

Sequential Abnormalities in Type 1 Diabetic Encephalopathy and the Effects of C-Peptide

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■ Abstract

Diabetic encephalopathy is a recently recognized complication in type 1 diabetes. In this review, we summarize a series of experimental results obtained longitudinally in the spontaneously type 1 diabetic BB/Wor-rat, and bringing out the beneficial effects of C-peptide replacement. It is increasingly clear that lack of insulin and C-peptide, and perturbations of their signaling cascades in type 1 diabetes are detrimental to the regulation of neurotrophic factors and their receptors. Other consequences of such deficits and perturbations are innate inflammatory responses with effects on synaptogenesis, neurite degeneration, and early behavioral abnormalities. Replacement of C-peptide, which does not effect hyperglycemia, has beneficial effects on a variety of pro-apoptotic stressors, oxidative stressors, and finally on apoptosis. Even-

tually, this cascade of events leads to neuronal loss and decreased densities of white matter myelinating cells, with more profound deficits in behavioral and cognitive function. Such changes are likely to underlie gray and white matter atrophy in type 1 diabetes, and are significantly prevented by full C-peptide replacement. Present data demonstrate that C-peptide replacement has beneficial effects on numerous sequential and partly interrelated pathogenetic mechanisms, resulting in prevention of neuronal and oligodendroglial cell loss, with significant prevention of neurobehavioral and cognitive functions.

Keywords: type 1 diabetes · C-peptide · encephalopathy · BB/Wor-rat · inflammation · hyperglycemia · neuronal loss · hippocampus · synaptic connectivity · cerebral atrophy · intracerebral insulin signaling · gray matter density

Introduction

Diabetes is an increasingly common metabolic disorder with well known serious secondary complications affecting kidney, retina, peripheral nerve and vasculature. Diabetic peripheral neuropathy (DPN) was long considered the only complication involving the nervous system. Whereas, the central nervous system (CNS) was believed to be relatively spared from the direct effects of diabetes. In the last decade, it has

become clear that diabetes may be both primarily and secondarily responsible for CNS complications, with adverse functional and cognitive effects. Primary diabetic encephalopathy is caused by direct metabolic perturbations due to hyperglycemia, insulin deficiency, or hyperinsulinemia. Secondary diabetic encephalopathy occurs as a result of micro- and macrovascular disorders, or severe and repeated episodes of hypoglycemia [1-3].

Primary diabetic encephalopathy affects both type 1 and type 2 diabetes. However, as in the

DPN occurring in the two types of diabetes, the underlying mechanisms seem to differ, and the cognitive outcomes are different in the two types of diabetes [2, 4].

In type 2 diabetes, several factors predispose to cognitive impairment such as hyperglycemia, insulin resistance, hyperinsulinemia, and age alone

Abbreviations:

8-OHdG - 8-Hydroxy-2'-deoxyguanosine
 ADP - adenosine diphosphate
 AGE - advanced glycation end products
 AMP - adenosine monophosphate
 cAMP - cyclic adenosine monophosphate
 AMPA - α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
 Bax - Bcl-2-associated X protein (promotes apoptosis)
 BB/Wor rat - BioBreeding/Worcester rat (animal model of spontaneous autoimmune diabetes)
 Bcl-2 - B-cell lymphoma 2
 BDNF - brain-derived neurotrophic factor
 Ca²⁺ - bivalent ionic calcium
 CA1 - *Cornu Ammonis* area 1 (also known as Ammon's horn)
 CNS - central nervous system
 C-peptide - connecting peptide
 CREB - cyclic AMP response element-binding protein
 DNA - deoxyribonucleic acid
 DPN - diabetic peripheral neuropathy
 EEG - Electroencephalogram
 Fas - transmembrane protein of the TNF family that induces apoptosis (also known as CD95L)
 FDG - ¹⁸F-fluorodeoxyglucose
 GFAP - glial fibrillary acidic protein
 GluR - postsynaptic glutamate receptor
 IGF - insulin-like growth factor
 IL - interleukin
 IQ - intelligence quotient
 MRI - magnetic resonance imaging
 Na⁺/K⁺-ATPase - sodium, potassium adenosinetriphosphatase, also sodium-potassium pump
 NGF - nerve growth factor
 NGFR-p75 - nerve growth factor receptor p75
 NGF-TrA - nerve growth factor tyrosine kinase A
 NF- κ B - nuclear factor-kappa light-chain enhancer of activated B cells
 PARP - poly (ADP-ribose) polymerase
 p-Akt - phosphorylated akt
 p-GSK-3 β - glycogen synthase kinase-3 beta phosphorylated on serine 9 (also known as phosphorylated stress kinase)
 RAGE - receptor for advanced glycation end products
 rMRG1c - regional metabolic rate of glucose 1c
 STZ - streptozotocin
 TNF α - tumor necrose factor alpha
 Trk - tropomyosin receptor kinase (also known as tyrosine kinase)
 TrkA - tyrosine kinase A (highly affine to the NGF receptor)
 TUNEL - terminal deoxynucleotidyl transferase (Tdt)-mediated dUTP-biotin nick end labeling

[2, 4, 5]. Additional components of the commonly associated metabolic syndrome, such as elevated cholesterol levels and hypertension, are likely to provide significant contributing factors [2, 4, 5]. Several studies have demonstrated an association between type 2 diabetes and Alzheimer's disease [6-11]. Such an association has also been corroborated in an animal model of type 2 diabetes [12].

