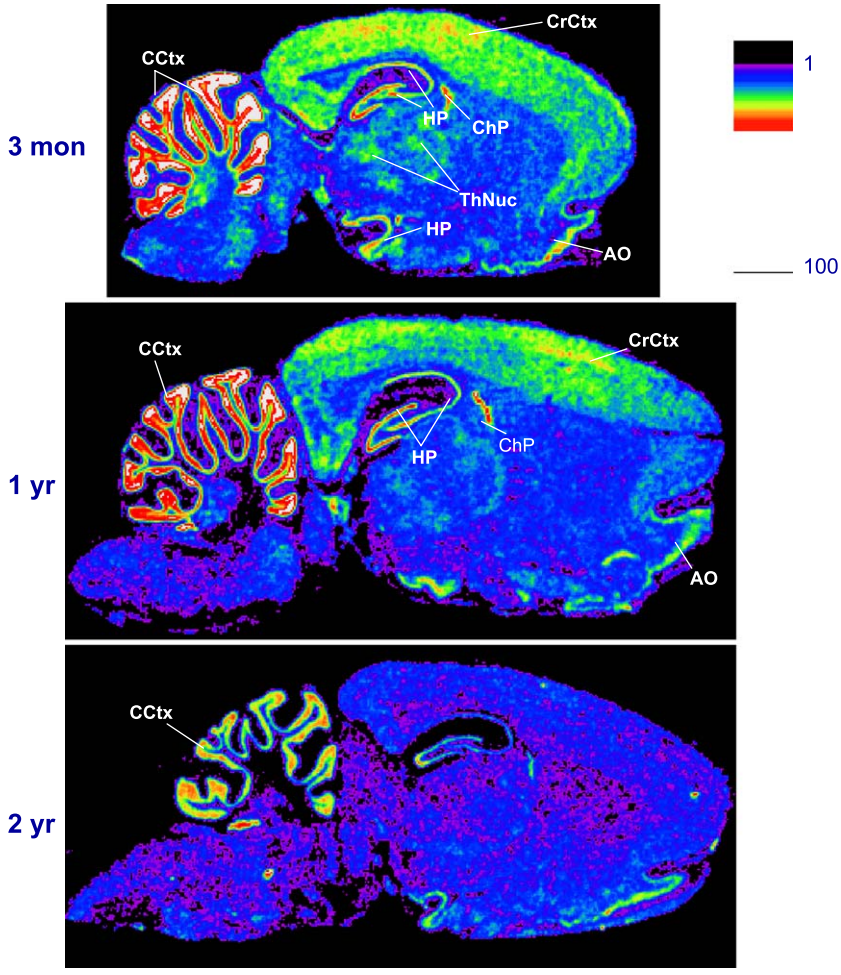

Fig. 1. Reductions of the brain insulin receptor mRNA by aging. Cryosections were prepared from rat brains of different ages. Distribution of insulin receptor mRNA was examined by in situ hybridization using an insulin receptor riboprobe (Zhao et al., 1999). Insulin receptor mRNA signals were revealed by film autoradiography. The brain insulin receptor mRNA level showed a significant reduction correlating with aging. Brain regions were defined according to The Rat Brain Stereotaxic Coordinates by Paxinos and Watson (1998). AO: olfactory bulb, anterior; CrCtx: cerebral cortex; CCtx: cerebellar cortex; chP: choroid plexus; HP: hippocampus; ThNuc: thalamic nuclei.

the disease (Cosway et al., 2001), correlates highly with aged subjects (Perlmuter et al., 1984, 1987; Mooradian et al., 1988; Ryan and Geckle, 2000) and is worsened by brain vascular complications such as stroke (Wu et al., 2003). In addition, type 2 diabetes has been more commonly reported to affect verbal learning and memory, tests of which generally require subjects to repeat verbatim a short story or to recall a list of words (Mooradian et al., 1988; Ryan and Geckle, 2000). These patients also show poor performance in the mini-mental state examination (MMSE), which is a general test for concentration, language and other memory components, used to screen for demented subjects (Folstein and McHugh, 1978). In parallel with poor memory performance, these patients also showed abnormal brain electroencephalograms (EEGs) in the cerebral cortex and subcortical areas that were not a result of hyperglycemia (Mooradian et al., 1988). Furthermore, the rate of cognitive decline associated with diabetes was increased by the presence of the apoE4 allele (Haan et al., 2003). All of these results suggest a link between aging-related insulin receptor failure and Alzheimer's dementia.

