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Increase of glial fibrillary acidic protein and S-100B in hippocampus and cortex of diabetic rats: effects of vitamin E

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Abstract

Glial interactions with neurones play vital roles during the ontogeny of the nervous system and in the adult brain. Physical and metabolic insults cause rapid changes in the glial cells and this phenomenon is called reactive gliosis. One of the important events during astrocyte differentiation is the increased expression of glial markers, glial fibrillary acidic protein (GFAP) and S-100B protein. Diabetes mellitus is the most common serious metabolic disorder, which is characterised by functional and structural changes in the peripheral as well as in the central nervous system. In the present study, we aimed to investigate glial reactivity in hippocampus, cortex and cerebellum of streptozotocin-induced diabetic rats by determining the expression of GFAP and S-100B and also to examine the protective effects of vitamin E against gliosis. Western blotting showed increases in total and degraded GFAP content and S-100B protein expression in brain tissues of diabetic rats compared with those of controls. In addition, there was a significant increase in lipid peroxidation in these brain regions of diabetic rats. Both glial markers and lipid peroxidation levels were reversed by vitamin E administration. These findings indicate that streptozotocin-induced diabetes alters degradation and production of GFAP and S-100B, which are markers of reactive astrocytosis. Thus, determination of GFAP and S-100B may provide a relevant marker in the central nervous system for studying neurodegenerative changes in experimental diabetes mellitus. This study also suggests that the gliosis that occurs in diabetes mellitus is mediated, at least indirectly, by free radical formation and antioxidants may prevent reactive gliosis possibly by reducing damaging effects of reactive oxygen species in the central nervous system.

Keywords: Diabetes; Streptozotocin; Lipid peroxidation; Hippocampus; Cortex; GFAP (glial fibrillary acidic protein)

1. Introduction

Diabetes mellitus is the most common serious metabolic disorder (Gispen and Biessels, 2000; McCall, 1992). Diabetes causes a variety of functional and structural disorders in the central as well as the peripheral nervous systems (Biessels et al., 1994). Streptozotocin-induced diabetes is a well-characterised experimental model for insulinopenic Type I diabetes mellitus and provides a relevant example of endogenous chronic stress (Scribner et al., 1991, 1993). During hyperglycaemia, enhanced formation of oxygen free radicals occurs in the tissues (Baydas et al., 2002). These oxidant radicals contribute to increased neuronal death by oxidising proteins, damaging DNA, and inducing the lipoperoxidation of cellular membranes (Hawkins and Davies, 2001; Luxford et al., 2000). For reducing damaging effects of reactive oxygen species, various antioxidant therapies have been proposed with variable results. The antioxidant, vitamin E, is present in normal diets and reduces lipid peroxidation (Ercel et al., 1999).

Neurones have been the primary focus of studies related to the effects of oxidative stress and antioxidants in the central nervous system. It is obvious that neuronal–glial interaction. Astrocytes possess physiological and metabolic properties that play a vital role in maintaining normal homeostasis in the brain. They involve the regulation of water, ions, neurotransmitters and pH in the

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neuronal environment (Montgomery, 1994). Chemical or mechanical insults to the brain cause permanent changes and astrocytes respond by a variety of reactions (Reier and Houle, 1988). Reactive astrocytes show extensive synthesis of glial fibrillary acidic protein (GFAP). GFAP is an intracellular intermediate filament protein. It has been suggested that GFAP is essential for the formation of stable astrocytic processes in response to neuronal damage and this may be critical for morphogenesis of the central nervous system (Liedtke et al., 1996). Change in GFAP levels have been proposed as an index of reactive gliosis (Janeczko, 1993; Norenberg, 1994).

Another astrocyte marker is the intracellular glycoprotein, S-100B, with a molecular wt. of 11,000 (Cerutti and Chadi, 2000). S-100B is an acidic Ca^{2+} binding protein, present mainly in astrocytes, that exerts paracrine trophic effects on several neuronal populations (Kligman and Marshak, 1985; Fano et al., 1995). S-100B elevates neuronal cytoplasmic free calcium levels, stimulates neurite outgrowth and promotes neuronal survival (Marshak et al., 1992; Bhattacharyya et al., 1992). In addition to the overexpression of GFAP, astrocytes express high levels of S-100B protein in response to the neuronal damage (Griffin et al., 1998).

