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**Review Article**

# Neurobiology of diabetic encephalopathy

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**Abstract**

Epidemiological evidence indicates that diabetes is a potential predisposing factor for neuropsychiatric deficits such as brain aging, stroke, cerebrovascular diseases, depression, anxiety and diabetic encephalopathy. These deficits are paralleled by neurophysiological and structural alterations in the brain. Diabetic encephalopathy, a relatively late and debilitating complication, is characterized by impaired cognitive function, neurochemical and structural abnormalities, and may involve direct neuronal damage caused by intracellular glucose. Behavioral, electrophysiological and biochemical evidence has unraveled the cellular and molecular basis for the central insulin/insulin receptor action in learning and memory. The present review focuses on the neurobiological role of insulin signaling, hyperglycemia, oxidative stress and neurotransmitter alterations in diabetes-associated learning and memory disruption.

## Introduction

Diabetes is a major endocrine disorder and growing health problem in most countries. The prevalence of diabetes in adults was estimated to be 4% in 1995 and is expected to rise to over 5% by the year 2025 worldwide. This implies an increase in the number of adults with diabetes from 135 million in 1995 to 300 million in 2025 (1). In recent years, India has witnessed a rapidly exploding epidemic of diabetes.

Both type 1 and type 2 diabetes can lead to several complications, such as retinopathy, nephropathy and peripheral neuropathy, and the characteristic clinical signs and symptoms, as well as the techniques to diagnose these complications, are well established (2). Now there is substantial evidence that acute hypo- and hyperglycemia have disruptive effects on the central nervous system (CNS) (3), although relatively less is known about the slowly developing end-organ damage to the CNS that may present itself as electrophysiological and structural changes and impairment of cognitive functioning (4). These cerebral complications of both type 1 and type 2 diabetes may be referred to as 'diabetic encephalopathy', a concept introduced several decades ago (5). Recently, Mijnhout *et al.* proposed a new term —'diabetes-associated cognitive decline' (DACD)— to facilitate research in this area and increase recognition of the disorder. This term is not suggestive of a particular pathogenesis, but merely describes a state of mild to moderate cognitive impairment, particularly psychomotor slowing and reduced mental flexibility, not attributable to other causes (6). The duration and age of onset of diabetes are the strongest predictors of low scores in psychomotor speed, memory, processing speed, attention, working memory, verbal ability, general intelligence and executive functions, and a low global score (4).

The present review focuses on the neurobiological role of cerebral insulin, insulin receptors, hyperglycemia, oxidative stress and neurotransmitter alterations in cognitive deficits associated with diabetes.

## Insulin and memory consolidation

The insulin receptor is a tetrameric membrane-spanning protein (7). There are two types of insulin receptors found in the adult mammalian brain: the peripheral type which is only found on glial cells and a neuron-specific brain type (8). However, both types appear to be similar in insulin signal-transducing properties. Binding of insulin to the receptor induces autophosphorylation of the intracellular domain, which in turn initiates the receptor's protein tyrosine kinase activity. Tyrosine phosphorylation of intracellular substrates such as the insulin receptor substrate (IRS) family (9) then leads to activation of multiple signals, including phosphatidylinositol 3-kinase (PI3K) and GTPase regulators (10).

Several studies have found high levels of insulin receptors in the CNS at specific locations. The highest concentrations of insulin receptors in the brain are in the olfactory bulb, cerebral cortex, hippocampus, cerebellum and hypothalamus (11, 12). Insulin modulates CNS levels of neuropeptides, monoamines and other neurotransmitters implicated in the pathophysiology of mood and dementias (13, 14). Insulin also inhibits the firing of neurons in the hippocampus and hypothalamus, modulates catecholamine signaling in the hypothalamus, stimulates phosphoinositol turnover in the hippocampus and regulates norepinephrine and dopamine transporter mRNA concentrations in neurons (13, 15-18). Furthermore, insulin also exerts important growth-regulatory (*e.g.*, promotion of neurite outgrowth and synaptogenesis) effects salient to CNS functions (13).

The presence of insulin and insulin receptors in the hippocampus and cerebral cortex and their functional involvement in brain cognition phenomena at behavioral, synaptic and molecular levels have been suggested. Insulin-sensitive biochemical systems exist which have the potential to affect various cognitive systems, and this process may be independent of or secondary to a glucoregulatory effect. Insulin has been shown to exert a memory-enhancing action in both humans and experimental animals. Microinjection of insulin into the CA1 region of rat hippocampus (12 MU, but not 0.5 and 6 MU) improved both memory retrieval and consolidation (19). Babri *et al.* (20) reported that intrahippocampal injections of insulin enhance memory in a simple learning task, which supports the concept that insulin possibly plays a role in memory formation. Administration of insulin into the third cerebral ventricle of rats shortly after a passive avoidance training experience resulted in greater memory retention compared to rats that received saline and a heat-inactivated insulin injection (21). Recently, a pilot study demonstrated the benefit of intranasal insulin in 25 patients with Alzheimer's disease (22, 23), as well as improved memory in healthy adults (24).

