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ORIGINAL RESEARCH

Diabetes Alters Aromatase Enzyme Levels in Sciatic Nerve and Hippocampus Tissues of Rats

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Abstract Diabetes mellitus (DM) is associated with increased risk of impaired cognitive function. Diabetic neuropathy is one of the most common and important complications of DM. Estrogens prevent neuronal loss in experimental models of neurodegeneration and accelerate nerve regeneration. Aromatase catalyzes the conversion of androgens to estrogens and expressed in a variety of tissues including neurons. Although insulin is known to regulate the activity of aromatase there is no study about the effects of diabetes on this enzyme. Present study was designed to investigate the effects of experimental diabetes on aromatase expression in nervous system. Gender-based differences were also investigated. Rats were injected with streptozotocin to induce diabetes. At the end of 4 and 12 weeks sciatic nerve and hippocampus homogenates were prepared and evaluated for aromatase proteins. Aromatase expressions in sciatic nerves of both genders were decreased in 4 weeks of diabetes, but in 12 weeks the enzyme levels were increased in females and reached to control levels in male animals. Aromatase levels were not altered in hippocampus at 4 weeks but increased at 12 weeks in female diabetic rats. No significant differences were observed at enzyme levels of hippocampus in male diabetic rats. Insulin therapy prevented all diabetes-induced changes. In conclusion, these results indicated for the first time that, DM altered the expression of aromatase both in central and peripheral nervous systems. Peripheral nervous system is more vulnerable to damage than central nervous system in diabetes. These effects of diabetes differ with gender and compensatory neuroprotective mechanisms are more efficient in female rats.

Keywords Diabetes mellitus · Aromatase · Hippocampus · Sciatic nerve · Neuroprotection · Gender difference · Rat

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Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia. Type 1 diabetes, which generally develops at young age, is characterized by an autoimmune destruction of the beta cells, resulting in an inability of the pancreas to secrete insulin and in turn in a dependency of exogenous insulin treatment. Type 2 diabetes is characterized with insulin resistance, leading to a relative insulin deficiency. The incidence of type 2 diabetes strongly increases with age. The primary goal of treatment in diabetes is to avoid hyperglycemia, which is responsible for the development of microvascular complications involving the eyes (retinopathy), kidneys (nephropathy) and nerves (neuropathy), and macrovascular complications involving the heart (Wessels et al. 2008). Diabetic neuropathy is also one of the most common and important complication (Said 2007). In addition DM is associated with damage to the central nervous system and cognitive deficits. DM leads to functional and structural changes in the brain, which appear to be most pronounced in the elderly. Impairment of learning and memory has been documented both in type 1 and 2 diabetes (Awad et al. 2004; Pasquier et al. 2006). It has been shown that insulin affects several brain functions including cognition and memory.

Aromatase (cytochrom P450 19A1) is a cytochrom P450 enzyme that catalyzes the biosynthesis of estrogens from

C19 steroids (androgens) and is expressed in several tissues including brain, gonads, adipose tissue, bone, and skin of most vertebrate species (Simpson et al. 1997). Aromatase is expressed in neurons under basal conditions and is upregulated after injury (Garcia-Ovejero et al. 2005). Extragonadal estrogen biosynthesis plays important physiological and pathophysiological roles (Simpson 2003). Estrogen synthesis is shown to take place in the hippocampus and estrogen concentrations in the hippocampus are found to be considerably higher than physiological serum levels (sixfold higher than in serum) (Hojo et al. 2004). Estrogens regulate gene expression, neuronal survival, neuronal and glial differentiation, synaptic transmission in many central nervous system areas (Bicknell 1998; Carrer and Cambiasso 2002; Garcia-Segura et al. 1999; Gorski 2002; Lee and McEwen 2001; Roof and Hall 2000) and have neurotrophic and neuroprotective properties (Garcia-Segura et al. 2001; Wise 2002). Strong biological evidence supports the benefits of estrogens in several experimental models of neurodegeneration (Garcia-Segura et al. 2001). Beneficial effects of estrogens in peripheral nervous system are also shown in several studies (Islamov et al. 2002; Islamov et al. 2003; Nachemson et al. 1985). Although insulin is known to regulate the activity of aromatase (Garzo and Dorrington 1984; Nestler 1993) there is no data about the immunoreactivity levels of this enzyme in diabetes and also the levels of aromatase in hippocampus and sciatic nerves of diabetic animals.

This study was designed to investigate the effects of experimentally induced diabetes on aromatase expression in nervous system. We also addressed the question whether gender-based difference alters the aromatase expression.

