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Insulin, C-peptide, hyperglycemia, and central nervous system complications in diabetes

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Abstract

Diabetes is an increasingly common disorder which causes and contributes to a variety of central nervous system (CNS) complications which are often associated with cognitive deficits. There appear to be two types of diabetic encephalopathy. *Primary diabetic encephalopathy* is caused by hyperglycemia and impaired insulin action, which evolves in a diabetes duration-related fashion and is associated with apoptotic neuronal loss and cognitive decline. This appears to be particularly associated with insulin-deficient diabetes. *Secondary diabetic encephalopathy* appears to arise from hypoxic–ischemic insults due to underlying microvascular disease or as a consequence of hypoglycemia. This type of cerebral diabetic complication is more common in the type 2 diabetic population. Here, we will review the clinical and experimental data supporting this conceptual division of diabetic CNS complications and discuss the underlying metabolic, molecular, and functional aberrations.

Keywords: CNS complication; Type 1 and type 2 diabetes; Insulin; C-peptide; Hyperglycemia

1. Introduction

Diabetes, an increasingly common metabolic disorder, causes complications affecting the retina, kidney, muscle and blood vessels, and the nervous system. Diabetic peripheral neuropathy has traditionally been considered the only primary nervous system complication in diabetes, whereas the central nervous system (CNS) was believed to be relatively spared from diabetes. However, in recent years it has become evident that diabetes causes both primary and secondary CNS complications with functional impairments. *Primary diabetic encephalopathy* being caused directly by either hyperglycemia or impaired insulin action, or both, whereas *secondary diabetic encephalopathy* being the result of diabetic vascular disease or from intensive insulin treatment, potentially causing hypoglycemic brain damage. Here we will review the evidence of CNS dysfunction in type 1 and type 2 diabetic subjects and the experimental evidence for underlying mechanisms causing both primary and secondary diabetic CNS complications, and the role of insulin action.

2. Clinical studies

Cognitive dysfunction in diabetic subjects has been recognized since the early 20th century (Miles and Root, 1922). Since then, a wealth of studies have described a series of neuropsychological and neurobehavioral changes in both type 1 and type 2 diabetic subjects, suggesting that "diabetic encephalopathy" should be recognized as a complication of diabetes.

2.1. CNS complications in type 1 diabetic patients

Impairments in learning, memory, problem solving and mental and motor speed are more common in type 1 diabetic patients than in the general population (McCarthy et al., 2002; Ryan, 1988; Ryan and Williams, 1993; Ryan et al., 1993). The cognitive impairments can be severe in

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rare cases (Deary et al., 1993; Gold et al., 1994). Variation in severity and pattern of impairments may be due to the subtle nature of the defects, the length of diabetes duration, the psychological tests employed and variations in glycemic controls among individual studies. A lower performance IQ was found in type 1 diabetic children with disease onset before the age of seven years and disease duration greater than or equal to 5 years (Holmes and Richman, 1985). Diabetic subjects suffered from more introversive symptoms than their healthy counterparts, especially with respect to somatic symptoms, sleeping disturbances, compulsions, and depressive moods (Lustman et al., 1988; Popkin et al., 1988; Blanz et al., 1993; Kovacs et al., 1997).

The neuropsychological changes are often accompanied by objective electrophysiological, structural and neurochemical abnormalities. Measurements of latencies of evoked potentials have been widely used to examine the functional integrities of the CNS in diabetic patients (Di Mario et al., 1995). Delayed conduction velocity and data processing time in the CNS was demonstrated in type 1 diabetic patients, including brainstem auditory evoked potential, somatosensory evoked potentials, visual evoked potentials and motor-evoked potentials (Cracco et al., 1984; Harkins et al., 1985; Pietravalle et al., 1993; Uberall et al., 1996; Comi, 1997; Verrotti et al., 2000). The latency of P100 of the visual evoked potential, believed to be generated in the visual cortex, is increased in diabetic patients (Ziegler et al., 1994; Parisi and Uccioli, 2001). Several studies have shown that the P300 latency, a neurophysiological correlate of information processing, such as stimulus evaluation, alertness and memory updating (Picton, 1992; Madl et al., 1994; Kramer et al., 1998), is increased in diabetic patients (Pozzessere et al., 1991; Uberall et al., 1996; Kramer et al., 1998), indicating impairment of higher brain function.