3. Mechanisms underlying roles of insulin/insulin receptor in learning and memory

3.1. The CNS insulin receptor is functionally different from those in classical insulin target tissues. Therefore, memory improvement by insulin/insulin receptor is not due to a direct effect on glucose metabolism

Although glucose is the major nutrient and energy source for brain cells and plays critical role in brain cognitive functions (McNay and Gold, 2002), its uptake, transport and utilization in the majority of brain regions do not depend on insulin. Firstly, the adult brain appears to express two main glucose transporters (GLUTs) that are not insulin-sensitive.

While GLUT-1 is expressed in the endothelium of cerebral microvessels and astrocytes, GLUT-3 is predominantly distributed in neurons (Vannucci et al., 1998; Simpson et al., 1999; Duelli and Kuschinsky, 2001). Different localizations of glucose transporter subtypes suggest different regulatory mechanisms underlying glucose uptake into different cell types. Low abundance of the insulin-sensitive GLUT-4 has been found in the brain associated with early development of the brain (Vannucci et al., 2000; Royer et al., 2000) and is predominantly expressed in the cerebellum of both developing and adult brains (Rayner et al., 1994; Kobayashi et al., 1996; Vannucci et al., 1998) This suggests that insulin may directly mediate glucose transport to cerebellar neurons. A recent study showed that although GLUT-4 gene expression could be detected in the hippocampus, its protein level, however, was very low compared with the level in cerebellar neurons (El Messari et al., 2002). Because the cerebellum controls balance and coordinates movement of the body, the insulin-sensitive glucose transport in the cerebellum may partially explain why type 1 diabetic subjects often suffer psychomotor (Ryan and Williams, 1993; Bohannon et al., 1995), and postural impairments (Di Nardo et al., 1999; Yamamoto et al., 2001).

Secondly, insulin receptor is not homogenously distributed throughout the brain, but concentrated in rather discrete regions such as the olfactory bulb, hypothalamus, hippocampus, choroid plexus and cerebellum (Adamo et al., 1989; Unger et al., 1991; Zhao et al., 1999). Similarly, entry of insulin into the brain also shows regional differences (Banks and Kastin, 1998). This brain regional distribution pattern of and insulin does not correlate well with a primary role in mediation of brain cell glucose metabolism. Thirdly, the brain insulin receptor displays structural and functional differences from that in the peripheral tissues. The brain insulin receptor has smaller molecular weights in both alpha- and beta-subunits compared with its peripheral counterparts as a result of alternative mRNA splicing of the exon-11, and differences in receptor glycosylation (Heidenreich et al., 1983; Goldstein and Dudley, 1992; Sugimoto et al., 2000). Unlike in the peripheral tissues, insulin receptor in the neuron lacks negative cooperativity (receptor down-regulation in response to prolonged and/or high concentrations of ligand reaction), which is otherwise shown in the peripheral insulin receptor, suggesting different insulin-binding specifics of the CNS insulin receptor (Boyd and Raizada, 1983; Gammeltoft et al., 1984). Furthermore, Heidenreich et al. (1983) reported that an anti-insulin receptor autoantiserum that blocked insulin binding of the peripheral insulin receptor had no effect on insulin binding to the brain insulin receptor. Unlike those from adipocytes, insulin receptor from brain did not bind to wheat germ agglutinin columns. These results again suggest that differences in structure and other molecular properties exist between the peripheral and CNS insulin receptors. Fourthly, evidence from the majority of experiments has consistently demonstrated insulin's lack of direct effects on glucose uptake and metabolism in brain cells (Goodner et al., 1980; Gorus et al., 1984; Hertz et al., 1981). For example, insulin infusion under euglycemia showed no effect on glucose transport across the blood–brain barrier, and net brain glucose uptake in humans (Hertz et al., 1981; Hasselbalch et al., 1999). Neither did it affect cerebral metabolism (Hasselbalch et al., 1999) and oxidation in cultured cortical cells (Gorus et al., 1984). Although some investigators report that insulin increased glucose uptake by glial but not neuronal cells (Werner et al., 1989), the extracellular glucose level exerted much stronger effects on glucose uptake into glial cells (Walker et al., 1988). Exposure of neurons to increased extracellular glucose also elevated glucose transport to neurons although to a much lesser extent compared to glial cells (Walker et al., 1988). Lastly, while the whole body homozygous insulin receptor knockout is lethal, specific deletion of the brain insulin receptor did not show an evident disruption in brain development and neuronal survival (Bruning et al., 2000).