Methods

Animals

Experimental diabetes was induced in male and female Sprague–Dawley rats (200–250 g). All animal procedures were conducted in accordance with the institutional guidelines for care and use of laboratory animals. Rats were housed under a 12 h light/dark cycle with food and water available continuously. The experimental protocols were approved by the Hacettepe University Animal Ethics Committee (2006/8-6).

Induction of Diabetes

Short (4 weeks)- and long (12 weeks)-terms of experimentally diabetic rats were used in experiments. Diabetes was induced by a single i.v. injection of streptozotocin (STZ) (35 mg/kg) in 0.1 M citrate buffer (pH: 4.5). Animals received 10% glucose in drinking water for the first 24 h. Non-diabetic rats were injected with the same volume of citrate buffer as control vehicle. Hyperglycemia was confirmed 72 h after STZ injection by measuring tail vein blood glucose levels using a glucometer. Animals with mean plasma glucose levels above 250 mg/dl were classified as diabetic. Glycemia was also confirmed at scheduled death, 4 or 12 weeks after STZ injection. 24 h before exsanguination, diethylstilbestrol (0.1 mg/kg, s.c.) were injected to female rats for initiating estrus cycle.

In insulin-treatment group 7 days after STZ injection, male and female diabetic rats received subcutaneous insulin implants (Linplants; LinShin Canada, Scarborough, Toronto, ON, Canada). The implants released insulin (in microrecrystallized palmitic acid) at $\sim$2 units/24 h for $\sim$50 days. The implants were inserted into the right abdominal region of rats through a trocar under light ketamine–xylazine (45:5 mg/kg, respectively) anesthesia. Within 24–72 h, rats with insulin implants that had glucose levels $\leq$110 mg/dl were considered to be normoglycemic and designated as the insulin-treatment group. Linplants were replaced every 6 weeks. The control rats were sham operated.

Western Analysis of Aromatase Protein Levels

At the end of 4 and 12 weeks the animals were killed by decapitation and the tissues were removed, rapidly frozen in liquid nitrogen and stored in -80°C deepfreezer until use. Hippocampus and sciatic nerve tissues were homogenated with lysis buffer on ice. The lysis buffer of hippocampus contained 20 mM Tris HCl (pH: 7.5), 1 mM EDTA, 5 mM $MgCl_2$, 5 mM DTT, 1 mM PMSF and protease inhibitor cocktail. The lysis buffer of sciatic nerve contained SDS (1%) and 1 mM PMSF. The lysates were centrifugated at 14,000$\times g$ for 20 min at 4°C. Total protein concentrations of the supernatants were determined by Lowry protein assay. Equal amount of total protein of each sample was diluted in sample buffer to perform the SDS-PAGE with 10% acrylamide/bisacrylamide gels. The proteins were transferred electrophoretically to polyvinylidendifluoride (PVDF) membranes. The equality of total protein loaded on each lane was confirmed by staining the membranes with Poncae S after transfer. PVDF membranes were blocked with 5% low-fat dried milk powder dissolved in Tris buffer saline that contained 0.1% Tween 20 (TBS-T) for 2 h at room temperature. The membranes were incubated with aromatase antibody (diluted 1:5000 in TBS-T contained 1% BSA) for 1 h and washed 3 times for 15 min in TBS-T. Finally membranes were incubated with goat anti-mouse antibody (diluted 1:5000 in TBS-T

contained 5% milk and 1% BSA) for 1 h and then washed with TBS-T 3 times for 15 min in room temperature. Immunoreactive bands were detected by the enhanced chemiluminescence visualization system.

The same samples were probed with β-actin antibody as an internal control to ensure equal protein loading in all lanes. Rat ovary extract is used as positive control.

Data Analysis and Statistical Procedures

Blots were measured by Scion Image (MD, USA) and data are expressed as relative density of control. One way ANOVA is used for statistical analysis. Tukey was used as post hoc test. Data are expressed as means ± SEM and n indicates the number of animals tested. $P<0.05$ was considered significant.

Drugs and Chemicals

Aromatase antibody was purchased from Acris, DE. Enhanced chemiluminescence kit (ECLplus) and films were obtained from Amersham International (Amersham, Buckinghamshire, UK) and Pierce. Bovine serum albumin (BSA) was purchased from Sigma (St. Louis, USA). Horseradish peroxidase-conjugated secondary antibody, goat anti-mouse IgG, β-actin antibody, PVDF membrane, enhanced chemiluminescence lighting system and protein molecular marker were products of Santa Cruz Biotechnology Inc. (Santa Cruz, USA). DC Protein assay reagents A and B for Lowry protein assay were obtained from Bio-Rad Laboratories (Richmond, USA). Coomassie Brilliant Blue G-250 was purchased from AppliChem GmbH, USA. Protease inhibitor cocktail tablets were obtained from Roche Diagnostics, USA.