In terms of structural changes, several abnormalities have been described in type 1 diabetic patients, such as diffuse and local degenerative changes of cerebral cortex (Reske-Nielsen and Lundbaek, 1964; Reske-Nielsen et al., 1965), neuronal loss, demyelination and gliosis, and infarction secondary to microangiopathy (DeJong, 1977). Magnetic resonance imaging and computed tomography have demonstrated indices of cerebral atrophy (Araki et al., 1994; Lunetta et al., 1994; Perros et al., 1997) and the occurrence of white matter hyperintensities (Araki et al., 1994; Perros et al., 1997) are more pronounced in diabetic patients than in age-matched control subjects.

Since hypoglycemia itself can cause brain damage (de Courten-Myers et al., 2000; Vannucci and Vannucci, 2001; Yager, 2002), it remains controversial as to whether the above CNS abnormalities are caused by diabetes per se (*primary*) or are the result of hypoglycemic episodes (*secondary*), which occur frequently in type 1 diabetic patients subjected to intensive insulin treatment. Alternatively, some of these structural defects are likely secondary to diabetic small vessel disease.

Recent studies have shown that a duration-dependent decline in cognitive function occurs in type 1 diabetic patients without a history of hypoglycemic episodes (Kramer et al., 1998), and that impaired intellectual and cognitive development occur in type 1 diabetic children, who have not experienced hypoglycemic episodes (Schoenle et al., 2002). These latter impairments correlate with diagnosis at young age, male sex and metabolic status at time of diagnosis. Therefore, CNS complications seem to be impacted by diabetes itself without implementing hypoglycemic episodes as a causative mechanism.

2.2. CNS complications in type 2 diabetic patients

Neuropsychologic studies in type 2 diabetes patients have shown more consistent results compared to type 1 diabetes patients. Cognitive deficits (Perlmuter et al., 1984; Tun et al., 1990; Gradman et al., 1993; Worrall et al., 1993; Strachan et al., 1997; Ryan and Geckle, 2000a) and poor performance in abstract reasoning and complex psychomotor functioning (Reaven et al., 1990) occur in type 2 diabetes. Complex cognitive tasks requiring storage and retrieval of new information are affected, whereas performances of less demanding tasks such as immediate memory recall and simple reaction time are not significantly altered (Tun et al., 1990). Electrophysiological studies have demonstrated delayed CNS conduction velocity (Donald et al., 1984; Verrotti et al., 2000; Varsik et al., 2001) and increased latency of P100 (Ziegler et al., 1994). Several studies have shown that the P300 latency is increased (Mooradian et al., 1988; Kurita et al., 1995; Dey et al., 1997; Mochizuki et al., 1998), indicating impairment of higher brain function. Recently, increased blood–brain barrier permeability was demonstrated by magnetic resonance imaging, suggesting that loss of blood–brain barrier integrity may play a role in CNS dysfunction in diabetes (Starr et al., 2003). In the All Wales Research into Elderly (AWARE) Diabetes Study, elderly type 2 diabetic patients demonstrated an excess of cognitive dysfunction associated with poor ability of self-care (Sinclair et al., 2000). However, no significant impairments in learning, memory and problem-solving skills were found in middle-aged type 2 diabetic patients compared to control subjects (Ryan and Geckle, 2000b). The current belief is that learning and memory dysfunction are more prominent in elderly type 2 diabetic subjects (Ryan and Geckle, 2000a). As to whether this is due to a potentiation of the normal aging process by superimposed diabetes or a function of diabetes duration or both is not settled.