However, accumulated evidence from a variety of experiments together with the results of brain specific deletion of insulin receptor has clearly indicated that the brain insulin/insulin receptor contributes importantly to glucose homeostasis. Particularly, the hypothalamic insulin/insulin receptor is shown to control peripheral insulin secretion and glucose levels in the circulation (Amir and Shechter, 1987; Chen et al., 1975; Chowers et al., 1966; Woods et al., 1968; Obici et al., 2002a, 2002b; Gerozissis, 2003) and plays a role in the peripheral insulin receptor resistance (Obici et al., 2002b). This action is likely to be mediated via the vagal system, as vagotomy and atropine block the CNS action of insulin on periphery plasma glucose regulation (Woods et al., 1972; Woods, 1991; Szabo et al., 1983). In addition, centrally administered insulin showed a Pavlovian conditioning effect on peripheral glucose levels in both humans and animals (Stochhorst et al., 2000; Woods et al., 1968, 1972). Based on the evidence above, it becomes obvious that the action of insulin/insulin receptor in the brain is predominantly mediated by its roles in neuromodulation.

3.2. Insulin/insulin receptor modulates synaptic plasticity by acting on glutamatergic and GABAergic receptors

Although hypothalamic insulin signaling may play an indirect role in regulation of peripheral glucose metabolism, actions of insulin/insulin receptor on learning and memory, particularly those located in the hippocampus (see previous sections), are more likely to be due to direct modulation of receptor activity in neurons and/or glial cells. Emerging evidence has shown that insulin receptor signaling plays a role in synaptic plasticity by acting on both glutamatergic and GABAergic transmissions. In *Xenopus* oocytes expressed with NMDA receptors, brief insulin exposure triggered a rapid and significant potentiation of responses to NMDA mediated by NMDA receptor subtypes (Liu et al., 1995; Chen and Leonard, 1996; Liao and Leonard, 1999).

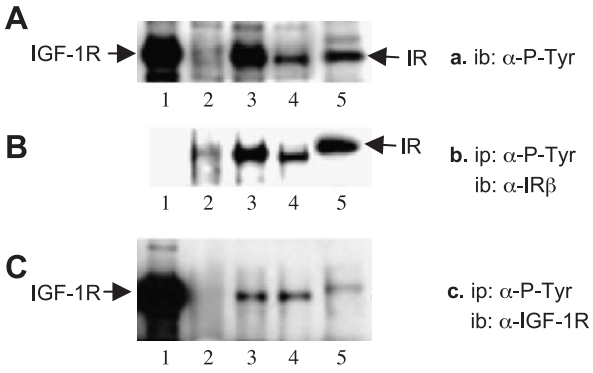

Fig. 2. Effect of Ca^{2+} on in vitro tyrosine phosphorylation of the hippocampal insulin receptor and IGF-1 receptor: (A) Tyrosine phosphorylated synaptic membrane proteins at ~95 kDa detected by an anti-phospho-tyrosine antibody. (B) Phosphorylated proteins were immunoprecipitated (ip) by the anti-phospho-tyrosine antibody, and then blotted (ib) by an anti-insulin receptor beta-subunit antibody. (C) Phosphorylated proteins were precipitated as described in (b), and blotted by an-anti IGF-1 receptor antibody. The results showed a Ca^{2+}-inhibited tyrosine phosphorylation of insulin receptor but not IGF-1 receptor. (1) Tyrosine-phosphorylated IGF-1 receptor overexpressed in NIH3T3 cells; (2) non-phosphorylated synaptic membrane fractions from rat hippocampus; (3) tyrosine phosphorylation in the absence of Ca^{2+}; (4) tyrosine phosphorylation in the presence of 1 mMCa^{2+}; (5) tyrosine phosphorylation of the human liver insulin receptor that is overexpressed in NIH3T3 cells.