Results

Body Weights and Blood Glucose Levels

At the end of 4 and 12 weeks periods, final blood glucose levels in STZ-treated rats were significantly higher than vehicle-treated control animals and insulin-treated animals (Table 1). The initial body weights of all groups were similar. However, final body weights of diabetic animals were significantly reduced when compared to non-diabetic control and insulin-treated animals (Table 1).

Short- and Long-Terms of Diabetes-Induced Changes on Aromatase Expression in Female Rats

Aromatase expression in sciatic nerve homogenates of 4 weeks diabetic female rats was significantly decreased when compared with age-matched non-diabetic group (Fig. 1; $n = 6$, $P < 0.05$) but was increased significantly in 12-weeks diabetic animals (Fig. 1; $n = 6$, $P < 0.05$). Insulin therapy reversed these changes to control levels (Fig. 1; $n = 4$). In hippocampal brain extracts aromatase levels were not altered at 4 weeks (Fig. 2; $n = 4$) but significantly increased at 12 weeks in female diabetic rats compared with age-matched non-diabetic group (Fig. 2; $n = 4$, $P < 0.05$). In insulin-treated animals there were no significant changes observed when compared with control animals (Fig. 2; $n = 5$).

Short- and Long-Terms of Diabetes-Induced Changes on Aromatase Expression in Male Rats

Aromatase expression in sciatic nerves of male rats was decreased at 4 weeks of diabetes (Fig. 3; $n = 5$, $P < 0.05$), and reached to control levels at 12 weeks (Fig. 3; $n = 3$).

Table 1 Body weights and plasma glucose concentrations for diabetic, age-matched non-diabetic, and insulin-treated animals

Groups	Blood glucose at sacrifice (mg/dl)	Body weight initial (g)	Body weight at sacrifice (g)
Non-diabetic group ♀, 4 weeks	93.1 ± 6.2	220 ± 3.6	248.7 ± 3.1
Diabetic group ♀, 4 weeks	349.6 ± 30.9[a]	225 ± 4.2	210 ± 10.8[a]
Non-diabetic group ♀, 12 weeks	99.8 ± 7.4	226.6 ± 5.42	280 ± 9.5
Diabetic group ♀, 12 weeks	448 ± 27[b,c]	210 ± 3.6	156.6 ± 9.5[b,c]
Insulin-treatment ♀, 12 weeks	72.5 ± 10.9	230 ± 7.3	293.3 ± 4.21
Non-diabetic group ♂, 4 weeks	89.8 ± 4.7	251.6 ± 3	258.7 ± 4.2
Diabetic group ♂, 4 weeks	406.6 ± 27.3[d]	250 ± 2.5	198.7 ± 4.2[d]
Non-diabetic group ♂, 12 weeks	93.5 ± 13.7	253.3 ± 7.1	405 ± 19.4
Diabetic group ♂, 12 weeks	416.6 ± 20.2[e,f]	245 ± 4.5	236 ± 15.8[e,f]
Insulin-treatment ♂, 12 weeks	88.2 ± 7.9	246 ± 4.2	398.3 ± 11.37

[a] Significantly different from 4 weeks non-diabetic ♀ group; [b] significantly different from 12 weeks non-diabetic ♀ group; [c] significantly different from insulin-treatment ♀ group; [d] significantly different from 4 weeks non-diabetic ♂ group; [e] significantly different from 12 weeks non-diabetic ♂ group; [f] significantly different from insulin-treatment ♂ group ($P < 0.05$). Data are shown as means ± SEM