The etiology of cognitive dysfunction in type 2 diabetes is not fully understood. It is likely that cognitive impairments are caused by an interaction between metabolic abnormalities intrinsic to diabetes and diabetes-specific vascular complications. In support of the former, Gradman et al. (1993) showed that memory and learning improved with glycemic control. The latter, on the other hand, may be

potentiated by the simultaneous presence of hypertensive microvasculopathy. The higher frequency of cerebral stroke in type 2 diabetes is likely to be associated with cognitive deficits. Cerebrovascular mortality rate is higher in patients with type 2 diabetes (Barrett-Connor and Khaw, 1988; Lehto et al., 1996) and the risk for developing stroke is increased two- to fivefold compared to non-diabetic control subjects (Manson et al., 1991; Stamler et al., 1993). Recently, 5102 patients with type 2 diabetes in the UK Prospective Diabetes Study (UKPDS) were analyzed which confirmed the higher risk of stroke in type 2 diabetes (Kothari et al., 2002). Although cerebrovascular disease is rarely considered in younger patients with type 1 diabetes, recent analyses of mortality from 23,000 patients with type 1 diabetes has shown for the first time that cerebrovascular mortality is raised at all ages in this patient group as well (Laing et al., 2003). Based on these reports, the continuing study of cerebral stroke in diabetes should provide useful insight into possible underlying mechanisms.

3. Experimental studies

3.1. Structural changes

A variety of structural changes has been described in the CNS of streptozotocin-induced diabetes in rats and Chinese hamsters (Luse, 1970; Bestetti and Rossi, 1980; Mukai et al., 1980; Bestetti and Rossi, 1982; Garris et al., 1982; Jakobsen et al., 1987). Structural alterations have been described in the ventromedial hypothalamus including accumulation of glycogen, degeneration of neurons and atrophy of tanycytes. Ultrastructural abnormalities such as dilated and fragmented endoplasmic reticulum, degranulated ergastoplasm, increased number of microtubuli, myelin figures, irregularities in the form of nuclei and appearance of chromatin occur. A significant loss of neocortical neurons was demonstrated in streptozotocin-diabetic rats compared to non-diabetic control rats (Jakobsen et al., 1987). Significant structural alterations in the blood–brain barrier were shown in experimental diabetic animals (Mooradian, 1997), consistent with the magnetic resonance imaging findings in diabetic patients (Starr et al., 2003).

3.2. Neurochemical changes

The concentration of neurotransmitters are altered in diabetic brains. A decrease in norepinephrine and serotonin content was shown in the neocortex and caudal segment of the brain stem in alloxan-induced diabetic rats (Kulikov et al., 1986) and in brains of streptozotocin-induced diabetic rats (Trulson et al., 1986). In contrast, an increase in the norepinephrine concentrations of the paraventricular nucleus, lateral hypothalamus, ventromedial hypothalamus and suprachiasmatic nucleus was reported. Increased concentrations of norepinephrine, dopamine and serotonin in the arcuate nucleus have been described in streptozotocin-diabetic rats (Barber et al., 2003), indicating that the monoaminergic system is affected in experimental diabetic animals. In this study insulin treatment reversed these changes, suggesting that insulin deficiency and/or hyperglycemia underlie the alterations of monoamine neurotransmission.

3.3. Electrophysiolgical changes

The latencies of auditory and visual potentials were found to be prolonged in streptozotocin-diabetic rats (Biessels et al., 1999; Rubini et al., 1992) and type 1 BB/Wor diabetic rats (Chakrabarti et al., 1991; Sima et al., 1992), indicating impaired CNS conduction velocities similar to those described in human diabetes. In hippocampal slices from streptozotocin-diabetic rats, long-term potentiation induced by 100 Hz stimulation is impaired, whereas long-term depression is enhanced compared with control rats (Biessels et al., 1996, 1998; Gispen and Biessels, 2000; Kamal et al., 2000), indicating that altered hippocampal synaptic plasticity occurs in type 1 diabetic streptozotocin rats. These changes correlate with the duration of diabetes (Kamal et al., 1999). Since changes in hippocampal synaptic plasticity in streptozotocin-diabetic rats occur in association with impairments of spatial learning function (Gispen and Biessels, 2000; Kamal et al., 2000), these studies provide a mechanism for cognitive dysfunction in diabetic animals. (These findings are described in more detail elsewhere in this issue.)