This insulin-induced potentiation was blocked by a tyrosine protein kinase inhibitor, genistein and a broad-spectrum protein kinase inhibitor staurosporine, suggesting involvement of tyrosine and possibly down-stream serine/threonine protein kinases such as protein kinase C (PKC) activities. In a similar experimental preparation, Skeberdis et al. (2001) demonstrated that application of insulin increases NMDA channel activities by recruiting NMDA receptors to the membrane surface. This process was blocked by a more specific insulin receptor tyrosine kinase inhibitor tyrphostin A47, and may involve function of SNAP-25 (synaptosomal associated protein 25), but does not seem to require tyrosine and serine/threonine phosphorylations at the NMDA receptor C-terminus. Other study, however, showed that incubation of rat hippocampal slices with insulin caused increases in tyrosine phosphorylation of the NR2A and 2B subunits of NMDA receptors (Christie et al., 1999). Given the important roles that NMDA receptors may play in synaptic plasticity and learning and memory formation (Huerta et al., 2000; Nakazawa et al., 2002), modulation of NMDA transmission may represent one of the synaptic bases for roles of insulin/insulin receptor signaling in learning and memory.

In addition, insulin plays a role in synaptic plasticity by acting on alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor trafficking. Redistribution of AMPA receptors has been proposed to regulate strength of glutamatergic synapses. A mature synaptic connection at glutamatergic synapses in the brain requires conversion of silent glutamatergic synapses into functional synapses during the course of postnatal brain development (Wu et al., 1996; Renger et al., 2001). A silent glutamatergic synapse that mediates only NMDA transmission is not functional unless AMPA receptors are delivered to such synapses (Malinow et al., 2000, Malinow, 2003). Conversion of a silent synapse to a functional synapse can be both development dependent (Wu et al., 1996; Renger et al., 2001) and activity dependent (Malinow et al., 2000; Malinow, 2003) that have been hypothesized as a synaptic basis for learning and memory formation. In cultured differentiating neurons, insulin promoted transfer of silent AMPA synapse to functional synapse and accelerated reduction of silent synapses (Plitzko et al., 2001). In the mature brain, insulin facilitated clathrin-dependent internalization of AMPA receptors leading to long-term depression of AMPA receptor-mediated synaptic transmission in hippocampal CA1 neurons (Man et al., 2000).

Insulin-mediated receptor trafficking has also been found in the gamma-aminobutyric acid (GABA) receptor, which

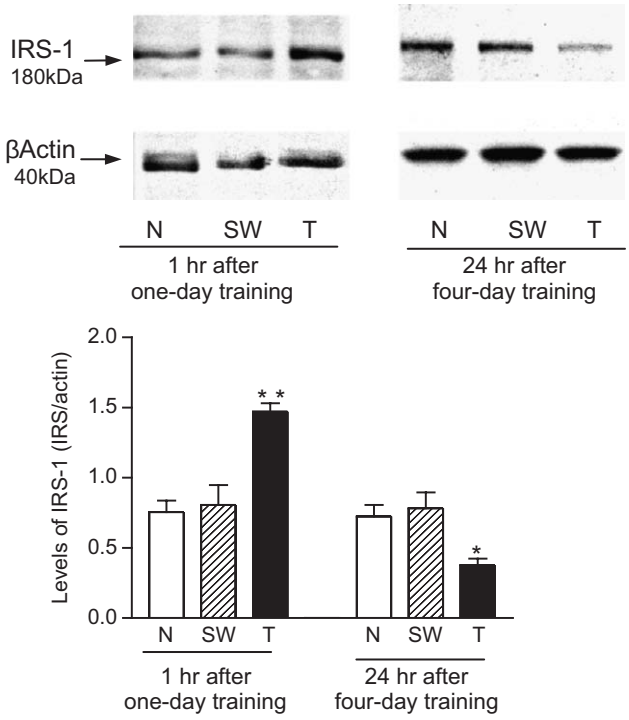

Fig. 3. Changes in amount of IRS-1 in the hippocampal synaptic membrane fractions after spatial learning: Synaptic membrane fractions were prepared from the hippocampus and the amount of IRS-1 was examined with Western blot by an anti-IRS-1 antibody and compared among trained and control animals. To investigate effects of training intensity (numbers of training trials that rats experienced) and stages of memory formation (short-term vs. long-term) on the amount of IRS-1, animals were subjected to two experimental conditions: (1) Rats were trained on a four-trial water maze task for a 1-day and sacrificed 1 h after training. (2) Animals were trained on four-trial water maze task per day for 4 consecutive days and sacrificed 24 h after the 4th day training. For each condition, groups of control rats for non-specific behavioral effects were designed. Immunoreactive signals of IRS-1 were normalized with that of β-actin from the same sample for a sample-loading variability control. N: Naïve animals that were used as the basal control. SW: Animals subjected to swimming only, but not training activities that were used to control for non-learning specific effects. T: Trained animals $^{**}p<0.01$, $^{*}p<0.05$ (one-way ANOVA), $n=4$.