Glial involvement in early pathological events in diabetic neuropathy has not been investigated. We proposed that if diabetes causes any structural and functional changes in brain tissue then it can induce astrocytic reactivity, such as promoting production and degradation of GFAP and S-100B as markers of brain injury. Therefore, we now aimed to study neurodegenerative effects of streptozotocin-induced diabetes by evaluating the expression of GFAP and S-100B and the levels of lipid peroxidation. The protective effects of vitamin E against reactive oxygen species and reactive gliosis in different brain regions were also examined.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Animal research unit, Firat University, Elazıg) weighing 200–250 g were used in this study. The rats were housed in a temperature-controlled room (22–25 °C) with a 12/12 h light/dark cycle. Water and food were given ad libitum.

2.2. Agents

Vitamin E (alpha tocopherol), olive oil and streptozotocin were purchased from Sigma (St. Louis, MO, USA).

2.3. Experimental diabetes

The animals were divided into three groups. A control group ($n=10$) was given saline via intraperitoneal (i.p.) injection. To induce experimental diabetes, streptozotocin was dissolved in sodium citrate buffer (pH: 4.5) and injected i.p. in a dose of 50 mg/kg to the remainder of the animals. The diabetic animals were injected with either saline (Streptozotocin group; $n=15$) or vitamin E (Vitamin E group; $n=15$) in a dose of 125 mg/kg/day. Vitamin E was dissolved in olive oil and injected i.p. All protocols described were reviewed and approved by the Local Institutional Committee for the Ethical Use of Animals.

All rats were then killed by decapitation after 6 weeks. The brain tissues were removed and the hippocampus, cerebral cortex and cerebellum were dissected. Samples were used fresh or kept at −70 °C.

2.4. Western blot

Fresh or frozen tissue samples were homogenised in 10 mM Tris–HCl (pH 7.4), 0.1 mM NaCl, 0.2 mM phenylmethylsulphonyl fluoride, 5 mM EDTA, 2 mM β-mercaptoethanol, 1% Triton X-100 containing protein inhibitors and centrifuged at 40,000 × *g* for 60 min at 4 °C, supernatants were collected, aliquoted and stored at −70 °C until used.

Sodium dodecyl sulfate (SDS)–polyacrylamide gradient gel electrophoresis was performed as described previously (Laemmli, 1970; Nedzvetskii et al., 1986). Samples and standard protein markers were submitted to SDS–polyacrylamide gradient gel and separated proteins were transferred to nitrocellulose filters (Schleich and Schuell, USA) by electrophoresis. Nonspecific binding was blocked by incubation with 1% bovine serum albumin. Primary antibody (rabbit anti-rat GFAP antibody) was diluted in a buffer containing 0.05% Tween-20. Blots were visualised using diaminobenzidine and peroxide-conjugated goat anti-rabbit immunoglobulin. S-100B protein was determined in the same supernatants by using anti S-100B antibody.

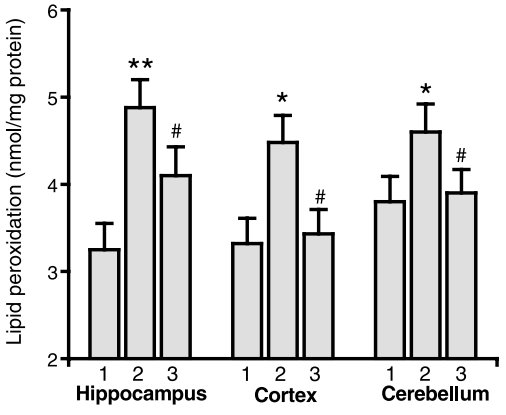

Fig. 1. Lipid peroxidation levels as malondialdehyde + 4-hydroxyalkenals in the various regions of rat brain (1 = Control; 2 = STZ-diabetic rats; 3 = STZ + Vitamin E group; *$P<0.05$; **$P<0.01$ vs. control; #$P<0.05$ vs. STZ-diabetic rats). Lipid peroxidation elevated in diabetic rats and vitamin E inhibited increases in lipid peroxidation level.