The brain insulin receptor is structurally and functionally different from that in peripheral tissues (25). The major actions of insulin and the insulin receptor in the brain are mediated by altering receptor trafficking during synaptic maturation and neuromodulation, which may modify the synaptic connections required for learning and memory. Insulin receptor-mediated signal trafficking provides a molecular basis underlying learning and memory. Binding of insulin activates the protein tyrosine kinase activity of the insulin receptor β-subunit, which triggers two major cascades of signal transduction through its downstream substrate molecules. These include insulin receptor substrate 1 (IRS-1)/PI3K/phosphoinositide-dependent kinase (PDPK)/protein kinase B (PKB/Akt) and the SH2 domain protein SHC/growth factor receptor-bound protein 2 (GRB2)/mitogen-activated protein (MAP) kinase pathways. Both IRS-1 and PI3K are abundantly expressed in the hippocampus, colocalizing with the insulin receptor (26). Increased IRS-1 at synaptic locations after learning may activate PI3K, leading to regulation of subsequent memory processing. It is known that insulin stimulates nitric oxide (NO) production (27, 28) via activation of endothelial nitric oxide synthase (eNOS), a process mediated by the IRS-1/PI3K/Akt pathway (29-31).

Cognitive impairment associated with diabetes caused by inadequate insulin/insulin receptor function has also been documented (32). Zhao *et al.* (25) hypothesized several mechanisms involved in insulin-mediated memory formation (Fig. 1):

- Insulin/insulin receptor potentiates NMDA channel activity, the function of which depends on the presence and activation of the AMPA receptor, which causes synaptic membrane depolarization and removal of the $Mg^{2+}$ blockade of the NMDA receptor, leading to long-term potentiation (LTP). Increased $Ca^{2+}$ influx via the NMDA receptor and neuronal activities may inhibit tyrosine phosphorylation of the insulin receptor via a feedback mechanism. Depending on the spatial and temporal specificity of information processing, insulin receptor signaling through PI3K may be involved in long-term depression (LTD) via internalization of AMPA receptors. The insulin receptor may also modulate GABA transmission by recruiting functional GABA receptors to the postsynaptic membrane. GABAergic neurons sense the excitatory transmission and regulate synaptic strength by sending feed-forward and/or feedback inhibitory inputs to the principal neurons. Regulation of synaptic efficacy by integrated excitatory and inhibitory transmission within specific neuronal networks is thought to underlie memory encoding and retrieval in the hippocampus (33).
- Activation of the insulin receptor/SHC/MAP kinase pathway after learning may lead to regulation of gene expression that is required for long-term memory storage (25).
- The insulin receptor may interact with G protein-coupled receptors and phospholipase C (PLC) to activate PKC, leading to facilitation of short-term memory encoding (25).
- The insulin receptor/IRS/PI3K pathway may trigger the synthesis of NO via eNOS activity. NO acts as a retrograde messenger for neurotransmitter release, and may also act intracellularly on memory processing (34).

Furthermore, insulin receptor signaling through the same pathway may promote neuronal survival, which is certainly beneficial for long-term memory consolidation.

## Neurobiology of diabetic encephalopathy

There are many pathophysiological mechanisms by which diabetes might affect the initiation and promotion of the many underlying pathologies associated with dementia (35). These mechanisms/factors include alterations in cerebral insulin, insulin receptors, insulin signaling, insulin-like growth factors (IGFs), C-peptide, glucose transporters (GLUTs), ischemic cerebrovascular disease, hyperglycemia, oxidative stress and alterations in neurotransmitter levels.