3.4. Cognitive functional changes

Perturbed spatial learning and memory have been demonstrated using the Morris water maze system in various diabetic animals including BB/Wor rats, streptozotocin rats, streptozotocin mice and Otsuka Long Evans Tokushima Fatty (OLETF) rats (Flood et al., 1990; Biessels et al., 1998; Kamal et al., 2000; Luesse et al., 2001; Li et al., 2002a,b). Multiple cognitive components are involved in this task, such as problem solving, enhanced selective attention, formation of internal representation of the external environments, and storage and retrieval of relevant information (Bannerman et al., 1995). The alterations in hippocampal synaptic plasticity are associated with defects in spatial learning and memory as detected by the Morris water maze (Biessels et al., 1996, 1998). In diabetic BB/Wor rats, we failed to show impaired spatial learning and memory in acutely diabetic rats (2 month), whereas significant deficits in the Morris water maze were demonstrated in 8-month diabetic BB/Wor rats at a time when apoptotic hippocampal neuronal loss was evident (see below) (Li et al., 2002b). These results suggest that progressive learning and memory deficiencies in type 1 diabetic rats occur in a duration-related fashion and are associated with alterations in synaptic connectivity and neuronal loss.

3.5. Neuronal loss/apoptosis in type 1 diabetes

A significant loss of neocortical neurons occurs in streptozotocin rats (Jakobsen et al., 1987). In the diabetic Chinese hamster, neuronal death occurs in the arcuate and ventromedial nuclei (Garris, 1984). The nature of cell death was not defined in these early studies, nor was it determined as to whether neuronal loss was associated with cognitive deficits. In the spontaneously diabetic BB/Wor rat, neuronal counts of hippocampal pyramidal cells were performed in the hippocampal CA_1–CA_4 regions. No significant differences were demonstrated at 2 months of diabetes. In 8-month diabetic animals, there were a 37% and 24% loss of pyramidal cells in the CA_1 and CA_2 regions, respectively, whereas other hippocampal regions showed non-significant decreases in neuronal densities (Li et al., 2002b). These data suggest that decreased neuronal density is diabetes duration-related. Comparisons with hyperglycemia- and duration-matched spontaneously type 2 diabetic BBDRZ/Wor rats revealed a milder but still significant neuronal loss of the hippocampal CA_1 region (Fig. 1) (Li and Sima, in press).

The above studies indicate that hippocampal neuronal loss occurs in type 1 diabetic animals and that this may be a major contributing mechanism to cognitive dysfunction. As to the nature of this neuronal loss, we have demonstrated that apoptosis plays an important role. In vitro and in vivo studies appear to support this notion (Li et al., 2002b, 2003). No apoptotic activity of hippocampal pyramidal neurons was demonstrated in 2-month diabetic BB/Wor rats, nor was any cognitive deficits detected by the Morris water maze procedure, nor was there any neuronal loss detectable. On the other hand, in 8-month diabetic rats, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeled (TUNEL) neurons, positive DNA laddering, increased Bax expression and caspase 3 activity were evident and associated with decreased neuronal density and impaired Morris water maze performances (Li et al., 2002b). Apoptosis as revealed by DNA laddering was also present in the prefrontal cortex of 8-month diabetic BB/Wor rats. Therefore, duration-related apoptosis is likely to account for the neuronal loss and the concomitant emergence of cognitive impairments in the type 1 diabetic BB/Wor rat.

3.6. Ischemic injury in diabetic animals

It is well known that diabetes aggravates brain damage in experimental and clinical stroke subjects. Diabetes accelerates maturation of neuronal damage, increases infarct volume, and induces post-ischemic seizures (Muranyi et al., 2003). A brief period of 30-min focal ischemia in normoglycemic rats leads to brain damage in a delayed fashion: infarction develops after 3 days and full maturation occurs by 2 weeks after recirculation (Du et al., 1996). In preischemic hyperglycemia or in streptozotocin-diabetic rats, the damage is more severe than that in non-diabetic animals (Muranyi et al., 2003) and the damage evolves faster: infarction develops after 2 h and is matured after 4–6 h after the ischemic insult (Li et al., 1998; Gisselsson et al., 1999). The mechanisms underlying diabetes-related aggravation of ischemic brain damage are unclear. Apoptosis, or programmed cell death, is believed to play an important role in the pathogenesis of various human disorders including diabetes (Barr and Tomei, 1994; Orrenius, 1995; Thompson, 1995; Fadeel et al., 1999) and a vast body of studies has shown that apoptosis is associated with cerebral ischemia and ischemia–reperfusion injury. It has been demonstrated that neurons bordering the maturing infarct exhibit TUNEL staining, and DNA prepared from the penumbral area of ischemic cortex show internucleosomal fragmentation (Du et al., 1996). Increased levels of either