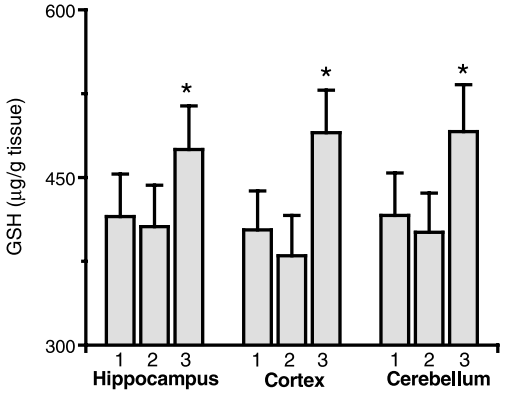

Fig. 2. Glutathione (GSH) levels in different brain areas of control, STZ-diabetic rats and vitamin E treated diabetic rats (*$P<0.05$; vs. STZ group). Vitamin E treatment markedly elevated GSH contents in all brain regions.

2.5. Tissue total protein, lipid peroxidation and reduced glutathione (GSH) concentration assay

Protein determinations were performed according to the Lowry procedure using a protein assay kit (Sigma, Deisenhofen, Germany). Tissue lipid peroxidation (as malondialdehyde + 4-hydroxyalkenals) was determined using an LPO-586 kit (Oxis, Int., OR, USA). GSH levels were determined according to the method of Ellman (1959).

2.6. Statistical analysis

The data are expressed as means ± S.D.; significance of differences between groups was evaluated by means of two-way analysis of variance in conjunction with Bonferroni's *t*-statistics, and *p*-values <0.05 were considered statistically significant.

3. Results

3.1. Effects of streptozotocin diabetes and vitamin E on the lipid peroxidation and GSH levels in different parts of the brain

Streptozotocin-induced diabetes caused a significant rise in malondialdehyde + 4-hydroxyalkenals levels in hippocampus, cortex and cerebellum as compared to the control values (Fig. 1). Administration of vitamin E to diabetic rats inhibited lipid peroxidation (Fig. 1). Furthermore, it induced a remarkable increase of GSH levels in all brain regions studied (Fig. 2).

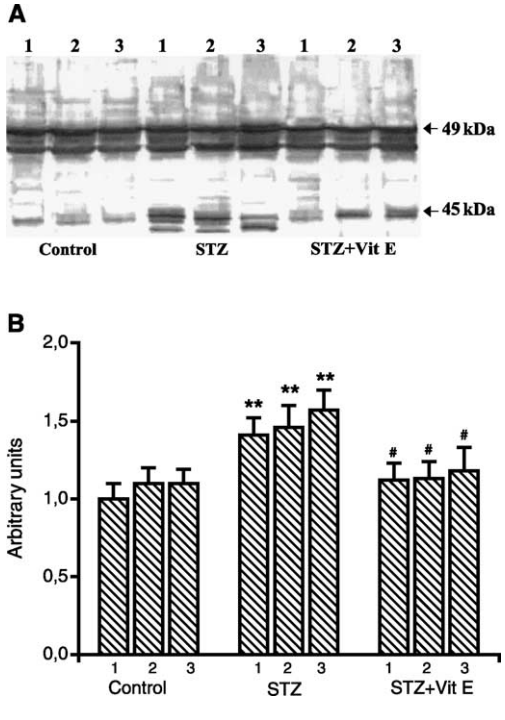

Fig. 3. (A) Western blot analysis of GFAP protein fraction prepared from cerebellar, cortical and hippocampal homogenates of control, STZ and STZ + vitamin E groups (lane 1 hippocampus, lane 2 cortex and lane 3 cerebellum). Densitometric analysis of total GFAP in the protein fraction from three brain regions is shown in (B). Significant elevation in total GFAP content was observed in the hippocampus, cortex and cerebellum in rats with diabetes. GFAP contents were decreased by administration of vitamin E (**$P<0.01$, vs. control values; #$P<0.05$ vs. STZ-diabetic rats).