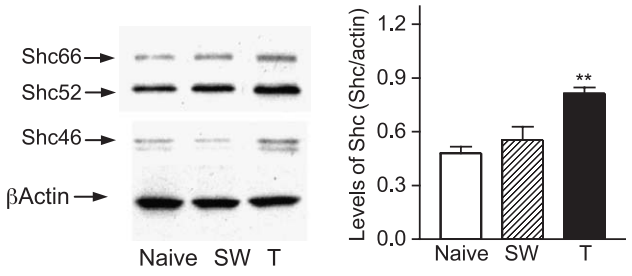

Fig. 4. Changes in the amount of Shc in the synaptic membrane during long-term memory formation: The synaptic membranes were prepared from hippocampi of different groups of rats after a 4-day water maze training experience. Shc protein was detected on Western blots with an anti-Shc antibody. β-Actin from the same sample was also measured for normalization of the Shc signal. Control groups were set as described in Fig. 3, and animals sacrificed 24 h after the 4th day of training. Naïve animals were used as the basal control, SW: swimming-only animals were used to control for non-specific effects-induced changes. T: Trained animals. $^{**}p < 0.01$, one-way ANOVA, $n = 4$.

mediates synaptic inhibition important for neuronal functions associated with learning (Paulsen and Moser, 1998; Chapouthier and Venault, 2002; McGaugh, 2002). When applied to HEK 293 cells transfected with the $GABA_A$ receptor, insulin caused rapid translocation of the $GABA_A$ receptors to the plasma membrane (Wan et al., 1997). Insulin also recruited functional $GABA_A$ receptors onto the postsynaptic and dendritic membranes of the CNS neurons, leading to augmented amplitudes of the $GABA_A$ receptor-mediated miniature inhibitory postsynaptic current (Wan et al., 1997). Furthermore, insulin activation of muscarinic transmission potentiated GABA receptor currents likely occurs via a phosphoinositide-3 (PI-3) kinase-dependent mechanism (Ma et al., 2003). Thus, insulin/insulin receptor plays a role in receptor trafficking during synaptic maturation and synaptic usage, and it may also mediate interactions of different neurotransmission systems during neuronal activation, all of which may underlie modification of synaptic connections required for higher brain functions such as learning and memory.

3.3. Insulin/insulin receptor-mediated signal transduction as a molecular basis underlying learning and memory

As in the periphery, insulin's action in the brain is mediated by insulin receptor, although at higher concentrations insulin also binds to insulin-like growth factor (IGF) receptors. Under an in vitro phosphorylation system, both insulin receptor and IGF-1 receptor can be tyrosine phosphorylated. In vitro tyrosine phosphorylation of hippocampal insulin receptor, but not IGF-1 receptor is inhibited by the presence of Ca^{2+} (Zhao et al., 1999; Fig. 2), suggesting that insulin receptor and IGF-1 receptor activities are differential regulated in response to Ca^{2+} signals such as those resulted from glutamatergic and GABAergic transmissions during synaptic activation or inhibition. Binding of insulin activates the protein tyrosine kinase activity of the insulin receptor β-subunit, which, in turn, triggers cascades of signal transduction through its downstream substrate