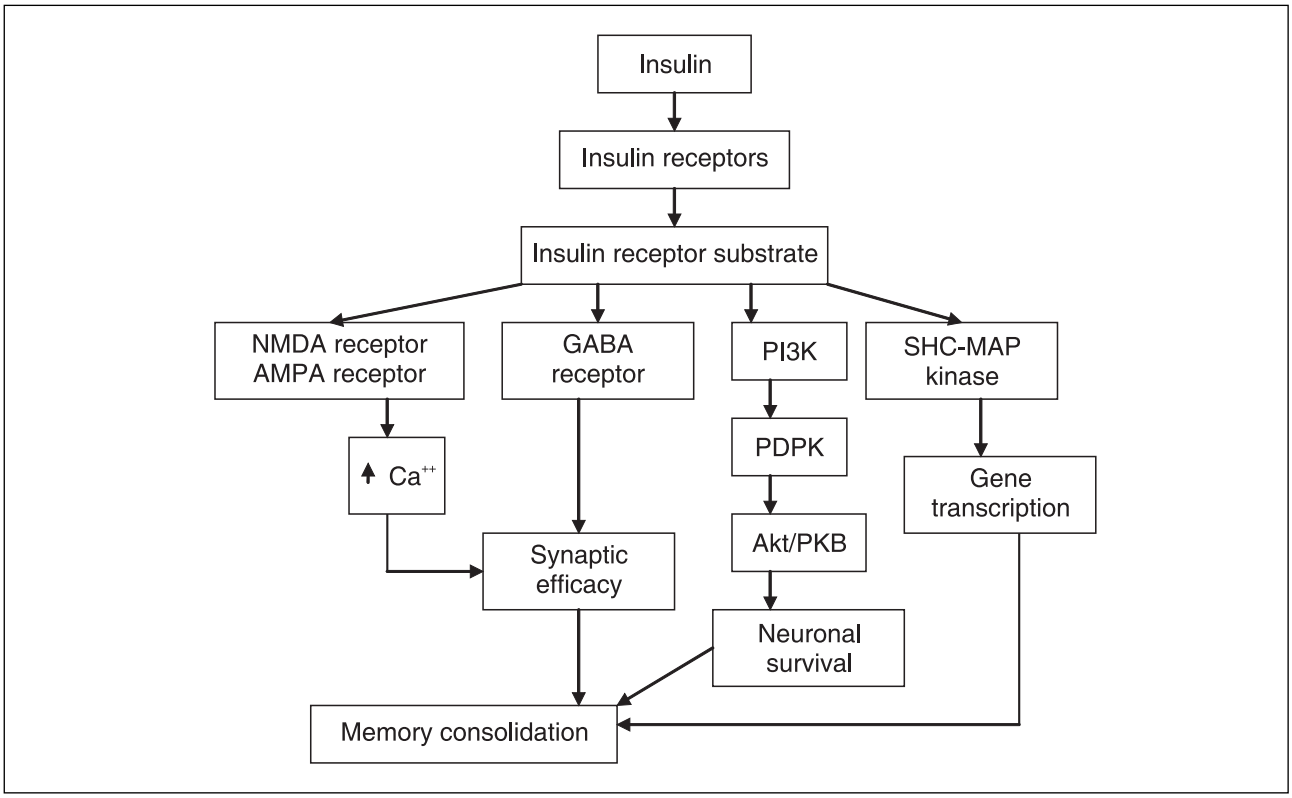


Fig. 1. Hypothetical scheme for insulin/insulin receptor modulation of memory-associated neuronal activities. PDPK, 3-phosphoinositide-dependent protein kinase; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B.

*Impairment in insulin signaling*

Insulin has to be transported across the blood–brain barrier to exert its effects on the brain, bind to cerebral insulin receptors and convey its signal through an intracellular signaling cascade (36). Each of these processes may be affected by diabetes. The transport of insulin across the blood–brain barrier, for example, was shown to be increased in hyperglycemic, hypoinsulinemic rodent models of type 1 diabetes (37), whereas it is decreased in hyperinsulinemic, hyperglycemic rat models of type 2 diabetes (38). The binding of insulin to receptors in brain tissue of hyperglycemic, hypoinsulinemic diabetic animals does not differ from controls (39, 40), whereas it appears to be decreased in the brains of hyperinsulinemic, hyperglycemic rats (41). Insulin signaling may be disturbed both in type 1 and type 2 diabetes, as type 1 diabetes is also associated with some degree of insulin resistance, albeit to a lesser degree than type 2 diabetes (42, 43).