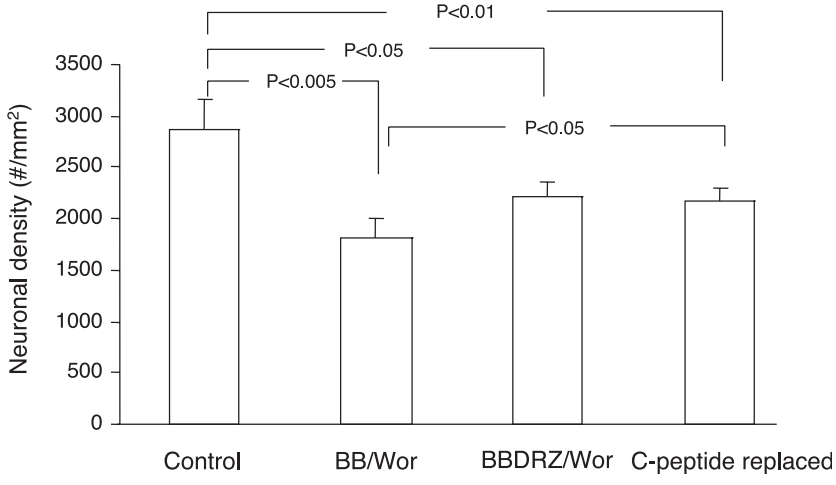

Fig. 1. Neuronal densities in hippocampal Ca_1 in 8-month type 1 diabetic BB/Wor rats, duration- and hyperglycemia-matched type 2 BBDRZ/Wor rats and BB/Wor rats replenished with C-peptide from onset of diabetes. Type 1 rats showed a 36% ($P<0.001$) neuronal loss, which was prevented by 45% ($P<0.05$) following C-peptide replacement. Type 2 normoinsulinemic BBZDR/Wor rats showed a milder neuronal loss that was similar to that of C-peptide-replaced type 1 rats. The neuronal density deficits in the two latter groups could potentially represent the hyperglycemia-induced apoptotic neuronal loss.

expression or activities of caspase-3 (Chen et al., 1998; Schulz et al., 1999) and induction of caspase-activated deoxyribonuclease activity (Luo et al., 2002) are detected in ischemic neurons. DNA fragmentation in ischemic brains has been demonstrated by either gel electrophoresis or TUNEL staining (Li et al., 1995; Charriaut-Marlangue et al., 1998; Endres et al., 1998). A strong correlation exists between caspase-3 activity, TUNEL staining, and the appearance of apoptotic neurons (Charriaut-Marlangue et al., 1998; Chen et al., 1998; Namura et al., 1998; Cao et al., 2001). Cytosolic cytochrome *c* release is markedly enhanced in both the ischemic focus and the penumbra in streptozotocin-diabetic rats, together with increased activation of caspase-3 and accelerated cleavage of poly-ADP ribose polymerase (Du et al., 1996), all of which suggest that apoptosis plays a prominent role in exaggerating the effect of cerebral ischemia in diabetes.

Recently, the effects of local ischemia on type 1 diabetic rats vs. normal control rats were reported. A significant increase in the number of TUNEL-positive and caspase-3-positive cell was demonstrated in selected brain regions (hypothalamic preoptic area, piriform cortex, and parietal cortex) of streptozotocin-diabetic rats subjected to middle cerebral artery occlusion for 24–48 h, compared to non-occluded diabetic rats (Britton et al., 2003). Consistent with these findings, preliminary data from our laboratory show that the ladder pattern of nucleosomal DNA fragmentation, assessed by ligand-mediated PCR, cannot be detected in unlesioned normal or streptozotocin-diabetic animals, but is evident in infarcted cortex of diabetic rats (Li and Sima, unpublished data). Bax expression is increased in normal infarcted cortex and is further increased in infarcted diabetic cortex. These data suggest that diabetes confers an expansion of stroke via increased apoptotic activity.