Several neurobehavioral studies in children with type 1 diabetes have demonstrated deficits in attention, processing speed, executive function, intelligence, and memory. Such deficits translate into lower IQs, worse school performances, and greater likelihood to fail grades [13-17]. In one study, early onset of diabetes correlated with lower IQ performance, and lower full scale IQ. In contrast, a positive history of hypoglycemic episodes correlated with lower verbal IQ [18]. It has been recognized repeatedly that early onset of diabetes leads to worse neuropsychological outcomes [14, 15, 19]. Also, males are more vulnerable than females [15, 20]. In contrast to earlier beliefs, most studies do not seem to associate cognitive dysfunctions with episodes of hypoglycemia due to intensive insulin treatment [21-23].

Neurostructural deficits in type 1 diabetic patients

Recently, neuroimaging studies of type 1 diabetes patients, have reported increased glucose levels in frontal white matter and cortex. These findings were associated with increased concentrations of myoinositol, and decreased levels of N-acetylaspartate [24]. Structural studies of patients with onset of diabetes before the age of six have revealed a high incidence of mesial temporal lobe sclerosis, which was not associated with a history of hypoglycemia [25]. Similarly, volumetric MRI assessments of patients with type 1 diabetes for 12 years showed significantly decreased gray matter volumes in thalami, hippocampal regions, insular cortex, and decreased white matter volumes in parahippocampus, temporal lobe, and in the middle frontal areas [18]. Voxel-based morphometry of a type 1 population with 15-25 years duration of diabetes demonstrated decreased gray matter densities of thalamus, superior and middle temporal gyri, and middle frontal gyri [26]. Based on these recent studies, it appears that cerebral atrophy does occur, and that limbic temporal structures and cortices are preferentially involved.

Only few neuropathological reports have described structural abnormalities. A recent study of

two young patients, who succumbed to the complications of ketoacidosis, reported severe neuronal loss in hippocampus and frontal cortex [27]. This was associated with white matter atrophy in the frontal and temporal regions, and was consistent with neuroimaging data. Such pathologies are likely to correlate with deficits in various cognitive domains, such as memory, information processing speed, executive function, attention and motor speed. Interestingly, deficits in such cognitive functions are associated with impaired functional connectivity, a measure of functional interactions between brain regions [28] and loss of fast β -frequency bands on quantitative EEG examinations [29]. These changes did not correlate with previous episodes of hypoglycemia.

Therefore, it is becoming accepted that type 1 diabetes leads to deficits in various cognitive domains associated with structural gray matter deficits, particularly in limbic structures and white matter atrophy. Such abnormalities appear to be more prevalent in patients with early onset of disease.

Experimental studies in animal models

Early studies in streptozotocin-induced diabetes in rats demonstrated neuronal loss in frontal cortex and white matter deficits [30]. Functional studies using the Morris water maze paradigm have demonstrated neurobehavioral deficits [31], which were prevented with insulin treatment from onset of diabetes [32]. Deficits in water maze learning were associated with impaired hippocampal long-term potentiation as a measure of synaptic plasticity [31]. This was also prevented by insulin treatment [32]. However, intervention with insulin to normalize hyperglycemic lev-

els did not reverse water maze learning deficits, but it had a partial effect on long-term potentiation [32]. These data suggest that both pre- and post-synaptic activities are affected in hippocampus [33].

Recent data obtained from streptozotocin-induced diabetes in Swiss Webster wild-type mice show cerebral atrophy associated with cognitive decline over time [34]. However, in this model the cerebral atrophy was not associated with neuronal loss, but was ascribed to atrophy of the white matter. One factor in this study associated with white matter atrophy was upregulation of the receptor for advanced glycation end products (RAGE) [35]. This occurred together with downregulation of insulin signaling intermediaries, transcription factor cyclic adenosine monophosphate (cAMP), response element binding protein (CREB), and synaptophysin. Interestingly, administration of intranasal insulin to this type 1 diabetic murine model, slowed the development of neurobehavioral deficits, ameliorated white matter atrophy, and corrected insulin signaling intermediaries [35]. These