Several mechanisms underlie the potential adverse effects of defective cerebral insulin signaling. Recent data point to changes in the insulin receptor cascade in obesity-related insulin resistance, suggesting that brain insulin receptors also become less sensitive to insulin, which could reduce synaptic plasticity (44). The expression of insulin-1 and -2 mRNA was significantly reduced, in intracerebroventricular streptozotocin (STZ)-treated rats, to 11% in hippocampus and 28% in frontoparietal cerebral cortex, respectively. Insulin receptor mRNA expression decreased significantly in frontoparietal cerebral cortex and hippocampus (16% and 33% of control, respectively). At the protein/activity level, different abnormalities of protein tyrosine kinase activity (increase in hippocampus), total insulin receptor β-subunit (decrease in hypothalamus) and phosphorylated insulin receptor tyrosine residues (increase) became apparent. The STZ-induced disturbance in learning and memory capacities was not abolished by i.c.v. application of GLUT inhibitors known to prevent STZ-induced diabetes. The discrepancy between the reduced insulin receptor gene expression and the increase in both phosphorylated insulin receptor tyrosine residues/protein tyrosine kinase activity may indicate an imbalance between phosphorylation/dephosphorylation of the insulin receptor β-subunit, causing its dysfunction. These abnormalities may point to a complex brain insulin system dysfunction after i.c.v. STZ application, which may lead to an increase in hyperphosphorylated tau protein concentrations. Furthermore, deterioration of insulin receptor signaling appears to be associated with aging-related brain degeneration such as Alzheimer's dementia and cognitive impairment in aged subjects with type 2 diabetes (25). Brain insulin system dysfunction is a possible pathological core in the generation of hyperphosphorylated tau protein as a morphological marker of sporadic Alzheimer's disease and diabetes-induced dementia (45).

One hypothesis is that the link between diabetes and Alzheimer's disease is related to the function of insulin-degrading enzyme (IDE), an enzyme that degrades not only insulin and pancreatic amylin, but also β-amyloid (Aβ). Thus, in diabetics, insulin and Aβ may compete for IDE and this may lead to an increase in Aβ (46). Dou *et al.* (47) investigated changes in long-term memory-associated expression of the insulin receptor and downstream molecules in the rat hippocampus and suggested that insulin/insulin receptor signaling plays a modulatory role in learning and memory processing, which may be compensated for by alternative pathways in the brain when an insulin deficit occurs.

### *Insulin-like growth factors*

Recent literature has addressed the role of growth factors in relation to a variety of neurological diseases, including Alzheimer's disease (48-50). In particular, IGF-1 has been a focus of both biological and epidemiological studies regarding its potential protective role in neurodegenerative diseases (51). The growth factors and their receptors are found in high levels in brain areas implicated as important for memory, including the amygdala, the hippocampus, the parahippocampal gyrus and the cerebellum (52-54). Raising blood IGF-1 levels via parenteral IGF-1 infusions has been shown to reduce brain Aβ in rodents (55, 56) and improve learning and memory in transgenic mice expressing the Alzheimer's disease phenotype (55). In addition, limited epidemiological data, largely from small-scale studies, suggest that higher total IGF-1 levels may be associated with better cognitive performance (57-61) and a lower risk of cognitive decline (62, 63) in older individuals. Nevertheless, bioavailable IGF-1 seems most likely to impact cognition (IGF-1 binds to brain IGF receptors in its free, or unbound, form) (64); thus, free IGF-1 levels, or perhaps the molar ratio of total IGF-1 to its principal binding protein (IGF-binding protein 3 [IGFBP-3]), are likely to be important in determining the full impact of IGF-1 (65). Only one previous study has explored the association between free IGF-1 and cognitive function (63), whereas none have specifically assessed the role of midlife free IGF-1 levels, although a large body of evidence indicates that cognitive decline takes decades to develop, and thus factors earlier in life may have the most significant impact (66, 67).

IGF-1 has effects on neurotransmitter synthetic pathways (68) and neurotransmitter release (53, 69). It can also affect calcium channel activity (70). Morley *et al.* (59) reported that cognitive function in aged humans was correlated with the serum ratio of IGF-1/growth hormone (GH). IGF-1 also stimulates the release of NO in endothelial cells (71), a possible retrograde messenger that has been implicated in the production of LTP (72).

Infusion of IGF-1 into the brain of aged rats improves performance on working memory and object recognition tasks (73). High serum IGF-1 levels were associated with a significant increase in cerebral blood flow in the left premotor cortex during a working memory test (for easier items) and in the left dorsolateral prefrontal cortex (for more difficult items), as measured by positron emission tomography (PET) (74). IGF-1 improves performance in different tests of either short- or long-term memory in rodents. Also, it may be that learning *per se* enhances *local* brain IGF-1, such as has been shown for brain-derived neurotrophic factor (BDNF) (75).