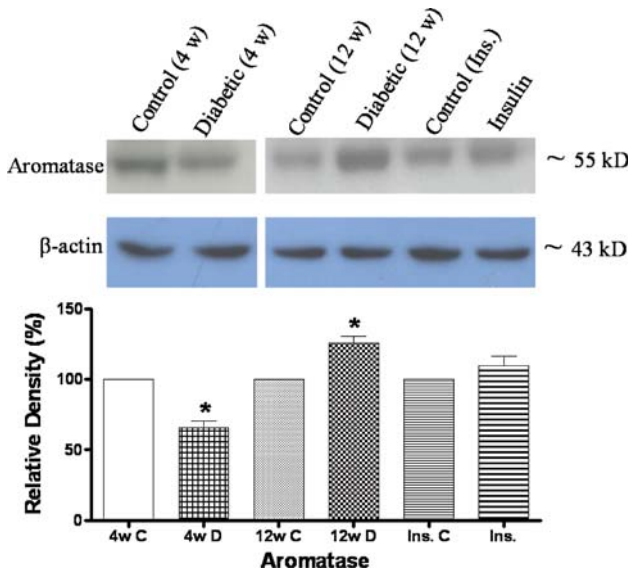

Fig. 1 Western blot analysis of aromatase protein expression in sciatic nerves of 4 weeks control (*4w C*), 4 weeks diabetic (*4w D*), 12 weeks control (*12w C*), 12 weeks diabetic (*12w D*), insulin control (*Ins. C*), and insulin-treated (*Ins.*) female rats. The immunoreactivity of aromatase was expressed as relative density of control group values. Data are expressed as means ± SEM. $n = 4$–6. * $P < 0.05$ was considered significant. β-Actin was used as a loading control

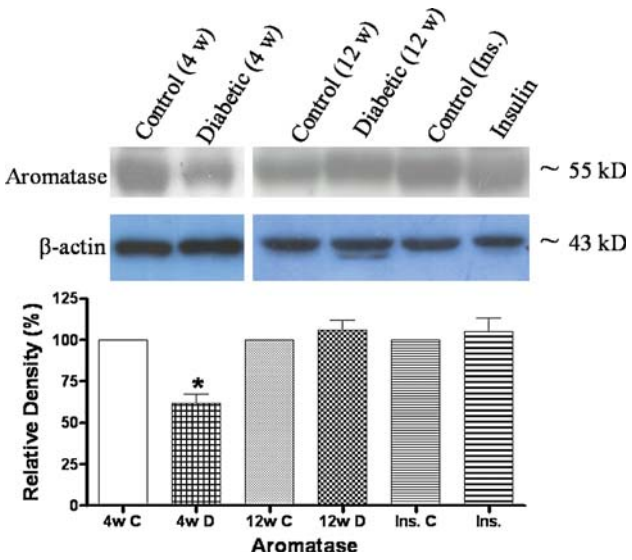

Fig. 3 Western blot analysis of aromatase protein expression in sciatic nerves of 4 weeks control (*4w C*), 4 weeks diabetic (*4w D*), 12 weeks control (*12w C*), 12 weeks diabetic (*12w D*), insulin control (*Ins. C*), and insulin-treated (*Ins.*) male rats. The immunoreactivity of aromatase was expressed as relative density of control group values. Data are expressed as means ± SEM. $n = 3$–5. * $P < 0.05$ was considered significant. β-Actin was used as a loading control

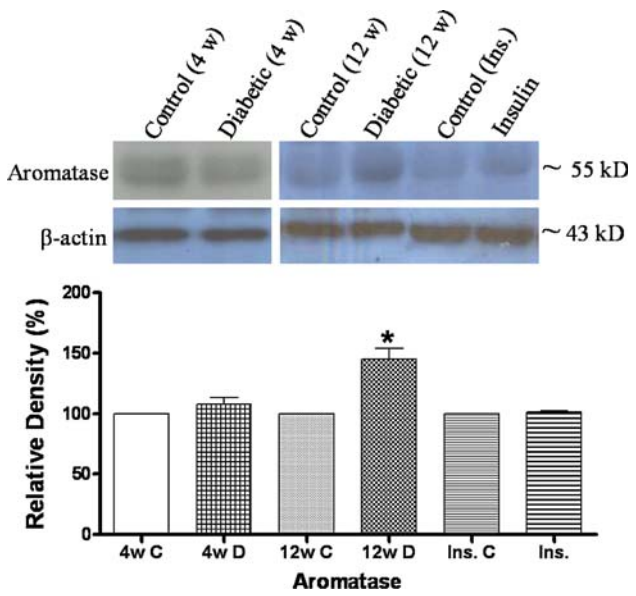

Fig. 2 Western blot analysis of aromatase protein expression in hippocampal tissues of 4 weeks control (*4w C*), 4 weeks diabetic (*4w D*), 12 weeks control (*12w C*), 12 weeks diabetic (*12w D*), insulin control (*Ins. C*), and insulin-treated (*Ins.*) female rats. The immunoreactivity of aromatase was expressed as relative density of control group values. Data are expressed as means ± SEM. $n = 4$–5. * $P < 0.05$ was considered significant. β-Actin was used as a loading control