In summary, experimental data obtained from type 1 diabetic animals show definite alterations in structure, neurotransmitters, electrophysiology, cognitive function, neuronal density and apoptotic activity. These results seem to suggest that "primary diabetic encephalopathy" is an identifiable complication in type 1 diabetes and that exaggerated brain damage occurs consequent to ischemic insults in type 1 diabetes.

4. Neuronal apoptotic mechanisms

The mechanisms underlying neuronal apoptosis and CNS dysfunction are not clear. We believe that contributing factors are insulin deficiency with concomitant C-peptide deficiency, as well as hyperglycemia and possibly the aging process itself.

4.1. Insulin/C-peptide deficiency

Insulin plays important roles in the regulation of brain metabolism and neurotrophism. Recent studies show that insulin exerts modulatory roles on brain functions such as feeding (Schwartz et al., 1992; Gerozissis et al., 2001), learning and memory (Gerozissis et al., 2001; Zhao and Alkon, 2001). In cerebral stroke, administration of insulin prevents neuronal death (Voll and Auer, 1991a,b) and reduces neurological disability (LeMay et al., 1988). These neuroprotective effects of insulin are associated with restoration of protein synthesis (Sullivan et al., 1999). In streptozotocin-diabetic rats, cognitive deficits are partially corrected by insulin (Biessels et al., 1998). In vitro studies also show that insulin has an anti-apoptotic effects (Lee-Kwon et al., 1998; Bertrand et al., 1999; Li et al., 2003). We have shown that the insulin receptor is downregulated in the hippocampus of type 1 diabetic BB/Wor rats (Li et al., 2002b), suggesting that impaired insulin action caused not only by insulinopenia but also by impaired insulin receptor expression may play a role in CNS apoptosis in diabetes.

In addition to insulin deficiency, C-peptide deficiency is a contributing pathogenic factor in type 1 diabetic complications (for reviews, see Sima, 2003a,b; in press). C-peptide is a 31-amino-acid peptide that is cleaved from proinsulin during the biosynthesis of insulin (Steiner and Rubenstein, 1997; Li et al., 2001). Recent studies have shown that C-peptide possesses physiological functions beyond that of providing structural support for proinsulin cleavage (Wahren et al., 1994, 1996; Wahren and Johansson, 1998; Grunberger et al., 2001; Sima, 2003a,b).

C-peptide shows specific binding to cell surfaces with high affinity (Rigler et al., 1999). No specific C-peptide receptor has been identified. However, there is evidence to suggest that C-peptide signals through the insulin signaling pathways (Grunberger et al., 2001; Li et al., 2001; Jensen and Messina, 1999; Kitamura et al., 2002; Sima, 2003a,b). It has insulinomimetic effects on its own by activating insulin receptor activity and increases glycogen synthesis and amino acid uptake. However, C-peptide has no glucose lowering effect. Furthermore, C-peptide promotes insulin action at low hormone concentration and inhibits it at high hormone levels (Grunberger et al., 2001; Grunberger and Sima, in press), suggesting a modulatory effect by C-peptide on insulin signaling. This would suggest that in hyperinsulinemic (and hyper-C-peptidemic) type 2 diabetes, C-peptide may have a dampening effect on insulin action. C-peptide does not compete with insulin for receptor binding, suggesting a different ligand site if it interacts with the insulin receptor. In patients with type 1 diabetes, C-peptide improves renal function, reduces urinary albumin excretion and glomerular filtration, and decreases blood retinal barrier leakage (Zierath et al., 1991, 1996; Johansson et al., 1992, 1996; Forst et al., 1998; Fernqvist-Forbes et al., 2001). Chronic C-peptide replacement prevents functional and structural peripheral nerve changes in type 1 diabetic rat models (Wu et al., 1996; Ido et al., 1997; Li et al., 1999; Samnegard et al., 2001; Zhang et al., 2001; Sima et al., 2001; Huang et al., 2002; Pierson et al., 2003), suggesting that C-peptide deficiency is a participating factor in the