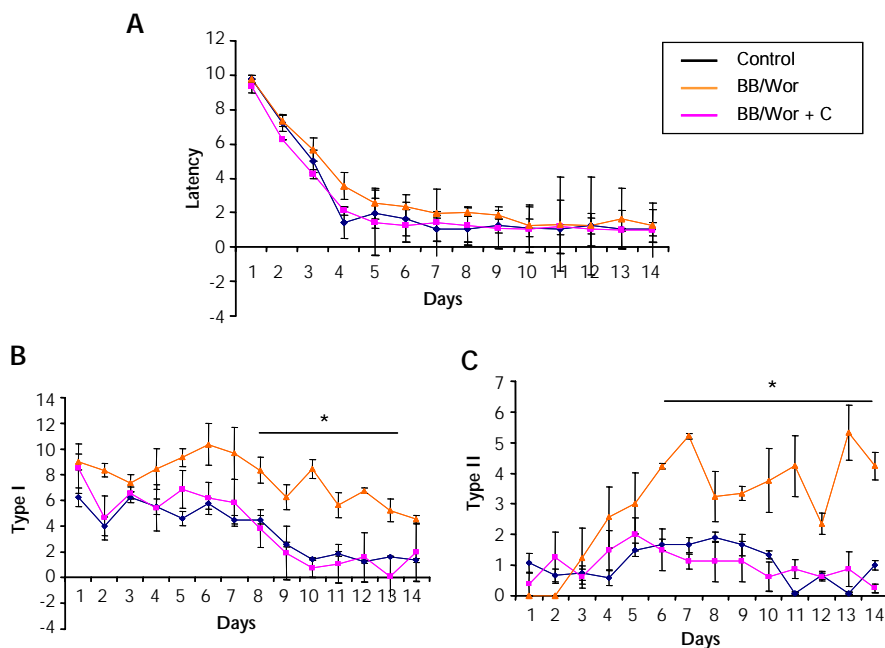


Figure 1. Results from behavioral testing of 2-mo control, diabetic, and C-peptide replaced diabetic BB/Wor-rats using the radial arm paradigm. **A:** Total latencies were not altered in diabetic or C-peptide-treated diabetic rats as compared to age-matched control animals. Type I (**B**) type II (**C**) errors were significantly more common in non-treated diabetic rats, signifying impaired reference and working memory, respectively. These error types were not recorded in C-peptide-treated diabetic rats (**B** and **C**).

results are consistent with, and strongly support, our previous data suggesting that insulin deficiency *per se* may play a pivotal role in the development of cognitive dysfunction in type 1 diabetes [1, 36].

In this review, we describe the sequential and longitudinal findings of cognitive deficits, metabolic, molecular, and structural abnormalities occurring in the encephalopathy in the BB/Wor-rat. This is a robust model that closely simulates human type 1 diabetes, which occurs spontaneously and secondary to an immune-mediated β -cell loss, with complete insulin and C-peptide deficiency [37]. Also, the model has been used widely to explore mechanisms underlying other type 1 diabetic complications [38]. For information regarding the metabolic profile of this model, the reader is referred to the accompanying paper on DPN in this issue [39].

The effect of C-peptide on early functional and neurotrophic deficits

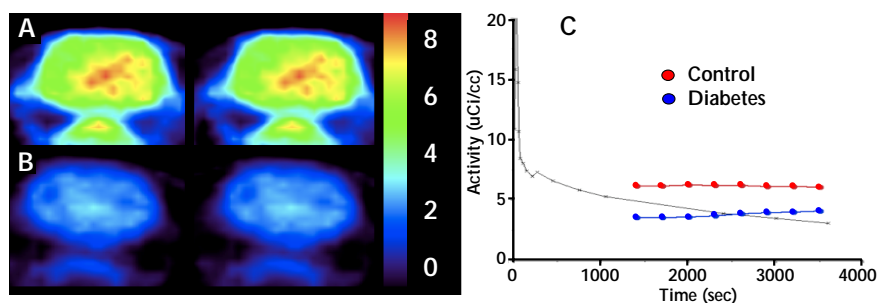
Changes in cerebral-evoked potentials reflected by somatosensory, visual, and auditory evoked potentials, occur in both STZ-induced diabetic rats and in the BB/Wor-rat. These changes are followed by progressive degenerative changes in the

dorsal columns of the spinal cord and the optic nerve [3, 38, 40, 41]. Such changes can be modified by insulin treatment [3].

Various neurobehavioral tests can be applied to rodents to examine different spheres of cognition. One paradigm that we have used for testing the BB/Wor-rat is the radial arm maze [42]. This consists of eight radial arms, of which three are baited with food and associated with visual cues. The animals are trained with three consecutive trials per day, over a period of one week. The setup has an integrated video camera to record latencies, type I and type II errors. Latency is the time it takes to retrieve all baits. Type I error is entrance into an arm without a bait (reference memory), and type II error is recorded when entering an arm from which the bait was already collected (working memory) (Figure 1). This paradigm has been closely related to hippocampal activity, long-term potentiation, and mossy fiber pathology [42-44]. Testing of 3-month diabetic BB/Wor-rats using this paradigm showed significantly increased frequencies of type I and type II errors, whereas the latencies were unaltered. Diabetic rats replaced with full substitution of C-peptide from onset of diabetes showed normal frequencies of type I and type II errors at the same duration of diabetes (Figure 1). This demonstrated

that deficits were preventable in both reference and working memories for spatial orientation abilities.

As previously shown, the findings suggest that correction of impaired insulin function alleviates early behavioral abnormalities, and long-term potentiation [32]. The behavioral abnormalities were preceded by impaired insulin signaling activities [36], and a 3-fold increase in glucose uptake in hippocampi of 2-month diabetic rats (Figure 2). At the same duration of diabetes, both the expression of the insulin-receptor, IGF-1-receptor, and



Region	K-cplx (min ⁻¹)			rMRGlc (umol/100g/min)		
	control	diabetic	D	control	diabetic	D
Cortex	0.0164 (5)	0.0111 (8)	-32%	17.95 (3)	51.12 (9)	283%
Hippocampus	0.0173 (9)	0.0136 (6)	-22%	19.08 (4)	62.15 (7)	326%
Whole brain	0.0166 (5)	0.0123 (6)	-26%	18.20 (3)	56.43 (9)	307%

Figure 2. Micro-PET data from three 3-month diabetic BB/Wor-rats (A) and age-matched control rats (B). The uptake rate constant of the FDA tracer (K-cplx) was decreased by 22-32% in diabetic rats as compared to control rats (C and Table). Despite this, the metabolic rate of glucose (rMRGlc) was increased approximately 3-fold in cerebral cortex, hippocampus, and whole brain (Table).