Infusion of an IGF-1 antisense oligonucleotide into the inferior olive impairs learning of the conditioned eye-blink response in rats (76). A perturbed IGF system has been demonstrated in the CNS of STZ-treated rats (77). After 2 weeks of diabetes, IGF-2 mRNA content is significantly decreased in the brain and spinal cord. Insulin replacement partially restores IGF-2 mRNA levels in cerebral cortex, medulla and spinal cord (78). Li *et al.* (79) have systematically examined the IGF system (IGF-1, IGF-2, IGF-1R and insulin receptor) in the BB/Wor model of type 1 diabetes and found significant reductions in the expression of IGF-1, IGF-2, IGF-IR and insulin receptor already in 2-month-old diabetic BB/Wor rats which persisted in 8-month-old diabetic rats, indicating that these abnormalities precede the functional cognitive impairment and the apoptotic neuronal loss in the hippocampus (79).

### *C-peptide*

C-peptide deficiency is a contributing pathogenic factor in type 1 diabetic complications (80-82). In patients with type 1 diabetes, C-peptide improves renal function, reduces urinary albumin excretion and glomerular filtration, and decreases blood–retinal barrier leakage (83-88). Chronic C-peptide replacement prevents functional and structural peripheral nerve changes in type 1 diabetic rat models, suggesting that C-peptide deficiency is a participating factor in the development of type 1 diabetic complications (89-96). C-peptide plays a prominent role in cognitive dysfunction and hippocampal apoptosis in type 1 diabetes (97). It has been shown that administration of C-peptide partially corrects perturbed IGF activity and insulin receptor expression, and partially but significantly prevents neuronal apoptosis in the hippocampus of type 1 diabetic BB/Wor rats, demonstrating a relationship between C-peptide deficiency, insulin action, IGF perturbation and neuronal apoptosis (98).

### *Cerebral GLUTs*

Although glucose is the major nutrient and energy source for brain cells and plays a critical role in brain cognitive functions (25), its uptake, transport and utilization in the majority of brain regions do not depend on insulin. The adult brain appears to express two main glucose transporters (GLUT-1 and GLUT-3) that are not insulin-sensitive. GLUT-1 is expressed in the endothelium of cerebral microvessels and astrocytes, and GLUT-3 is predominantly distributed in neurons (99, 100). *GLUT4* and *GLUT8* gene expression was detected in the hippocampus (101, 102). Insulin-stimulated trafficking of GLUT-4 and GLUT-8 may provide rapid and localized assessment

of neuronal glucose levels and energy homeostasis in the hippocampus and contribute to the rapid fluctuation in hippocampal glucose levels that occurs during task learning. Winocur *et al.* (103) reported that plasma membrane association of the insulin-sensitive glucose transporter GLUT-4 was reduced in the hippocampus of obese rats in the absence of changes in total GLUT-4 and insulin receptor expression. These results parallel those of human studies in pointing to the susceptibility of the hippocampus and related structures to the adverse environment of diabetes.

*Cerebrovascular alterations*

Epidemiological studies of vascular risk factors provide proof of concept that cerebral hypoperfusion is one of the earliest pathological signs in the development of cognitive failure. Vascular risk factors, including heart disease and stroke in elderly individuals who already possess a declining cerebrovascular reserve due to advancing age, contribute to a further decline in cerebral blood flow, resulting in unrelenting brain hypoperfusion. Brain hypoperfusion, in turn, can reach a critical threshold, giving rise to a neuronal energy crisis via reduced ATP synthesis. The ensuing metabolic energy crisis initially carves up ischemia-sensitive neurons in the hippocampus and posterior parietal cortex, leading to cognitive meltdown and progressive neurodegenerative and atrophic changes in the brain (104). Neuronal energy compromise accelerates oxidative stress, aberrant protein synthesis, ionic membrane pump dysfunction, signal transduction impairment, neurotransmitter failure and abnormal processing of amyloid precursor protein, resulting in Aβ deposition and axonal microtubule disruption due to tau hyperphosphorylation. The high-energy metabolic changes leading to oxidative stress and cellular hypometabolism precede the clinical expression of Alzheimer's disease.

Diabetes is associated with both structural and functional alterations of the cerebrovascular system, which increase the risk of stroke (36, 105, 106) and may also affect cognitive functioning. Atherosclerotic disease is the main manifestation of structural alterations of the large extra- and intracranial arteries in diabetic patients (5, 105). Age, duration of diabetes, male gender, triglycerides and nephropathy are important determinants of atherosclerosis, assessed by ultrasonographic measurement of carotid intima–media wall thickness (107, 108). Structural abnormalities at the microvascular level include thickening of capillary basement membranes and decreased capillary density, as has been shown in brain autopsy studies of diabetic patients (5, 109). Functional alterations in the cerebrovascular system that have been associated with type 1 diabetes include regional alterations in cerebral blood flow and disturbances of vascular reactivity. Cerebral blood flow has been reported to be decreased (110), with some degree of regional variation (111). Others, however, report increased cerebral blood flow in diabetic subjects (112), and it has been suggested that the decrease in blood flow that is reported in studies using PET (110) possibly reflects an artifact due to concomitant atrophy (113). Regional cerebral blood flow measurements using neuroimaging techniques can predict diabetes-induced memory deficits and Alzheimer's disease preclinically at the mild cognitive impairment stage, or even before any clinical manifestation of dementia (104).