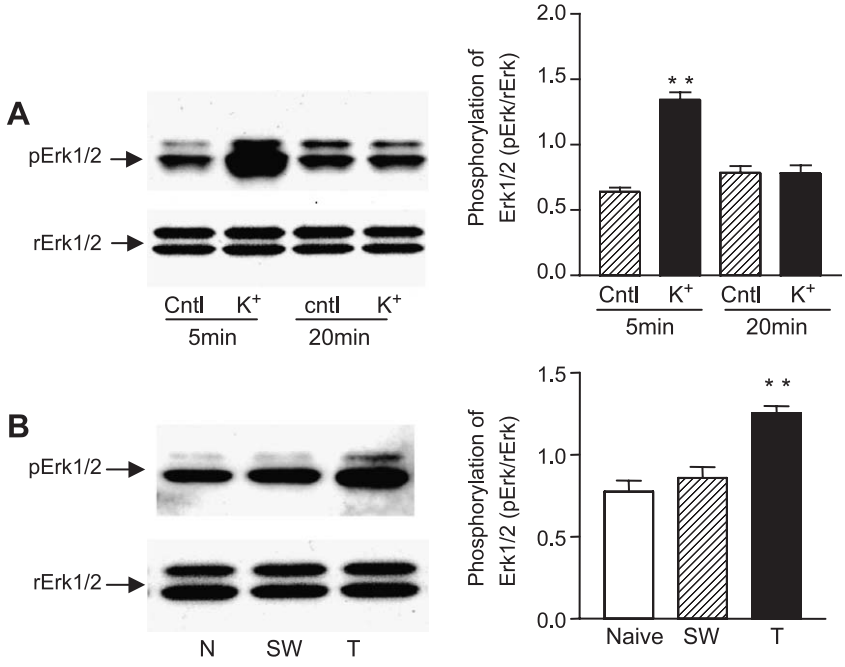

Fig. 5. Effects of neuronal depolarization and learning on phosphorylation of Erk1/2: (A) Primary cortical neuronal cultures were depolarized with high concentration (55 mM) KCl for 5 or 20 min. Phosphorylation of Erk1/2 was detected with an anti-phospho-Erk antibody. Phosphorylation extents were normalized with the total amount of Erk detected with an anti-regular Erk antibody. Erk1/2 was activated by depolarization, but returned to the control level when depolarization prolonged. (B) Synaptic membranes were obtained from rat hippocampi as described in Fig. 4. A significant learning-specific increase in Erk1/2 phosphorylation was observed during long-term memory formation. $^{**}p < 0.01$, one-way ANOVA, $n = 4$.

molecules. Several signaling pathways activated by insulin receptor have been identified (Olefsky, 1990; White and Kahn, 1996; Bevan, 2001), among which the insulin receptor substrate-1 (IRS-1)/PI-3 kinase/phosphoinositide-dependent kinase (PDK)/protein kinase B (PKB/Akt), and the SH2 and collagen containing protein (Shc)/growth factor receptor-bound protein-2 (Grb2)/mitogen-activated protein (MAP) kinase pathways have been most intensively studied. These two signaling pathways also constitute insulin receptor downstream cascades in the brain (Unger et al., 1991; Wozniak et al., 1993; Adamo et al., 1993). When rats were trained in a spatial learning task (Morris water maze training), a learning-specific increase in IRS-1 was detected in the hippocampal synaptic membranes shortly after training (Fig. 3). However, after exhaustive training experience, from which rats normal demonstrated stable spatial memory (Morris, 1984), the amount of IRS-1 in the synaptic membranes was reduced (Fig. 3). The increased IRS-1 in the synaptic membrane may be due to an increase in local expression, or a translocation of IRS-1 from cytosol to the synaptic membrane. These results suggest that IRS-1 may participate in an early stage of memory processing. Tyrosine phosphorylated IRS-1 transduces the insulin signal by binding to the p85 subunit of PI-3 kinase, leading to activation of the p110 catalytic subunit (Combettes-Souverain and Issad, 1998). Both IRS-1 and PI-3 kinase are abundantly expressed in the hippocampus, colocalizing with insulin receptor (Folli et al., 1994). Involvement of PI-3