NGF and NGF-TrA receptor were significantly suppressed in diabetic BB/Wor-rats (Figure 3). On the other hand, IGF-I expression was only modestly suppressed, whereas that of IGF-II was severely decreased to about 30% of that of control rats (Figure 3). These abnormalities were significantly, if not fully, prevented in C-peptide replaced rats (Figure 3). Simultaneously, insulin signal intermediaries such as p-Akt and p-GSK-3 β were significantly decreased and increased respectively. These abnormalities were significantly prevented in C-peptide-replaced diabetic rats. Thus, C-peptide replacement corrects the insulin signaling cascade in type 1 diabetic hippocampus.

Both insulin and NGF provide potent neurotrophic support in hippocampus [45, 46]. Insulin is closely involved in neurotransmitter synthesis including acetylcholine and glutamate [45], and NGF secretion exerts a protective effect of cholinergic neurons [46, 47]. Interestingly, IGF-II expression was markedly suppressed in diabetic rats, which was largely prevented following C-peptide supplementation. On the other hand, IGF-I expression was only mildly decreased. It is known that endogenous IGF-I inhibits hippocampal acetylcholine release, whereas IGF-II has the opposite effect [48, 49].

Although not examined in the BB/Wor-rat, brain-derived neurotrophic factor (BDNF) is known to modulate pre- and post-synaptic transmission and plasticity [50]. The early perturbations of trophic factor activities were followed by presynaptic degeneration in hippocampi of 4-month diabetic rats. This was demonstrated by decreased synaptophysin expression, and decreased numbers of presynaptic terminals in strata oriens of Ammon's horn area 1 (CA1), a region rich of pyramidal cells. The degenerative changes of presynaptic terminal neurites, and expression of synapto-

physin, were fully prevented by C-peptide replacement (Figure 4). Therefore, it is highly likely that the perturbations of various neurotrophic factors, including insulin itself, are involved in neurite degeneration, and abnormalities in hippocampal plasticity. This is evidenced by suppressed long-term potentiation [31, 32], and spatial learning defects, as shown here (Figure 1). Importantly, full replacement of C-peptide, which exerts insulin-like effects [51], prevents this series of early degenerative events.

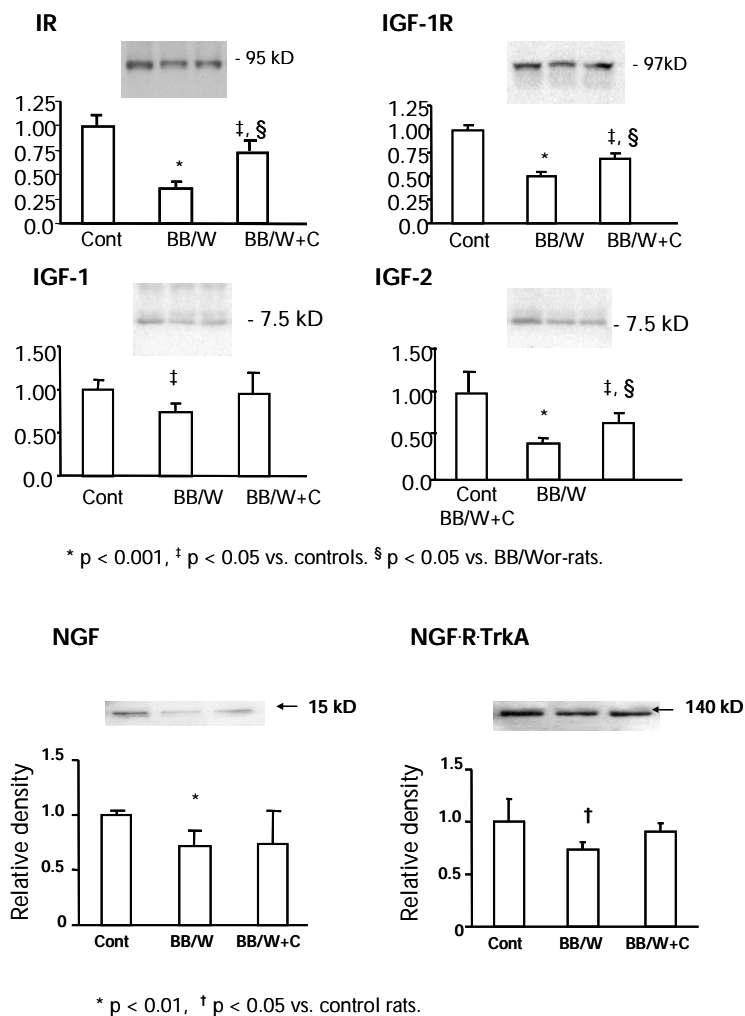


Figure 3. Neurotrophic profiles in hippocami of 2-month diabetic and C-peptide treated rats (IR, IGF-R, IGF-1 and IGF-2). Note the significant suppression of the expression of these factors and receptors in diabetic rats, abnormalities that were significantly prevented by C-peptide replacement. Also, NGF and its receptor Trk A showed decreased expression in hippocampi of 8-month diabetic BB/Wor-rat with modest prevention in C-peptide treated rats. Equal loading of lanes was evaluated by staining of membranes with Ponceau reagent.

