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EFFECTS OF 17β-ESTRADIOL ON THE CYTOARCHITECTURE OF PYRAMIDAL CA1 NEURONS IN NORMOGLYCEMIC AND DIABETIC MALE SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract—Previous work has shown a reduction of apical dendritic length and spine density in neurons from the CA1 hippocampus subfield of spontaneously hypertensive rats (SHRs). These abnormalities are prevented by treatment for 2 weeks with 17β-estradiol. In view of the fact that diabetes and hypertension are comorbid diseases, we have now studied the effect of Streptozotocin-induced diabetes on the dendritic tree and spines of CA1 hippocampus neurons, and also compared the regulation of these parameters by 17β-estradiol in diabetic and normoglycemic SHR. Twenty-week-old male SHR received iv 40-mg/kg Streptozotocin or vehicle and studied 1 month afterward. A group of normoglycemic and hyperglycemic SHR also received sc a single 17β-estradiol pellet or vehicle for 2 weeks. Hippocampus sections were impregnated with silver nitrate following a modified Golgi's method and the arbor of CA1 pyramidal neurons analyzed by Sholl's method. 17β-Estradiol treatment of normoglycemic SHR reversed the reduced length of apical dendrites, the low spine density and additionally decreased blood pressure (BP). Diabetic SHR showed increased length of apical and basal dendrites but reduced spine density compared to normoglycemic SHR. Diabetes also decreased BP of SHR. Treatment with 17β-estradiol of diabetic SHR enhanced dendritic length, increased dendritic spine density and further decreased BP. Thus, changes of cytoarchitecture of CA1 neurons due to 17β-estradiol treatment of normoglycemic SHR persisted after diabetes induction. A decrease of BP may also contribute to the central effects of 17β-estradiol in SHR diabetic rats.

Key words: hippocampus, 17β-estradiol, dendrites, spines, diabetes mellitus, spontaneously hypertensive rat.

INTRODUCTION

Increased hippocampus vulnerability is an important consequence of hypertensive encephalopathy (Oppenheimer and Fishberg, 1928). Chronic elevation of BP causes atrophic changes, microvascular thickening with ischemia, cytotoxic edema, demyelination, beta-amyloid deposits and tau pathology of the hippocampus. These changes are accompanied by cognitive decline and increased risk of dementia (Skoog et al., 1996; Petrovitch et al., 2000; Mulvany, 2002; Korf et al., 2004; Wiseman et al., 2004; Paglieri et al., 2008).

The spontaneously hypertensive rat (SHR) model of primary hypertension shows a pronounced hippocampus pathology characterized by astrogliosis, neuronal loss, demyelination, decreased growth factor expression, decreased neurogenesis and enhanced mRNA expression of the mineralocorticoid receptor and aromatase (Sabbatini et al., 1999, 2000; Tomassoni et al., 2004; Pietranera et al., 2006, 2010, 2011, 2012). Changes of learning and memory have made SHR models for dementia and the attention-deficit hyperactivity syndrome (Paglieri et al., 2008). Functionally, the hippocampus is highly dependent on the integrity of connections within the trisynaptic circuit (Lorente de No, 1934). This neuronal connectivity of the hippocampus is compromised in SHR, as shown by the abnormal dendritic morphology of pyramidal neurons compared to normotensive Wistar-Kyoto rats (Sánchez et al., 2011; Brocca et al., 2013).

Uncontrolled diabetes mellitus also damages the hippocampus. This is reflected as disturbed memory, impaired neurogenesis, changes of gene expression, altered signaling cascades, decreased energy metabolism and poor cell survival (Saravia et al., 2002, 2004; Reagan, 2005; Revsin et al., 2005; Stranahan et al., 2008; Thomas et al., 2013). Morphological abnormalities also appear in the hippocampus of diabetic animals. Magariños et al. (2000) using the Golgi method and electron microscopy, have observed that Streptozotocin-induced diabetes causes retraction of the presynaptic mossy fiber terminals contacting the CA3 apical dendrites, in addition to synaptic vesicle depletion. They suggest that diabetes is an endogenous stressor and accelerates the effect of exogenous stress. Nitta et al. (2002) have reported in the hippocampus of diabetic rats a pronounced synaptic dysfunction, revealed by a decreased number of basal dendrites and abnormal spine structure. Lastly, impaired insulin and insulin growth factor 1 (IGF1) in BB rats is associated with neuronal apoptosis

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Abbreviations: ANOVA, analysis of variance; BP, blood pressure; GPER, G-protein-coupled receptor 30; IGF1, insulin growth factor 1; RAS, renin–angiotensin–aldosterone system; SHR, spontaneously hypertensive rats.

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and increased Bax/Bcl-x ratio in the hippocampus (Li et al., 2002).

Analysis of different parameters has shown a multifactorial derangement of the diabetic hippocampus, in which the advanced glycation end products, changes of adrenal steroid secretion and their brain receptors, increased oxidative stress, excess production of proinflammatory cytokines, loss of cholinergic neurons, development of microvasculopathy and impaired brain glucose transport play important roles (Wang et al., 2009; Ye et al., 2011; Ceretta et al., 2012; Sherin et al., 2012; Jing et al., 2013; Rocco et al., 2013; Zhang et al., 2013). These findings strengthen the view that encephalopathy of diabetes mellitus (Rowlands and Bellush, 1989; Gispen and Biessels 2000; Artola et al., 2002; Biessels et al., 2002) increases hippocampus vulnerability, resembling hypertension-induced damage.