*Hyperglycemia-induced toxicity*

Hypoglycemia is an alarming and even life-threatening condition, but exposure to chronic hyperglycemia has a greater detrimental effect on the brain than recurrent exposure to severe hypoglycemia (36, 114-116). The active neural response to hyperglycemia induces changes in gene expression and function. The first responses to hyperosmolality are initially adaptive, but later hyperactivation of the hypothalamic magnocellular neurosecretory cells leads to their structural damage. Changes in hippocampal gene transcription are partially implicated in the deterioration of declarative memory. Neurologically passive shunting of excess glucose through alternative cellular metabolic pathways induces atherogenic vascular lesions, free radicals, leukoencephalopathy and atrophy of the brain, leading to cognitive deficits.

Hyperglycemia irreversibly modifies long-lived macromolecules by forming advanced glycation end products (AGEs) as a function of glucose concentration and time (117, 118). Increased amounts of AGEs have been demonstrated in the brain and spinal cord of diabetic rats, albeit at lower levels than in peripheral nerves (119, 120). AGEs have been reported to exert several CNS neurotoxic actions, including increased oxidative stress and acceleration of aging and cross-linking of soluble Aβ peptide (121, 122). Araki *et al.* (123) studied several domains of cognitive function in 198 diabetic patients with a mean age of 74 years. Among the diabetic patients, glycosylated hemoglobin (HbA1c), a marker of hyperglycemia, was associated with cognitive impairment, and short-term glucose control partially improved the cognitive impairment. Serum AGE levels were significantly associated with the impairment of complex psychomotor skills, independent of HbA1c. In conclusion, hyperglycemia and increased AGE accumulation were associated with cognitive impairment in patients with diabetes as a model of accelerated aging (124).

*Oxidative stress*

Both micro- and macrovascular cerebral diseases occurring in diabetic patients and the direct neuronal damage caused by chronically elevated intracellular glucose concentrations are implicated in diabetic encephalopathy. However, it remains unclear to what extent the neuronal impairment is caused directly by intracellular glucose. The direct glucose toxicity in neurons is mainly due to increased intracellular glucose oxi-

dation (125, 126), which leads to an increase in reactive species production (127, 128): in both man and experimentally diabetic rats, oxidative stress seems to play a central role in brain damage (129-131).

Li and Sima (98) demonstrated hippocampal neuronal apoptosis in type 2 hyperinsulinemic and C-peptidemic BBDRZ/Wor rats. Oxidative damage to various brain regions contributes to long-term complications, morphological abnormalities and memory impairment (132-134). The increased oxidative stress in diabetes produces oxidative damage in many regions of rat brain, including the hippocampus. Enhanced formation of oxygen free radicals occurs in tissues during hyperglycemia (135). These oxidant radicals contribute to increased neuronal death through protein oxidation, DNA damage and peroxidation of membrane lipids (136). The activity of superoxide dismutase and catalase, enzymes involved in the antioxidant defense of the brain, appears to be decreased in STZ-diabetic rats (137), and antioxidants such as curcumin, vitamin E and sesamol ameliorated diabetic encephalopathy in STZ-treated rats (132, 133).

*Neurotransmitter modulation*

The most well-known effect of diabetes on the CNS is neurotransmitter dysfunction, which is secondary to metabolic disorders such as hyperglycemia and acidosis (138). Other CNS abnormalities such as neuronal atrophy and axonal degeneration (139, 140) are also associated with diabetes. The altered levels of neurotransmitters in specific brain areas in patients with diabetes and in animals with experimental diabetes have been documented and implicated in CNS disorders (141-143).