Glucose metabolism, prevention of RAGE, and innate inflammatory responses by C-peptide

The diabetic BB/Wor-rats were maintained at high glycemic levels, but free of ketonuria. Blood glucose levels were not influenced by C-peptide treatment [36, 39]. MicroPet imaging of diabetic BB/Wor-rats revealed an approximate 30% decrease in the uptake rate constant of ^{18}F -fluorodeoxyglucose (FDG) into hippocampus and cerebral cortex (Figure 2). However, the regional metabolic rate of glucose (rMRG1c) showed a three-fold increase in whole brain, as well as in hippocampus and cerebral cortex (Figure 2). These data are consistent with those reported in humans [24]. Increased cerebral glucose was associated with an increased expression of RAGE [49]. In the hippocampus, RAGE was mainly colocalized with glial fibrillary acidic protein (GFAP)-positive proliferating astrocytes. To a lesser extent it was lo-

calized with hippocampal pyramidal cells and white matter oligodendrocytes [52]. These findings are similar to those reported in studies of brains of human type 1 diabetes subjects [27], and in a STZ-induced diabetic mouse model [34]. RAGE is a multi-ligand receptor for AGE, formed as a consequence of elevated glucose or oxidative stress. It is also a receptor for β -amyloid, and even prion proteins [53]. RAGE activation stimulates NF- κ B, which is upregulated in the hippocampus of BB/Wor-rats. It should be mentioned that increased expression of NF- κ B also occurs as a consequence of impaired insulin signaling via phosphorylation of I κ B [53-55].

Interestingly and unexpectedly, C-peptide replacement prevented the upregulations of RAGE and NF- κ B in diabetic hippocampi [52]. NF- κ B is also known to upregulate RAGE itself, thereby providing a self-perpetuating loop. These data suggest that upregulation of RAGE and its response to C-peptide supplementation may largely be due to an impaired insulin signaling mechanism [51, 54, 57] rather than a downstream effect of hyperglycemia. RAGE was localized to activated astrocytes, which together with activated microglia play central roles in the activation of inflammatory mediators. Upregulation of RAGE and NF- κ B, was accompanied by upregulation of TNF- α , IL-1 β , IL-2, and IL-6. Whereas, the anti-inflammatory interleukin IL-10 was downregulated [52, 58]. Consequent to the normalization of RAGE and NF- κ B after C-peptide replacement, TNF- α as well as the pro- and anti-inflammatory interleukins were normalized in the hippocampus [52, 58]. As discussed, this cascade of partly interactive and self-perpetuating activities of inflammatory factors 1. affect the already compromised insulin-signaling cascade [35], 2. enhance oxidative stress, and 3. promote apoptotic stress.

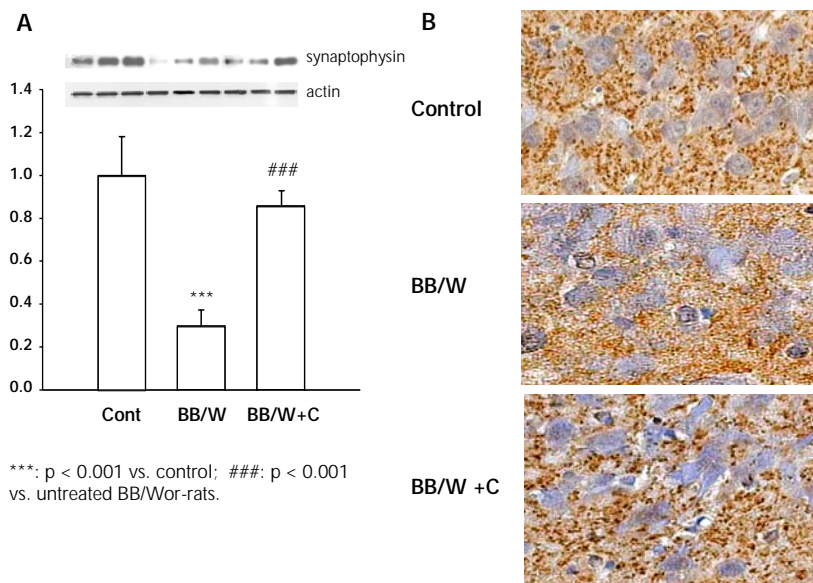


Figure 4. A: The expression of hippocampal presynaptic synaptophysin was significantly decreased in 4-month diabetic rats, and was prevented by C-peptide replacement from onset of diabetes. **B:** This corresponded to a decreased density of synaptophysin stained synapses in the CA1 region of diabetic rat hippocampi, suggesting presynaptic degeneration. The normal density of presynaptic buttons was preserved in C-peptide treated rats.

The effect of C-peptide on late cognitive function and structural deficits

As mentioned above, deficits in reference and working memory occur relatively early after onset of diabetes. Longitudinal testing in the Morris water maze system [59] revealed normal performances in diabetic rats of 4-month duration of diabetes. Significant deficits in latencies were evident only after 6-month of diabetes (Figure 5). At 8-month duration of diabetes, C-peptide replacement from onset showed significant preventative effects on Morris water maze latencies [1, 36] (Figure 5). Multiple cognitive components are involved in this task such as problem solving, formation of internal representation of the environment, and storage and retrieval of relevant information [1, 59]. The data indicate that progressive learning and memory deficiencies occur in a duration-related fashion, and are significantly prevented by C-peptide. Such changes are indicative of altered synaptic plasticity and cognitive deficits. They can be associated with depression of long-term potentiation, and enhanced long-term depression [31, 32, 60].