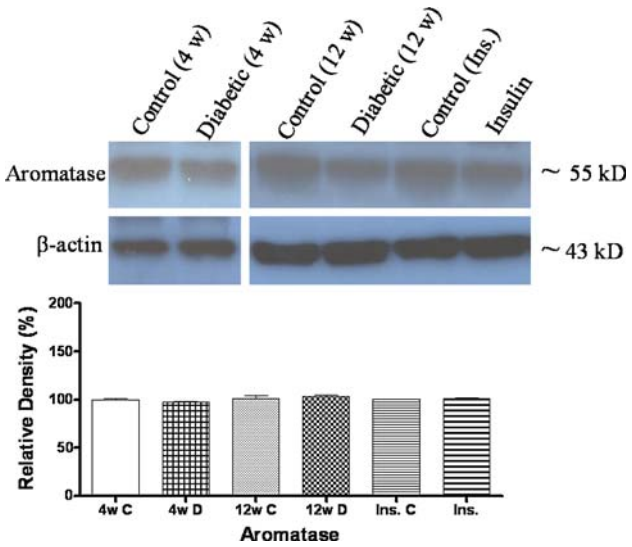

Fig. 4 Western blot analysis of aromatase protein expression in hippocampal tissues of 4 weeks control (*4w C*), 4 weeks diabetic (*4w D*), 12 weeks control (*12w C*), 12 weeks diabetic (*12w D*), insulin control (*Ins. C*), and insulin-treated (*Ins.*) male rats. The immunoreactivity of aromatase was expressed as relative density of control group values. Data are expressed as means ± SEM. $n = 4$. β-Actin was used as a loading control

Insulin therapy reversed these changes to control levels (Fig. 3; $n = 5$). In hippocampal brain extracts of male rats aromatase levels were not altered in both 4 and 12 weeks diabetic animals compared with age-matched non-diabetic group (Fig. 4; $n = 4$). There were no significant changes observed in insulin-treated diabetic rats compared with non-diabetic sham operated control animals (Fig. 4; $n = 4$).

Discussion

Diabetes mellitus is characterized by hyperglycemia and associated with neurodegeneration, neuropathy, and impaired cognitive function. Central conversion of androgens to estrogens is known to play an important role in many physiological processes. Estrogens have numerous reproductive and non-reproductive functions in the brain. It has been reported that, after brain injury, aromatase and estrogen receptor expressions are up-regulated and also shown that local estrogen synthesis is involved in neuroprotection (Azcoitia et al. 2001; Garcia-Segura et al. 2001; Veiga et al. 2003). Aromatase is responsible for catalyzing the conversion of androgens to estrogens and is the rate-limiting step in estrogen biosynthesis (Simpson et al. 1997). This study was designed to investigate the effects of experimental diabetes on aromatase expression in hippocampus and sciatic nerve.

It has been demonstrated that insulin could alter aromatase activity (Garzo and Dorrington 1984; Nestler 1993). However, the effects of DM on aromatase enzyme have been investigated only in a few studies. In those studies aromatase activity or expression levels have not been measured directly, only the estrogen and/or testosterone (androgen) levels have been measured assuming that aromatase levels were altered (Garzo and Dorrington 1984; McRobie et al. 1997; Stamataki et al. 1996). To our knowledge this is the first study that investigated the aromatase expression levels directly in DM. Insulin exert a selective inhibitory effect on cytotrophoblastic aromatase activity in diabetic pregnant women (Nestler 1993) and conversely has been shown to augment the aromatase activity of human granulosa cells (Garzo and Dorrington 1984). It is possible that insulin might have affected various cell types differently. Garzo and Dorrington (1984) have also shown that insulin stimulates the activity of the enzyme aromatase in vitro. McRobie et al. (1997) have investigated the placental aromatase activity in diabetes and have found that the presence of diabetes during pregnancy does not affect placental aromatase activity. In another study Stamataki et al. (1996) have suggested that, in women with NIDDM the ovaries have a reduced ability to convert androgens to estrogens, probably due to a reduction of ovarian aromatase activity. Also in another part of our study we found that the aromatase expression in ovary was reduced in diabetic rats (unpublished data).

Brain is susceptible for the effects of diabetes. It is well-known that cognitive impairments are more common in diabetic patients than non-diabetics (Brands et al. 2005; Ryan et al. 1992, 2003). Numerous neuropsychological studies have delineated the nature and extent of these cognitive changes, but little is known about the underlying mechanism that may explain the cognitive decline. We hypothesized that aromatase enzyme might be one of the important molecules in DM-induced cognitive disorders.