1. Cholinergic modulation

Acetylcholine (ACh) output increases in the hippocampus of rats performing a spatial alternation task, and peripheral and hippocampal injections of glucose enhance ACh release along with increasing scores on the behavioral task (21, 144, 145). Similarly, peripheral administration of insulin also acts to increase ACh levels in the amygdala (146), while intracerebroventricular administration increases ACh levels in the midbrain, caudate nucleus and pons medulla (147). Messier has reported that peripheral insulin administration in mice attenuates scopolamine-induced deficits in an operant task (148), suggesting an increase in cholinergic activity. On the other hand, Kopf and Baratti (149) have reported that hyperinsulinemia in mice results in impaired retention of an inhibitory avoidance task and this appears to be mediated by a decrease in cholinergic activity. However, there appears to be an inverted-U dose–effect curve for the glucose effect on memory (150) and ACh release (144), and a difference in dose–effect may explain the apparent contradiction.

Very recently it was reported that a few natural agents, such as ginseng, oleanoic acid and Hon Chi, have the ability to increase the release of ACh from nerve terminals, which in turn stimulates muscarinic $M_3$ receptors in pancreatic cells and increases insulin release, resulting in plasma glucose lowering (151-153). In parallel to the reduction in plasma glucose, an increase in plasma levels of insulin or C-peptide was also observed. Moreover, disruption of synaptically available ACh using an inhibitor of choline uptake, hemicholinium-3, or an inhibitor of vesicular acetylcholine transport, vesamicol, abolished these actions. Physostigmine at concentrations sufficient to inhibit acetylcholinesterase enhanced the actions of these substances on ACh release from the nerve terminals to enhance insulin secretion. Both the plasma glucose-lowering action and the raised plasma levels of insulin and C-peptide induced by these agents were also inhibited by 4-diphenylacetoxy-*N*-methylpiperidine methiodide, but were not affected by the ganglionic nicotinic antagonist pentolinium or hexamethonium, indicating the involvement of muscarinic $M_3$ receptors (151-153). It can be concluded that enhancement of ACh secretion can improve learning and memory deficits associated with diabetes.

2. Glutamatergic modulation

Abnormal regulation of glutamate receptors appears to play an important role in diabetes-induced impairment of synaptic plasticity and may therefore contribute to the development of cognitive deficits in diabetic patients. Trudeau *et al.* (154) discussed the possibility that deficits in LTP during chronic diabetes might arise from dysfunction of the NMDA subtype of glutamate receptors in early stages of the disease. Biochemical experiments in nonobese diabetic mice suggest that upregulation of NMDA receptors is associated with the early stages of diabetes. There is, of course, a need for further studies on how these changes in NMDA receptor properties may accentuate glutamate toxicity. Preliminary investigations using experimental models of type 2 diabetes and NMDA receptors provide evidence that hyperinsulinemia might be able to limit NMDA-mediated toxicity.

In *Xenopus* oocytes expressing NMDA receptors, a brief insulin exposure triggered a rapid and significant potentiation of responses to NMDA mediated by NMDA receptor subtypes (155, 156). This insulin-induced potentiation was blocked by the protein tyrosine kinase inhibitor genistein and the broad-spectrum protein kinase inhibitor staurosporine, suggesting the involvement of tyrosine and possibly downstream serine/threonine protein kinases such as PKC. In a similar experiment, Skeberdis *et al.* (157) 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 the function of SNAP-25 (synaptosomal-associated protein 25), but does not appear to require tyrosine and serine/threonine phosphorylation at the NMDA receptor *C*-terminus. Another 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 (158). Given the important role that NMDA receptors may

play in synaptic plasticity, learning and memory formation (159, 160), modulation of NMDA transmission may represent one of the synaptic bases for the role of insulin/insulin receptor signaling in learning and memory.

In addition, insulin plays a role in synaptic plasticity by acting on AMPA receptor trafficking. Redistribution of AMPA receptors has been proposed to regulate the strength of glutamatergic synapses. A mature synaptic connection at glutamatergic synapses in the brain requires conversion of silent glutamatergic synapses to functional synapses during the course of postnatal brain development (161, 162). A silent glutamatergic synapse that mediates only NMDA transmission is not functional unless AMPA receptors are delivered to such synapses (163, 164). Conversion of a silent synapse to a functional synapse can be both development- (161, 162) and activity-dependent (163, 164), which has been hypothesized as the synaptic basis for learning and memory formation. In cultured differentiating neurons, insulin promoted the transfer of silent AMPA synapses to functional synapses and accelerated the reduction of silent synapses (165). 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 (166).

3. GABAergic modulation

Insulin-mediated receptor trafficking has also been found in the GABA receptor, which mediates synaptic inhibition important for neuronal functions associated with learning (25, 33, 167, 168). When applied to HEK 293 cells transfected with the $GABA_A$ receptor, insulin caused rapid translocation of $GABA_A$ receptors to the plasma membrane (169). Insulin also recruited functional $GABA_A$ receptors to the postsynaptic and dendritic membranes of CNS neurons, leading to increased amplitudes of the $GABA_A$ receptor-mediated miniature inhibitory postsynaptic current (169). Furthermore, insulin activation of muscarinic transmission-potentiated GABA receptor currents likely occurs via a PI3K-dependent mechanism (170). 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 modifications in synaptic connections required for higher brain functions such as learning and memory.