Seven-month diabetic rats showed increased expression of the post-synaptic glutamate receptor 2 (GluR2) and 4 (GluR4) subunits (Figure 6), of which GluR2 is the most prevalent of the subunits in the fore-brain and hippocampus [61, 62]. The GluR2 subunit controls Ca^{2+} permeability of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor channels, and plays a crucial role in several forms of long-term synaptic plasticity [63]. Increased presence of GluR2 decreases, or abolishes, Ca^{2+} permeability [64]. C-peptide replacement fully prevented the change in GluR2, and partially prevented that of the GluR4 subunit (Figure 7). Future stud-

ies have to explore whether these changes relate to changes in long-term depression. At this stage of the encephalopathy, hippocampal pre-synaptic synaptophysin remained significantly suppressed in both hippocampus and frontal cortex, and was significantly, but not fully, prevented by C-peptide (data not shown). These findings will inevitably have profound effects on our understanding of the intrinsic hippocampal neuronal networking. Therefore, it is apparent that numerous factors emanating from impaired insulin-signaling, and possibly hyperglycemia, lead to sequential and progressive behavioral deficits, perturbations in expression of neurotrophic factors, and alterations in synaptic connectivity.

Both insulin and C-peptide display strong anti-apoptotic effects. In previous studies [1, 36, 54, 65], we have shown that insulinopenic diabetes in the BB/Wor-rat is associated with upregulation of a number of pro-apoptotic factors such as NGFR-p75, Fas, Bax, PARP, oxidative stress-induced DNA damage, caspase 12, and active caspase 3 expression [36, 65, 66]. Activation of such stress-

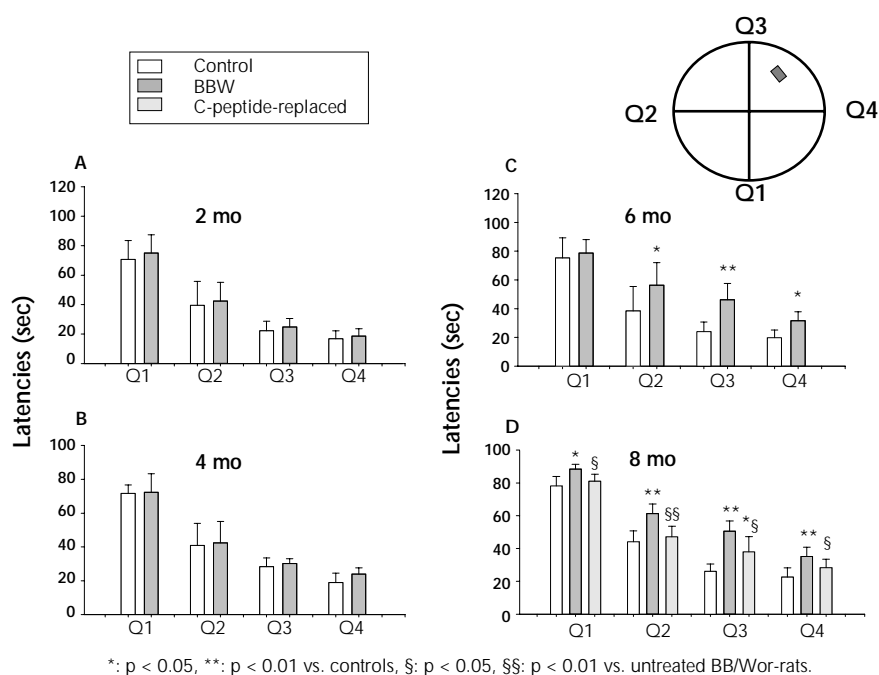


Figure 5. Latencies in the Morris water maze set up (right upper corner) in control and diabetic BB/Wor-rats at 2 (A); 4 (B); 6 (C), and 8 months (D) of diabetes. Q₁-Q₄ signify the four quadrants of the water maze. Note, significantly prolonged latencies are seen only after 6 months of diabetes (C). Diabetic rats treated with C-peptide from onset of diabetes showed significant prevention in the latencies at 8 months (D), suggesting prevention of spatial memory and long-term memory deficits.

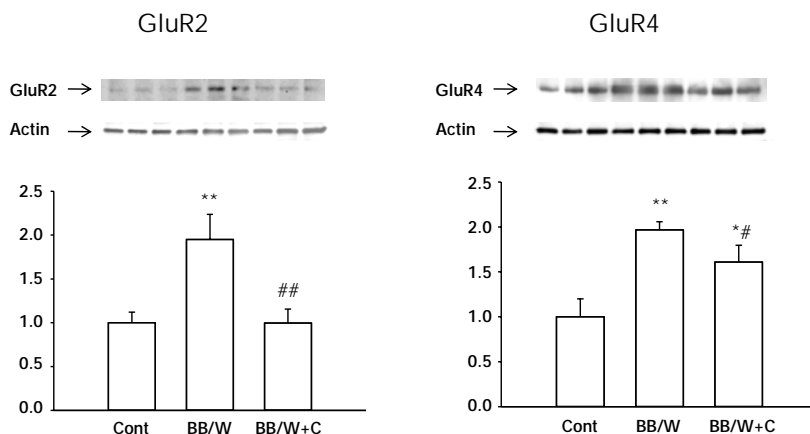


Figure 6. Expression of post-synaptic excitatory GluR2 and GluR4 in hippocampus were both significantly increased in 7-month diabetic BB/Wor-rats. C-peptide substitution from diabetes onset fully prevented the more abundant GluR2 subunit, whereas the effect was less striking with respect to the less abundant GluR4.

ors was accompanied by increased TUNEL staining of hippocampal pyramidal cell neurons, increased DNA laddering of hippocampus, and decreased density of pyramidal cell neurons, particularly in the CA1 region [36]. C-peptide replacement for the duration of 8 months of diabetes, significantly prevented pro-apoptotic factors (except for Fas), partially prevented hippocampal pyramidal cell loss, and normalized associated behavioral deficits, as reflected by the latencies of the Morris water maze [36] (Figure 5).