It is well-established by numerous studies that estrogens have neurotrophic and neuroprotective properties (Bisagno et al. 2003; Garcia-Segura et al. 2001; Green et al. 1997; Wise 2002). It increases survival of neurons in vitro (Green et al. 1997), and protects neurons in vivo (Garcia-Segura et al. 2001). Moreover, it has been shown that the brain regulates estrogen synthesis at sites of injury. Findings of Rune and Frotscher (2005) confirmed that estradiol regulates synapse formation and hippocampus-derived estradiol is essential for the maintenance of hippocampal spine synapses and synaptic plasticity. Estradiol and testosterone prevent neuronal loss in several experimental animal models of neurodegeneration in the central nervous system (Garcia-Segura et al. 2001). In the study of Azcoita et al. (2001) aromatase knock-out male mice and their wild type control group have been injected with a low dose neurotoxic agent domoic acid that does not induce neurodegeneration in the hippocampus of normal mice and the number of neurons was than assessed in the hilus of dentate gyrus. They have found that aromatase deficiency increased the vulnerability of hilar neurons to neurotoxic degeneration. In our study aromatase levels in hippocampal brain extracts were not altered at 4 weeks both in female and male rats but conversely increased significantly at 12 weeks only in female diabetic rats compared with age-matched non-diabetic animals. Insulin treatment prevented the changes observed in aromatase levels. It could be suggested that in female rats the enhanced levels of aromatase and accordingly estradiol levels in hippocampus might be a compensatory mechanism against diabetes-induced neurodegeneration in central nervous system. In male animals this compensatory mechanism is not active at least in 12-weeks diabetes.

Diabetic neuropathy, is one of the most common and important complications of DM occurring in more than 50% of patients and affects their quality of life (Said 2007). Although a number of studies have examined the mechanisms underlying hyperglycemia-induced nerve damage, the pathogenesis of diabetic neuropathy remains unclear. Multiple physiopathologic mechanisms have been proposed including; microvascular damage of the nerve, increased activity of aldose reductase, generation of free radicals, and decreased levels of neural growth factor in serum (Faradji and Sotelo 1990; Leinninger et al. 2006; Said 2007). Recent observations have indicated that peripheral nerves are able to synthesize and metabolize neuroactive steroids (Roglio et al. 2007, 2008). The effects of 17β-estradiol on the rate of regeneration of nerve fibers and the functional recovery after crush injury of the sciatic nerve in ovariectomized mice have been investigated

(Islamov et al. 2002). In estrogen-treated mice they have observed enhanced functional recovery, and accelerated growth and maturation of regenerating nerve fibers. Their results have demonstrated that estrogen promotes regeneration of the sciatic nerve after crush injury. The data of Islamov et al. (2003) suggest that estrogen could be one of the critical factors regulating transcription of genes involved in nerve regeneration and also estrogen promotes regeneration locally. In another study it is shown that after injury of sciatic nerve, estrogen suppresses the scar reaction (Nachemson et al. 1985). In our study aromatase expression in sciatic nerve homogenates of 4 weeks diabetic rats was significantly decreased when compared with age-matched non-diabetic group. In 12 weeks of diabetes enzyme levels were increased significantly in diabetic female rats and reached to control levels in diabetic male animals. Insulin therapy reversed these changes to control levels. Although the aromatase levels were decreased in short-term diabetes the enzyme levels were increased in following time period (12 weeks). According to these results it is possible that enhanced aromatase levels in long-term diabetes might be a protective mechanism against the induction of peripheral neuropathy in diabetes. Since this protective mechanism was not active in short term (4 weeks) of diabetes it can be speculated that in short term, direct effects of diabetes was potent on aromatase expression in sciatic nerves and a time period is required for the development of adaptive mechanisms. Aromatase may represent a new molecular target for the therapy or prevention of neuropathy seen in diabetes.

Gender may also be an important factor in the development of diabetic neurologic complications. Few studies have evaluated gender as a potential risk factor for the development of neurologic symptoms in patients with diabetes and several studies reviewed the effects of gender on the severity of the signs and symptoms and the frequency of diabetic neuropathy (Aaberg et al. 2008). It is clear from previous studies that males have more severe symptoms related to diabetic neuropathy as compared to females and that males have a higher frequency of diabetic neuropathy. Female animals are protected from many forms of neurological injury and degeneration relative to their male counterparts, in part attributable to their estrogens (Alkayed et al. 1998). Furthermore, the investigation of Aaberg et al. (2008) demonstrated that males developed diabetic neuropathic complications earlier than did females. As estrogens have neuroprotective effects we decided to investigate how gender differences affect the aromatase levels in experimentally induced type-1 DM. Similarly in our study we have shown that DM affected aromatase expression levels differently in female and male rats. In the present study, in long term of experimental DM aromatase levels were increased in female rats both in central and peripheral nervous systems. In male rats aromatase levels in peripheral nervous system were significantly enhanced in 12 weeks compared to 4 weeks of DM but the enzyme levels were not different from control levels at the end of 12 weeks. Aromatase levels of hippocampus were similar both in short- and long-terms of diabetes in male animals. According to these results we have suggested that the reason of the vulnerability of males to diabetic neurodegeneration than females might be related with the difference of aromatase expression of both genders in DM. The increased levels of aromatase might be a defense mechanism against neurodegeneration and these compensatory neuroprotective mechanisms are more efficient in female rats.