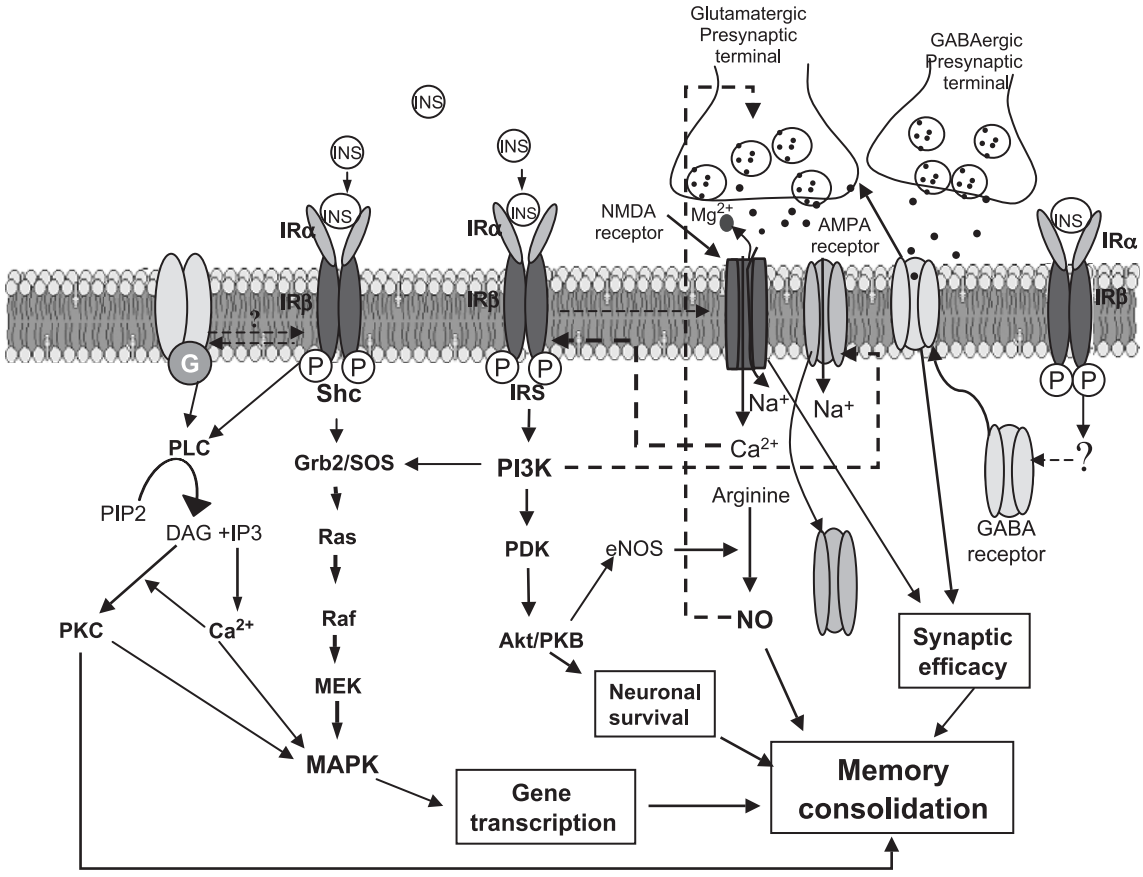

Fig. 6. Hypothetic schema for insulin/insulin receptor modulation of memory-associated neuronal activities. During learning, insulin binds to the a-subunit of insulin receptor and cause activation of the tyrosine kinase activity of the b-subunit. Activated insulin receptor may be involved memory formation via several mechanisms: (1) via modulation of glutamatergic and GABAergic transmission. Insulin/insulin receptor potentiates NMDA channel activity, functions of which depend on the presence and activation of AMPA receptor that cause synaptic membrane depolarization and removal of the Mg^{2+} blockage of the NMDA receptor leading to long-term potentiation. Increased Ca^{2+} influx via the NMDA receptor and neuronal activities may inhibit tyrosine phosphorylation of insulin receptor via a feedback mechanism. Depending on spatial and temporal specificity of information processing, insulin receptor signaling through PI-3 kinase may be involved in long-term depression via internalization of AMPA receptors. Insulin receptor may also modulate GABA transmission by recruiting functional GABA receptor to the postsynaptic membrane. GABAergic neurons sense the excitatory transmission and regulate synaptic strength by sending feedforward and/or feedback inhibitory inputs to the principal neurons. Regulation of synaptic efficacy by integrated excitatory and inhibitory transmissions within specific neuronal network is thought to underlie memory encoding and retrieval in the hippocampus (Paulsen and Moser, 1998). (2) Activation of nsulin receptor-Shc-MAP kinase pathway after learning may lead to regulation of gene expression that is required for long-term memory storage. (3) Insulin receptor may interact with G-protein coupled receptor and PLC to activate PKC leading to facilitation of short-term memory encoding. (4) The insulin receptor/IRS/PI-3 kinase pathway may trigger synthesis of NO via eNOS activity. NO acts as a retrograde messenger for neurotransmitter release, and may also act intracellularly on memory processing. Furthermore, insulin receptor signaling with the same pathway may promote neuronal survival that is certainly beneficial for long-term memory consolidation.