These findings underline the pivotal role of impaired insulin signaling activities in the development of type 1 diabetic encephalopathy, which are normalized by C-peptide substitution [36, 51]. Recently, this concept was supported by the beneficial effects of direct intranasal insulin delivery to the CNS, which was observed to influence cerebral atrophy, cognitive deficits, and intracerebral insulin signaling intermediaries in STZ-diabetic mice [35]. Neither intranasal insulin administration, nor C-peptide replacement, alter systemic hyperglycemia. These results may shed light on mechanisms underlying hippocampal and cortical neuron loss in human type 1 diabetes [27].

White matter changes in the BB/Wor-rat

Preliminary studies have revealed early deficits in temporal white matter consisting of in-

creased RAGE expression, and pro-inflammatory TNF- α and IL-6. RAGE colocalized with GFAP-positive proliferating astrocytes (Figure 7). These changes were associated with increased expression of Bax, cleaved poly-ADP-ribose polymerase (PARP), and increased expression of active caspase 3 in white matter. As an indicator of oxidative DNA damage, there was increased stainability of 8-OHdG in white matter oligodendrocytes. Quantitative analyses of myelinating oligodendroglia cells and white matter astrocytes, demonstrated a significant decrease in oli-

godendroglia cell densities in 2-month diabetic rats. A progressive increase was seen in the density of astrocytes from 2- to 8-month duration of diabetes (Figure 7). These changes were significantly prevented by C-peptide replacement, and interestingly, partly reversed by C-peptide treatment between 4 and 7 months of diabetes. These preliminary data suggest that factors similar to those operative in gray matter structures also affect the myelinating white matter. Furthermore, it appears that the white matter deficits, such as loss of myelinating oligodendroglia, occur earlier than the neuronal deficits described in hippocampus [34, 35]. One may speculate that this apparent difference in susceptibility to the metabolic insults caused by insulin and C-peptide deficiencies, may relate to the later occurring maturation of white matter structures, both in rats and humans [67].

Summary and conclusions

It is now established that type 1 diabetes may result in intellectual and cognitive deficits. In this regard, the timing of diabetes onset is critical, since it appears that a developing brain is more vulnerable to the deleterious effects of diabetes than a developed brain [68, 69]. Given that the type 1 diabetic population is globally increasing, and the age of diabetes onset is becoming progressively younger, patients will be more frequently affected at ages when the brain is still developing [70-72].

As outlined in this review, it is apparent that the abnormalities underlying type 1 diabetic encephalopathy are complex, and not well understood. It is clear though that hyperglycemia and its consequences, like non-enzymatic glycation and oxidative stress, are playing important pathogenetic roles, as in other complications. However, recently it is being increasingly recognized that impaired insulin action plays an equally important role, and that C-peptide replacement may be important in correcting the defects caused by insulin deficiency. The potential mechanisms by which C-peptide exerts its insulin-like effects are detailed in the accompanying review on the effect of C-peptide on diabetic polyneuropathy. Insulin replacement alone is not likely to fully normalize these defects. Therefore, type 1 diabetes appears to be a defect in the interaction between two failing hormones, insulin and C-peptide [73].

As demonstrated in this review, diabetic encephalopathy evolves with duration of insulin and C-peptide deficiencies. A conceptual construct of the progressive and interrelated abnormalities underlying the development of encephalopathy in type 1 diabetes, as we understand it at the present time, is illustrated in Figure 8. A major initiating event appears to be impaired insulin signaling, with consequences for the expression of other neurotrophic factors. Such defects are likely to impact on neurotransmitter synthesis, expression of neuroskeletal proteins, and to enhance activation of innate inflammatory factors. The latter may be further enhanced by hyperglycemia *per se* with upregulation of RAGE. Interestingly though, whilst full replacement of C-peptide

does not correct hyperglycemia, it prevents, and in some instances corrects, these early metabolic perturbations. Additionally, it has beneficial effects on trophic factor deficits and perturbations of pre-synaptic connectivity, leading to correction of early