causation of type 1 diabetic complications. We recently showed that administration of C-peptide partially corrects perturbed insulin-like growth factor (IGF) activity and insulin receptor expression and partially but significantly prevents neuronal apoptosis in the hippocampus of type 1 diabetic BB/Wor rats (Li et al., 2002c; Li and Sima, in press), demonstrating a relationship between C-peptide deficiency, insulin action, IGF perturbation and neuronal apoptosis. In neuroblastoma SH-SY5Y cells, C-peptide provides a dose-dependent stimulatory effect on cell proliferation, and neurite outgrowth, whereas no effects were evident with the same concentration of scrambled C-peptide. In the same in vitro cell cultures, apoptosis was induced by high concentrations of glucose mimicking hyperglycemia. Apoptosis of SH-SY5Y cells is inhibited by addition of insulin alone, whereas the combination of insulin and C-peptide shows an enhanced anti-apoptotic effect (Fig. 2), as assessed by nuclear condensation and shrinkage, reduction in the number of apoptotic cells, and stimulation of Bcl2 expression and nuclear factor kappa B (NF-κB) (Li et al., 2003). The inactivated form NF-κB exists in the cytoplasm as a complex consisting of p50, p65 subunits of inhibitor-κB (IκB). On stimulation, I-κB is phosphorylated and degraded, resulting in activation and translocation of NF-κB to the nucleus, where it activates target gene transcription. It is known that NF-κB and the genes regulated by this transcription factor, such as those coding for TNF receptor-associated factor 1 (TRAF1), TRAF2, inhibitor-of-apoptosis (IAP) proteins c-IAP1 and c-IAP2, manganese superoxide dismutase, Bcl2 and BclxL, play important roles in the regulation of apoptosis (Aggarwal, 2000; Bours et al., 2000). In the SH-SY5Y cells grown under elevated glucose concentrations, C-peptide plus insulin gives rise to a significant increase in nuclear NF-κB staining, suggesting that insulin/C-peptide plays effective anti-apoptotic roles via activation and translocation of NF-κB (Li et al., 2003). In summary, the data obtained from in vitro system are consistent with the in vivo results obtained from experimental diabetes and support the notion that insulin/C-peptide deficiency plays important roles in type 1 diabetes-induced neuronal apoptosis.

4.2. Impairments of the IGF system

Perturbed IGF system has been shown in the CNS of STZ rats. After 2 weeks of diabetes, IGF-II mRNA content is significantly decreased in the brain and spinal cord. Insulin replacement partially restores IGF-II mRNA levels in cerebral, cortex, medulla, and spinal cord (Wuarin et al., 1996). We have systematically examined the IGF system (IGF-1, II, IGF-IR and IR) in the BB/Wor model of type 1 diabetes. We found significant reductions in the expression of IGF-I, IGF-II, IGF-IR and insulin receptor already in 2-month diabetic BB/Wor rats which persisted in 8-month diabetic rats, indicating that these abnormalities precede the functional cognitive impairments and the apoptotic neuronal loss in hippocampus (Li et al., 2002b). These abnormalities were largely prevented by