It is known that Alzheimer's disease (AD) is associated with peripheral and central insulin abnormalities. Insulin has been shown to regulate the metabolism of Aβ degradation and the phosphorylation state of tau protein (Gasparini et al. 2002; Hoyer 2002). Several studies implicate a biological relationship between diabetes and AD which are two major disorders affecting a large population world-wide (Awad et al. 2004; Moreira et al. 2007). Together with these results, it can be speculated that altered levels and/or activity of aromatase in hippocampus may be an important link between AD and DM. Since aromatase is expressed in the adult human brain, including the hippocampus (Stoffel-Wagner 2001), besides the control of hyperglycemia, therapies focused on aromatase and estrogen might be beneficial against the neurodegeneration, impaired cognitive function and development of AD especially in elderly diabetic patients.

In conclusion, these results indicated for the first time that; DM alters the expression of aromatase both in central and peripheral nervous systems and these effects of diabetes differ with gender. The increased levels of aromatase might be a defense mechanism against to neurodegeneration and these compensatory neuroprotective mechanisms are more efficient in female rats. In the future aromatase might be an important target molecule for the treatment of the neurodegeneration and neuropathy seen in DM.

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References

Aaberg ML, Burch DM, Hud ZR, Zacharias MP (2008) Gender differences in the onset of diabetic neuropathy. J Diabetes Complicat 22:83–87

Alkayed NJ, Harukuni I, Kimes AS, London ED, Traystman RJ, Hurn PD (1998) Gender-linked brain injury in experimental stroke. Stroke 29:159–165, discussion 66

Awad N, Gagnon M, Messier C (2004) The relationship between impaired glucose tolerance, type 2 diabetes, and cognitive function. J Clin Exp Neuropsychol 26:1044–1080

Azcoitia I, Sierra A, Veiga S, Honda S, Harada N, Garcia-Segura LM (2001) Brain aromatase is neuroprotective. J Neurobiol 47: 318–329

Bicknell RJ (1998) Sex-steroid actions on neurotransmission. Curr Opin Neurol 11:667–671

Bisagno V, Bowman R, Luine V (2003) Functional aspects of estrogen neuroprotection. Endocrine 21:33–41

Brands AM, Biessels GJ, de Haan EH, Kappelle LJ, Kessels RP (2005) The effects of type 1 diabetes on cognitive performance: a meta-analysis. Diabetes Care 28:726–735

Carrer HF, Cambiasso MJ (2002) Sexual differentiation of the brain: genes, estrogen, and neurotrophic factors. Cell Mol Neurobiol 22:479–500

Faradji V, Sotelo J (1990) Low serum levels of nerve growth factor in diabetic neuropathy. Acta Neurol Scand 81:402–406

Garcia-Ovejero D, Azcoitia I, Doncarlos LL, Melcangi RC, Garcia-Segura LM (2005) Glia-neuron crosstalk in the neuroprotective mechanisms of sex steroid hormones. Brain Res Brain Res Rev 48:273–286

Garcia-Segura LM, Naftolin F, Hutchison JB, Azcoitia I, Chowen JA (1999) Role of astroglia in estrogen regulation of synaptic plasticity and brain repair. J Neurobiol 40:574–584

Garcia-Segura LM, Azcoitia I, DonCarlos LL (2001) Neuroprotection by estradiol. Prog Neurobiol 63:29–60

Garzo VG, Dorrington JH (1984) Aromatase activity in human granulosa cells during follicular development and the modulation by follicle-stimulating hormone and insulin. Am J Obstet Gynecol 148:657–662

Gasparini L, Netzer WJ, Greengard P, Xu H (2002) Does insulin dysfunction play a role in Alzheimer's disease? Trends Pharmacol Sci 23:288–293

Gorski RA (2002) Hypothalamic imprinting by gonadal steroid hormones. Adv Exp Med Biol 511:57–70, discussion 3

Green PS, Bishop J, Simpkins JW (1997) 17 alpha-estradiol exerts neuroprotective effects on SK-N-SH cells. J Neurosci 17:511–515

Hojo Y, Hattori TA, Enami T, Furukawa A, Suzuki K et al (2004) Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017alpha and P450 aromatase localized in neurons. Proc Natl Acad Sci USA 101:865–870