kinase has been reported in learning and memory formation using different experimental paradigms (Lin et al., 2001; Barros et al., 2001), as well as in regulation of synaptic plasticity such as long-term potentiation and long-term depression (Ma et al., 2003; Daw et al., 2002; Sanna et al., 2002; Kelly and Lynch, 2000). Thus, increased IRS-1 at synaptic locations after learning may activate PI-3 kinase leading to regulation of subsequent memory processing. Other downstream molecules in the context of insulin receptor-activated IRS-1/PI-3 kinase pathway may include production of nitric oxide (NO) through nitric oxide synthase (NOS) activities. It has been known that insulin stimulates NO production (Montagnani et al., 2001; Vincent et al., 2003) via activation of endothelial NOS (eNOS), an IRS-1/PI-3 kinase/Akt pathway mediates this process (Zeng and Quon, 1996; Zeng et al., 2000; Montagnani et al., 2002). eNOS is expressed in the hippocampus, and has been shown to be involved in learning and synaptic plasticity (Zhuo et al., 1993; O'Dell et al., 1994; Arancio et al., 1996; Rickard et al., 1999; Doreulee et al., 2003). However, whether activation of eNOS occurs in learning downstream of insulin receptor action has not yet been explored.

We have also reported learning-induced changes in Shc, another substrate molecule for insulin receptor that contains a SH2 domain and a collagen-homologous region (Pelicci et al., 1992) Shc comprises three isoforms, namely Shc66, Shc52 and Shc46, due to alternative splicing of the primary Shc transcript (Pelicci et al., 1992; Migliaccio et al., 1997). Shc can be tyrosine phosphorylated by insulin receptor and epidermal growth factor (EGF) receptor. While the EGF receptor similarly phosphorylates all three isoforms, insulin receptor preferentially phosphorylates Shc52, and Shc46 to a lesser extent (Sasaoka et al., 1994). Shc52 is a potent activator of Ras21. We have shown that spatial learning increases expression of Shc isoforms in the hippocampal synaptic membrane fraction. While increases in Shc-66 and Shc-52 were seen during short-term memory formation (Zhao et al., 1999), Shc52 was the major isoform that showed significant increases in long-term memory, although a small increase was also seen in Shc46 and Shc66, respectively (Fig. 4). These results suggest that increases of Shc52 and shc46 in the hippocampal synaptic membrane may be associated with insulin receptor activity after training. The downstream cascades of Shc52 include activation of Ras21 leading to activation of MAP kinases, such as extracellular signal-regulated kinase 1/2 (Erk1/2). In our experimental systems, Erk1/2 has been shown to respond to learning and neuronal depolarization (Fig. 5A), with high sensitivity and specificity. Indeed, a lasting activation of Erk1/2 has been observed after water maze training (Zhao et al., 1999; Fig. 5B). Interestingly, when hippocampal synaptic membranes were treated with insulin under an in vitro phosphorylation condition, only those from trained but not control animals demonstrated an insulin-induced increase in total amount of Erk1/2 and its phosphorylation (Zhao et al., 1999). These results suggest that learning input has specifically sensitized Erk1/2 expression and phosphorylation in response to insulin, probably due to facilitation of signal transduction involving Shc and other upstream molecules.

In summary, research in recent years has significantly advanced our knowledge about roles of insulin/insulin receptor in functions of the central nervous system, including learning and memory at behavioral, synaptic and molecular levels. Based on evidence accumulated to date, a hypothetical schema for involvement of insulin/insulin receptor in learning and memory consolidation processes is summarized in Fig. 6. However, precise molecular mechanisms underlying insulin's roles in learning and memory, and in the pathogenesis of memory degenerative disorders are still far from understood. Many substrates of insulin receptor, and interactions between insulin receptor and other receptor and kinase molecules directly associated with learning experiences have not been explored. Future in-depth studies at cellular and molecular levels, particularly those with gene interference, and specific deletions of insulin receptor and insulin receptor substrates in the brain, are expected to provide meaningful answers.

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