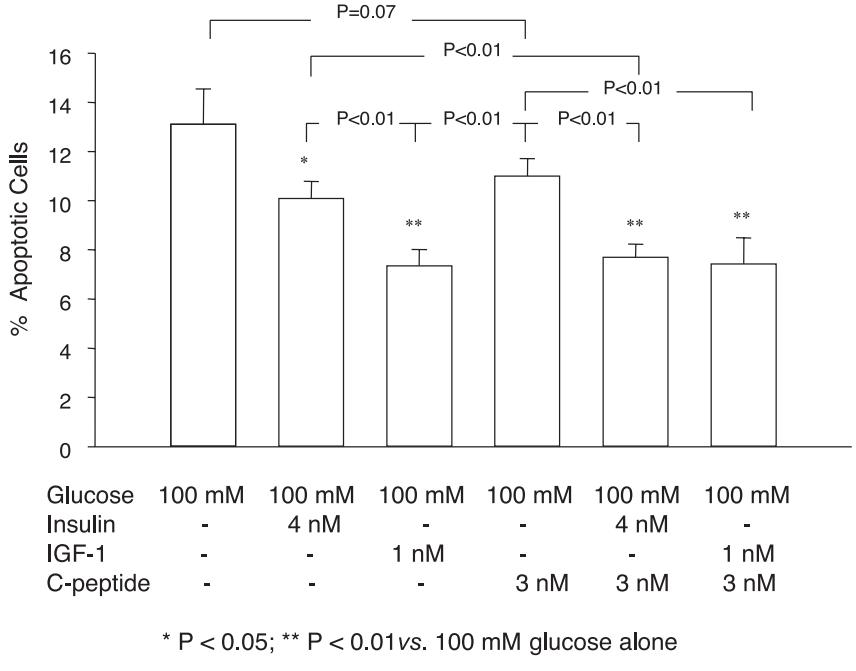

Fig. 2. The effect of C-peptide, insulin and IGF-1 or their combinations on apoptosis of SH-SY5Y cells after 4 days exposure to 100 mM glucose. The percentage of apoptotic cells was determined by flow cytometry. Insulin and IGF-1 demonstrated significant anti-apoptotic effects. The addition of C-peptide enhanced ($P<0.01$) the anti-apoptotic effects of insulin, but not that of IGF-1. Reproduced from Li et al. (2003), by permission from Diabetes/Metabolism Research and Reviews.

replenishing C-peptide in BB/Wor rats (Li et al., 2002c; Li and Sima, in press).

4.3. Hyperglycemia

Hyperglycemia causes increased glucose levels in the brain. It is well established that hyperglycemia causes oxidative stress via the polyol pathway, enhanced advanced glycation end-products, increased lipid peroxidation and imbalances in the generation of reactive oxygen species and their scavengers (Ceriello et al., 1993; Mercuri et al., 2000; Lipinski, 2001; Opara, 2002; Sima, 2003a). Oxidative stress is also associated with cerebral ischemic injury (Love, 1999; Kaminski et al., 2002) and neuronal apoptosis (Gorman et al., 1996; Nicotera et al., 1997; Sastre et al., 2000). It is therefore likely that hyperglycemia per se contributes to apoptotic activities, most likely via oxidative stress, as indicated by hippocampal apoptosis with neuronal loss in the type 2 hyperinsulinemic and C-peptidemic BBDRZ/Wor rat (Li and Sima, unpublished data). This model shows a normal expression of the hippocampal insulin receptor and insulin-like growth factor 1 (IGF-1) expression. However despite this, it exhibits significant apoptosis of the hippocampus, although the resulting neuronal loss is significantly milder than in duration-matched and isohyperglycemic type 1 BB/Wor rats. In support of hyperglycemia-induced oxidative stress, the BBDRZ/Wor rats exhibit increased expression of caspase 12, poly-ADP ribose polymerase and Fas (Li and Sima, unpublished data). Therefore, comparing these two models of type 1 and type 2 diabetes, it appears that both hyperglycemia as well as impaired insulin-action contribute to duration-related apoptotic phenomena in the CNS.

5. Concluding remarks

It is becoming increasingly evident that diabetes per se impairs CNS functions in a seemingly duration-dependent manner in both humans and experimental models, which we propose as a "primary diabetic encephalopathy." Experimental data to date suggest that both impaired insulin action as well as hyperglycemia are of pathogenetic significance. This is consistent with both human and animal studies showing more severe abnormalities under type 1 diabetic conditions. These duration-related phenomena appear to be coupled to apoptotic activities, which is supported by in vitro data.

"Secondary diabetic encephalopathy", caused by ischemic events secondary to vascular disease or by hypoglycemic events, exhibits exaggerated ischemic lesions which at least in part appear to be caused by penumbral apoptotic activities. Secondary diabetic encephalopathy tends to be more common in elderly type 2 patients. As to whether the apoptotic phenomena leading up to neuronal death in primary and secondary diabetic encephalopathy are the same is not known at the present time. In summary therefore, a multitude of CNS complications have been established in both types of diabetes, and are potentially preventable by tight hyperglycemic control combined with replacement of insulinomimetic C-peptide in the case of type 1 diabetes.

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