## Conclusions

The escalating diabetes epidemic, together with its neurological consequences, may have crucial socioeconomic ramifications. Diabetes itself is not a neurological disease, but ensuing hyperglycemia exerts an adverse impact on brain function and cognition. Insulin deficiency, reduced insulin sensitivity, impairment of insulin-sensitive neurotransmitter modulation, cerebrovascular alterations and oxidative stress render neurons susceptible to neurotoxic insults, with resulting neurodegeneration and cognitive decline (Fig. 2). Research in recent years has significantly advanced our knowledge regarding physiological as well as pathological roles of

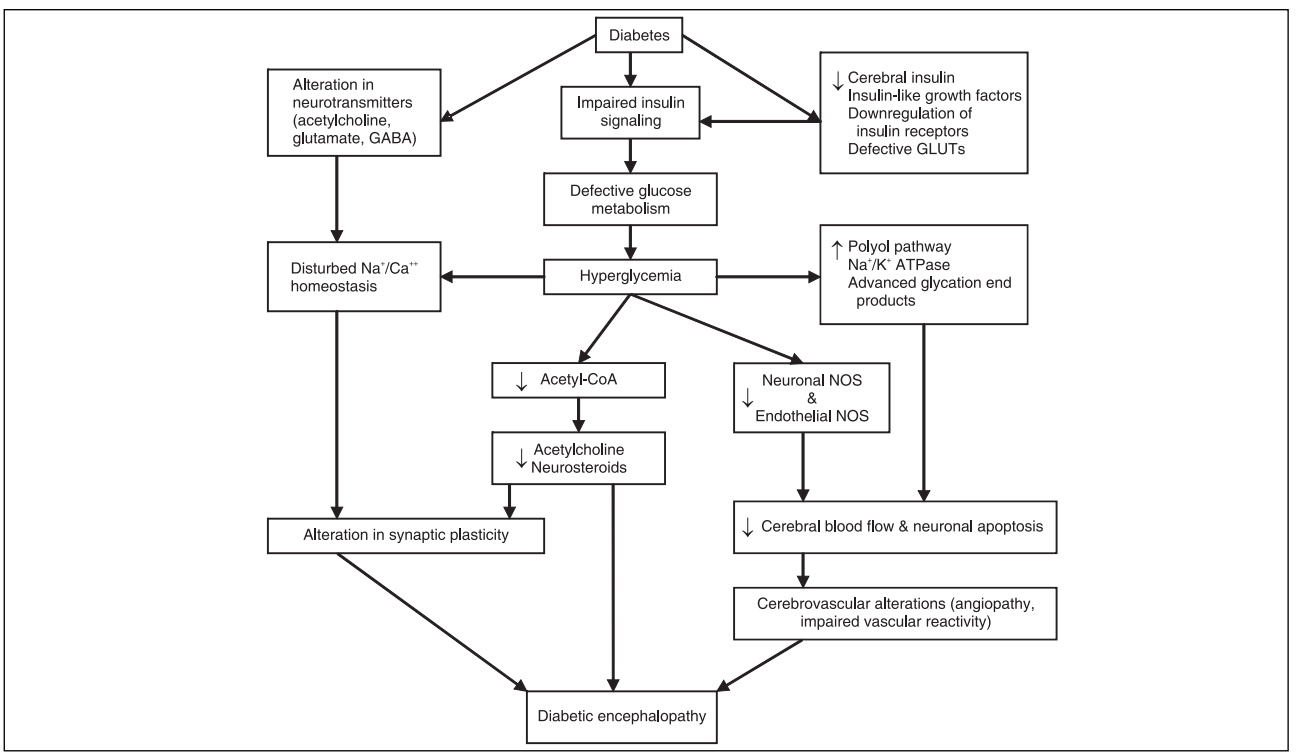


Fig. 2. Schematic presentation of the neurobiology of diabetic encephalopathy.

insulin/insulin receptor in diabetes-associated neuropsychiatric dysfunction, especially learning and memory deficits. However, future in-depth studies will be necessary to unravel the complex interactions among various pathogenic factors, and this may foster the development of preventive measures or treatment strategies to restore these deficits.

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**Disclosure**

The authors claim no conflicts of interest.

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