Hoyer S (2002) The brain insulin signal transduction system and sporadic (type II) Alzheimer disease: an update. J Neural Transm 109:341–360

Islamov RR, Hendricks WA, Jones RJ, Lyall GJ, Spanier NS, Murashov AK (2002) 17Beta-estradiol stimulates regeneration of sciatic nerve in female mice. Brain Res 943:283–286

Islamov RR, Hendricks WA, Katwa LC, McMurray RJ, Pak ES et al (2003) Effect of 17 beta-estradiol on gene expression in lumbar spinal cord following sciatic nerve crush injury in ovariectomized mice. Brain Res 966:65–75

Lee SJ, McEwen BS (2001) Neurotrophic and neuroprotective actions of estrogens and their therapeutic implications. Annu Rev Pharmacol Toxicol 41:569–591

Leinninger GM, Edwards JL, Lipshaw MJ, Feldman EL (2006) Mechanisms of disease: mitochondria as new therapeutic targets in diabetic neuropathy. Nat Clin Pract Neurol 2:620–628

McRobie DJ, Korzekwa KR, Glover DD, Tracy TS (1997) The effects of diabetes on placental aromatase activity. J Steroid Biochem Mol Biol 63:147–153

Moreira PI, Santos MS, Seica R, Oliveira CR (2007) Brain mitochondrial dysfunction as a link between Alzheimer's disease and diabetes. J Neurol Sci 257:206–214

Nachemson AK, Lundborg G, Myrhage R, Rank F (1985) Nerve regeneration and pharmacological suppression of the scar reaction at the suture site. An experimental study on the effect of estrogen-progesterone, methylprednisoloneacetate and cis-hydroxyproline in rat sciatic nerve. Scand J Plast Reconstr Surg 19:255–260

Nestler JE (1993) Regulation of the aromatase activity of human placental cytotrophoblasts by insulin, insulin-like growth factor-I, and -II. J Steroid Biochem Mol Biol 44:449–457

Pasquier F, Boulogne A, Leys D, Fontaine P (2006) Diabetes mellitus and dementia. Diabetes Metab 32:403–414

Roglio I, Bianchi R, Giatti S, Cavaletti G, Caruso D et al (2007) Testosterone derivatives are neuroprotective agents in experimental diabetic neuropathy. Cell Mol Life Sci 64:1158–1168

Roglio I, Giatti S, Pesaresi M, Bianchi R, Cavaletti G et al (2008) Neuroactive steroids and peripheral neuropathy. Brain Res Rev 57:460–469

Roof RL, Hall ED (2000) Gender differences in acute CNS trauma and stroke: neuroprotective effects of estrogen and progesterone. J Neurotrauma 17:367–388

Rune GM, Frotscher M (2005) Neurosteroid synthesis in the hippocampus: role in synaptic plasticity. Neuroscience 136: 833–842

Ryan CM, Williams TM, Orchard TJ, Finegold DN (1992) Psychomotor slowing is associated with distal symmetrical polyneuropathy in adults with diabetes mellitus. Diabetes 41:107–113

Ryan CM, Geckle MO, Orchard TJ (2003) Cognitive efficiency declines over time in adults with type 1 diabetes: effects of micro- and macrovascular complications. Diabetologia 46:940–948

Said G (2007) Diabetic neuropathy—a review. Nat Clin Pract Neurol 3:331–340

Simpson ER (2003) Sources of estrogen and their importance. J Steroid Biochem Mol Biol 86:225–230

Simpson ER, Zhao Y, Agarwal VR, Michael MD, Bulun SE et al (1997) Aromatase expression in health and disease. Recent Prog Horm Res 52:185–213, discussion 4

Stamataki KE, Spina J, Rangou DB, Chlouverakis CS, Piaditis GP (1996) Ovarian function in women with non-insulin dependent diabetes mellitus. Clin Endocrinol (Oxf) 45:615–621

Stoffel-Wagner B (2001) Neurosteroid metabolism in the human brain. Eur J Endocrinol 145:669–679

Veiga S, Garcia-Segura LM, Azcoitia I (2003) Neuroprotection by the steroids pregnenolone and dehydroepiandrosterone is mediated by the enzyme aromatase. J Neurobiol 56:398–406

Wessels AM, Scheltens P, Barkhof F, Heine RJ (2008) Hyperglycaemia as a determinant of cognitive decline in patients with type 1 diabetes. Eur J Pharmacol 585:88–96

Wise PM (2002) Estrogens and neuroprotection. Trends Endocrinol Metab 13:229–230