The combined effects of hypertension plus diabetes on peripheral and central organ damage have been the subject of several studies. In humans, high BP and diabetes mellitus are considered comorbid diseases reaching an epidemic status (Yang et al., 2011). Thus, patients with hypertension are at a two- to threefold higher risk of developing diabetes mellitus than normotensive patients and viceversa (Mancia, 2005). Hypertensive patients with diabetes mellitus are more prone to developing severe cerebrovascular disease and cognitive impairment (Lago et al., 2007). Working with diabetic SHR, Tomassoni et al. (2004) have shown a potentiation of damage to the cerebrovascular tree with increased brain pathology. Yang et al. (2011) and DeVisser et al. (2011) have studied the differential impact of diabetes and hypertension in gray and white matter regions of the brain of SHR with or without Streptozotocin-induced diabetes. They have determined that white matter abnormalities are more common in diabetic animals, whereas neuronal loss requires both pathologies.

Estrogens are recognized protective factors for neurodegenerative diseases. In connection with these properties, treatment of SHR with 17β-estradiol normalizes dendritic arborization and spine number of the CA1 subfield (Brocca et al., 2013), and prevents development of abnormalities involving neurogenesis, growth factor expression, hilus neuronal number and astrocyte reactivity of the hippocampus (Pietranera et al., 2008, 2010, 2011). Therefore, 17β-estradiol protects the hippocampus from hypertensive encephalopathy. Likewise, some hippocampus parameters injured by diabetes are reversed by treatment with 17β-estradiol, which increases cell proliferation and doublecortin-positive neuroblasts in the dentate gyrus, and decreases astrogliosis of type I diabetic rodents (Saravia et al., 2004, 2006). In the cerebral cortex, 17β-estradiol reduces lipid peroxidation, and strengthens the antioxidant systems of diabetic-ovariectomized rats (Ulas and Cay, 2010), whereas chronic 17β-estradiol treatment reduces cortical and striatal infarct volume in male diabetic rats with middle cerebral artery occlusion (Toung et al., 2000).

Since previous studies have addressed the regulatory effects of 17β-estradiol in the hippocampus of hypertensive or diabetic models separately, we first aimed to compare the morphology of dendritic arbor and spine density in normoglycemic and hyperglycemic SHR with 1-month-long diabetes. Once this objective was accomplished, we investigated if 17β-estradiol modulated the hippocampus cytoarchitecture in a combined hypertensive + diabetic model. The results may shed light on the therapeutic value of sex steroid hormones in hypertensive encephalopathy comorbid with diabetes mellitus.

EXPERIMENTAL PROCEDURES

Animals

Male SHRs were obtained from the Institute of Biology and Experimental Medicine Animal facility. Animals were 20 weeks old at the beginning of the experiment. All rats were housed under controlled conditions of temperature (22 °C) and lighting conditions (lights on 07:00–19.00 h) with free access to food and water.

Mean BP was measured by an indirect tail-cuff method (Blood pressure system, Kent Scientific Corporation: Torrington, Connecticut, USA). For steroid treatment, a group of SHR were anesthetized using a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) given ip and implanted sc with a pellet containing 12 mg of 17β-estradiol benzoate (Sigma–Aldrich, St. Louis, MO, U.S.A.) dissolved in cholesterol during the last 2 weeks of the experiment. Another group of SHR was implanted with cholesterol pellets only. This 17β-estradiol treatment provides neuroprotection in different experimental conditions (Ferrini et al., 1999; Pietranera et al., 2008, 2010, 2011; Brocca et al., 2013). For diabetes induction, SHR received via the tail vein 40-mg/kg Streptozotocin (Sigma–Aldrich, St. Louis, MO, U.S.A.) dissolved in 0.5 M sodium citrate buffer. Two days after the injection glycosuria was determined using Keto-Diastix (Bayer Diagnostics, Buenos Aires, Argentina). Glycemia was determined at the time of killing using a one-touch ULTRA (Johnson and Johnson, Milpitas, CX, U.S.A.). Normoglycemic and hyperglycemia SHR were used 1 month after diabetes induction.

Animal experiments followed the NIH Guide for the Care and Use of Laboratory Animals and were approve by the Ethics Committee of the Institute of Biology and Experimental Medicine. Efforts were made to minimize animal suffering and to reduce the number of animals used in the different experiments.

Golgi staining for analysis of dendrite length and spine number in CA1 hippocampus neurons of normoglycemic and diabetic hypertensive rats

The procedures followed for perfusion of rats intracardially and fixation of brains for preparation for Golgi staining were already described (Brocca et al., 2013). We employed a variant of the Golgi procedure thoroughly described in previous publications (Beauquis et al., 2010; González-Burgos et al., 2012; Brocca et al., 2013). Neurons impregnated with silver nitrate were studied in the CA1 region of the dorsal hippocampus at