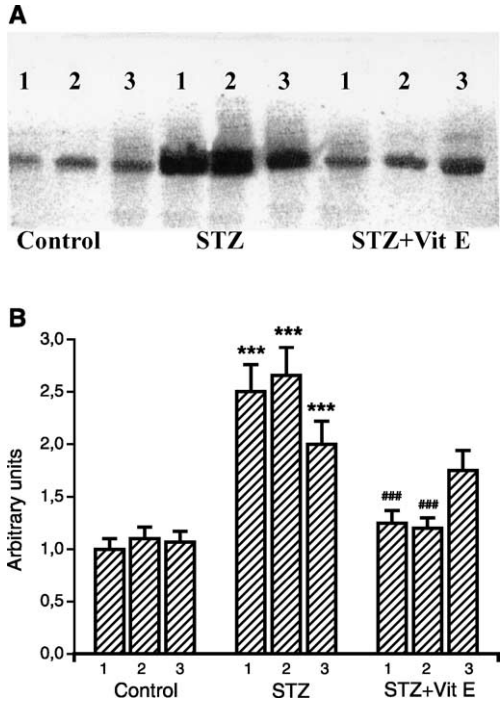

Fig. 4. (A) Western blot of S-100B protein from hippocampus, cortex and cerebellum of control, STZ and STZ + vitamin E groups (lane 1 hippocampus, lane 2 cortex and lane 3 cerebellum). Densitometric analysis of S-100B protein bands from three brain regions is shown in (B). Significant elevation in S-100B content was observed in the different brain parts. Treatment with vitamin E reduced S-100B protein contents (***$P<0.01$, vs. control values; ###$P<0.001$ vs. STZ-diabetic rats).

Table 1
Correlation between densitometric results of glial markers (GFAP and S-100B) and lipid peroxidation levels of different brain parts of streptozotocin- and vitamin E-treated rats

		GFAP–lipid peroxidation		S-100B–lipid peroxidation	
		r	*P*	*r*	*P*
STZ group	Hippocampus	0.680	0.001	0.570	0.01
(*n* = 15)	Cortex	0.640	0.01	0.610	0.01
	Cerebellum	0.590	0.01	0.540	0.05
Vitamin E group	Hippocampus	0.637	0.01	0.605	0.01
(*n* = 15)	Cortex	0.602	0.01	0.680	0.001
	Cerebellum	0.435	0.05	0.235	N.S.

STZ group: streptozotocin-induced diabetic rats; Vitamin E group: vitamin E-treated diabetic rats; N.S.: nonsignificant.

3.2. Effects of experimental diabetes and vitamin E treatment on GFAP contents and its degradation products

Western blotting analysis of GFAP demonstrated significantly higher over expression in the hippocampus, cortex and cerebellum of streptozotocin-diabetic rats in comparison with the control group. In addition to the main GFAP 49 kDa protein bands, degradation products of GFAP were also significantly increased in these brain regions of streptozotocin group. The beneficial effects of the antioxidant vitamin E treatment were also manifested by down-regulation of GFAP and its degradation products (Fig. 3A, B).

3.3. Effects of experimental diabetes and vitamin E on S-100B protein levels in brain tissues

S-100B contents were also significantly elevated in protein homogenates of hippocampus, cortex and cerebellum in streptozotocin-induced diabetic rat brain. Like those of GFAP, S-100B protein levels were reduced by the treatment with vitamin E (Fig. 4A, B). The reduction in S-100B protein levels in cerebellum by vitamin E treatment was less than that in other parts of the brain. The results from densitometric analysis of GFAP and S-100B protein bands were correlated with the levels of malondialdehyde + 4-hydroxyalkenals and a significantly positive correlation was found between these two glial markers and lipid peroxidation levels in both streptozotocin- and vitamin E-treated groups (Table 1), a slight but nonsignificant correlation was only found between S-100B protein levels and lipid peroxidation in the cerebellum ($r = 0.235$; $p > 0.05$).

4. Discussion

The current study expanded findings demonstrating that diabetes significantly elevates lipid peroxidation levels in many brain regions. These findings are in agreement with our previous results (Baydas et al., 2002; Celik et al., 2002; Ercel et al., 1999). Furthermore, we now found that diabetes induces glial reactivity by increasing expression and degradation of GFAP and S-100B protein in the many parts of brain tissue.