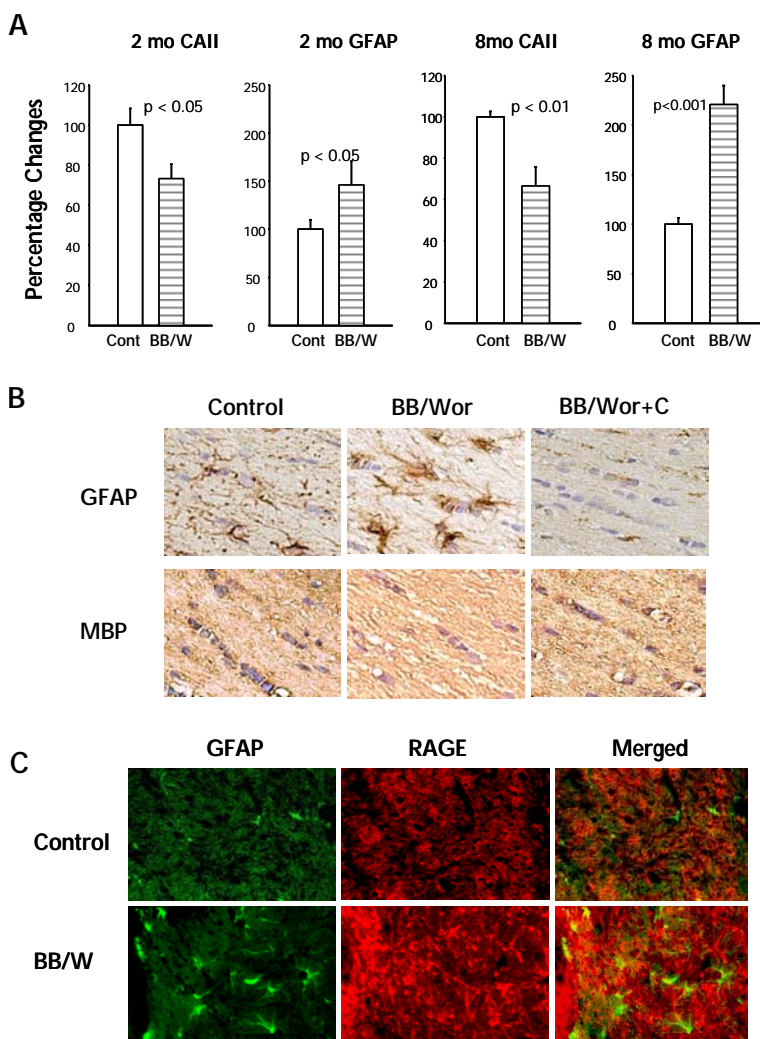


Figure 7. White matter changes in control, diabetic, and C-peptide replaced diabetic rats. **A:** Already in 2-month diabetic rats, there is a significant decrease in the number of oligodendroglial cells (identified by the oligo marker CAII) in temporal white matter, which is associated with increased proliferation of astrocytes. These differences are sustained and increased in 8-month diabetic rats. **B:** Immunocytochemical identification of astrocytes (GFAP), which are increased in 4-month diabetic rats, which simultaneously show decreased stainability for the oligo marker myelin basic protein (MBP). There is close to normal stainability of astrocytes and oligodendroglia cells in C-peptide treated animals. **C:** RAGE is markedly upregulated in the white matter of 8-month diabetic rats, and colocalizes with GFAP-positive astrocytes.

behavioral deficits in reference and working memory.

Upregulation of RAGE and induction of inflammatory interleukins have downstream effects on oxidative and apoptotic stressors. Somewhat unexpectedly, C-peptide substitution largely corrected the inflammatory responses and subsequent oxidative and apoptotic DNA damage. The latter benefits are likely (at least in part) due to the normalization of the insulin-signaling cascade by C-peptide. Both insulin and C-peptide have documented anti-apoptotic effects. Oxidative and apoptotic activities eventually result in neuronal cell loss in gray matter structures, such as the hippocampus, and early loss of myelinating oligodendroglia in the white matter. Inevitably, these changes lead to disturbances in brain interconnectivity and disruption of regional circuitry systems, such as the hippocampus, with consequences for storage and retrieval of information, and long-term memory.

In summary, C-peptide replacement from onset of type 1 diabetes prevents the neuronal consequences of the disease. The case of early onset diabetes is particularly important, because the de-

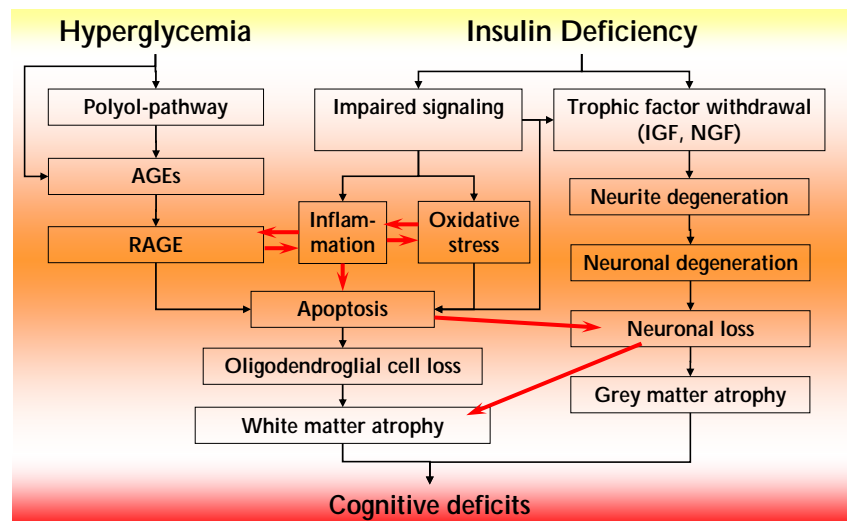


Figure 8. Conceptual depiction of the temporal inter-relationships between pathogenetic mechanisms emanating from hyperglycemia and insulin deficiency in type 1 diabetic encephalopathy. Hyperglycemia leads to activation of the polyol pathway and AGEs, with upregulation of RAGE and activation of innate inflammatory factors. Inflammation and RAGE activation are also induced by increased expression of NF- κ B secondary to impaired insulin signaling activity. Suppressed insulin signaling leads to decreased expression of IGF, NFG, and their respective receptors, with consequent neurite degeneration, and loss of presynaptic connections. This results in early (radial-arm paradigm) neuro behavioral deficits. Innate inflammatory activities and suppressed insulin signaling result in oxidative and apoptotic stresses. This results in apoptosis and cell loss of both neurons and oligodendroglial cells, with consequent gray and white matter atrophy and cognitive deficits. As outlined in this review, full substitution of C-peptide has significant beneficial effects on several of these pathogenetic components, resulting in substantial prevention of behavioral and cognitive deficits.

veloping brain is more vulnerable to damage by diabetes effects than the matured brain. The data we obtained from the representative model of type 1 diabetes strongly suggest that replacement of C-peptide in type 1 diabetes will have marked beneficial effects on type 1 diabetic encephalopathy.

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