In neurodegenerative diseases, oxidative stress both initiates and drives the progression of the pathogenic process (Coyle and Puttfarcken, 1993) and many of the diabetic complications such as diabetic neuropathy are believed to be a result of excessive accumulation of reactive oxygen species and of a decreased antioxidant defense system (Kesavulu et al., 2000).

Many studies have evaluated the effects of oxidative stress and antioxidant systems in the central and peripheral nervous system in diabetes mellitus (Baydas et al., 2002; Celik et al., 2002; Ercel et al., 1999). Excessive production of free radicals is believed to be involved in many diabetic complications, including diabetic neuropathy in diabetes mellitus (Sima and Sugimoto, 1999). It is known that, like other cells, neurones can protect themselves against excitotoxic and oxidative insults. Thus, in general, studies have been focused on neurones in the central nervous system to evaluate the antioxidant defense system of the brain. On the other hand, it is obvious that glial cells express a variety of neurotrophic factors and cytokines that protect neurones from reactive oxygen species-induced neurotoxicity. Astrocytes are also known to have more antioxidant capacity than do neurones (Makar et al., 1994; Raps et al., 1989; Savolainen, 1978). Thus, they protect neurones against oxidative stress and promote neuronal survival.

The present findings show that streptozotocin-induced diabetes causes glial reactivity in many parts of the brain. Several mechanisms may account for the astrocyte reaction in streptozotocin-induced diabetes. These mechanisms include increases in the polyol pathway, protein glycation, disturbed calcium homeostasis and oxidative stress (Gispen and Biessels, 2000). Our findings support the hypothesis that the increase of GFAP content and its degradation products and also elevation of S-100B levels are responses of astrocytes to oxidative stress. A significant positive correlation between glial markers and lipid peroxidation in the brain homogenates supports this idea. In a previous study (Morgan et al., 1997), increased expression of GFAP during aging was found to be related to oxidative stress. Accumulation of reactive oxygen species promotes astrocyte reactivity and may stimulate the production of trophic factors, thus protecting neurones and/or favouring neuronal recovery.

Protective effects of vitamin E as an antioxidant in diabetes have been studied extensively (Baydas et al., 2002; Celik et al., 2002). On the other hand, glial involvement in early pathological events in diabetes mellitus and antioxidant effects against reactive gliosis has not been clarified. The current study showed glial cells respond to the diabetes by overexpression and degradation of GFAP and elevation of S-100B in a few weeks after the onset of diabetes induced with streptozotocin and administration of vitamin E showed beneficial effects via decreasing lipid peroxidation and preventing reactive gliosis. It has been reported that alpha

tocopherol decreased reactive gliosis by scavenging free radicals in cultures of rat brain (Halks-Miller et al., 1986).

Astrocytes exhibit the earliest cellular responses by overexpression of GFAP following an insult to the central nervous system (Norenberg, 1994). The present findings demonstrate that, in addition to its direct protective effects on neurones, vitamin E also has beneficial effects on glial cell lines against chemical and/or metabolic insults to the brain. Its protective effects on nerve tissues are scavenging free radicals and stabilising neuronal and glial cell membranes. Vitamin E exerts its effect in at least two ways; first, it inhibits lipid peroxidation and protects membranes. Second, as we have shown here, vitamin E administration increases levels of GSH, an intracellular antioxidant, and the activity of enzymes involved in GSH metabolism (Baydas et al. 2002). These findings with the present results support the notion that vitamin E exerts neuroprotection by enhancing the defense system of glial cells, which envelop neurones.

In conclusion, the increases in levels of lipid peroxidation parallel the elevation and degradation of glial markers in streptozotocin-induced diabetic rats, proving that elevated glial reactivity may be associated with oxidative stress in diabetes. Furthermore, the fact that reactive gliosis as indicated by increased levels of GFAP was prevented by vitamin E administration also suggests that this response is probably mediated by oxidative stress and antioxidants are beneficial to prevent reactive gliosis in diabetes mellitus.

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