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Streptozotocin diabetic mice display depressive-like behavior and alterations in the structure, neurotransmission and plasticity of medial prefrontal cortex interneurons

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ABSTRACT

Diabetes mellitus patients are at increased risk of developing depression, although the neurobiological bases of this comorbidity are not yet fully understood. These patients show CNS alterations, similar to those found in major depression, including changes in the structure and neurotransmission of excitatory neurons. However, although depressive patients and animal models also display alterations in inhibitory networks, little is known about the effects of diabetes on interneurons. Our main objective was to study the impact of diabetes on interneurons of the medial prefrontal cortex (mPFC), one of the regions most affected by major depression. For this purpose we have induced diabetes with high-dose streptozotozin in transgenic mice displaying fluorescent interneurons. These animals showed a depressive-like behavior (increased immobility time in tail suspension test) in parallel with reductions in interneuronal dendritic arborization and in the expression of GAD67, the enzyme that synthetizes the inhibitory neurotransmitter GABA. However, the levels of PSA-NCAM, a plasticity-related molecule exclusively expressed by interneurons in the mPFC, were unaltered in the different regions and layers of this cortical area. Interestingly, diabetic mice also showed increased levels of synaptophysin, a synaptic vesicle protein. These results indicate that the structure and neurotransmission of interneurons is altered in the mPFC of diabetic mice and suggest that these changes may play a key role in the depressive symptoms associated to diabetes.

1. Introduction

Type 1 diabetes mellitus (T1DM) is an endocrine disorder of carbohydrate metabolism characterized by a decline in and later an absence of insulin production by the pancreas, which leads to chronic insulin deficiency. Despite insulin injections, diabetic individuals typically develop complications associated with aberrant glucose metabolism. This type of diabetes is characterized by the autoimmune mediated destruction of the insulin producing cells of the pancreatic islets of Langerhans. Animal models have enormously contributed to the study of T1DM (Reagan, 2012; Rees and Alcolado, 2005); particularly streptozotocin (STZ) is widely used as a T1DM-inducer in experimental animals, through its toxic effect on insulin producing β-cells.

DM has been associated with a variety of neuropsychiatric disorders, especially with major depression. There is evidence that patients with diabetes are at increased risk of developing this disorder (Anderson et al., 2001; Doyle et al., 2014), although a bidirectional relationship might also exist. Although the impact of diabetes has been extensively studied in animal models, the neurobiological bases of this neuro–psycho-endocrinologic interaction are not yet fully understood. However, various cellular and molecular alterations have been reported (Biessels et al., 1996; Gardoni et al., 2002; Kamal et al., 2006; Piroli et al., 2004; Reagan and

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McEwen, 2002; Reagan, 2012; Reagan et al., 2000), which are similar to those proposed as etiological factors for depression (Krishnan and Nestler, 2008). These changes have been described repeatedly in the limbic system and the medial prefrontal cortex (mPFC), which are crucial cerebral regions in the experience of emotions and in memory storage, and are among the most affected in depressive disorders. Interestingly, these alterations are similar to those found in animals subjected to chronic stress, an aversive experience known to be a precipitating factor for depression, which has been extensively used as a model of this psychiatric disorder in experimental animals (McEwen, 2003). Different lines of evidence suggest that diabetes is a chronic metabolic stressor and, consequently, this endocrine disorder may produce its effects on the CNS through pathways similar to those found altered in chronically stressed animals (see Reagan, 2012 for review). These pathways include altered glutamatergic (Guyot et al., 2001; Moghaddam et al., 2013) and monoaminergic (Figlewicz et al., 1996; Rowland and Bellush, 1989; Sandrini et al., 1997) neurotransmission, increased activity of the hypothalamic-pituitary-adrenal axis (HPA) (De Nicola et al., 1977), decreases in BDNF expression (Nitta et al., 2002) and alterations in dendritic arborization and dendritic spine density of cortical excitatory neurons (Joghataie et al., 2007; Martínez-Tellez et al., 2005; Nitta et al., 2002).

During recent years, increasing evidence indicates the involvement of the GABAergic system in the pathophysiology of major depression (Luscher et al., 2011). Patients suffering from major depression and experimental animals subjected to chronic stress display alterations in inhibitory neurotransmission: GABA and GAD-67 levels as well as the density and size of certain GABAergic interneurons are significantly reduced in the PFC of depressed subjects (Hasler et al., 2007; Karolewicz et al., 2010; Krystal et al., 2002; Oh et al., 2012; Rajkowska et al., 2007). In a similar way, chronic stress in experimental animals also affects GABA levels and inhibitory neurotransmission in the mPFC (Gilabert-Juan et al., 2013; Shalaby and Kamal, 2009). Interestingly, interneurons, as pyramidal neurons, in the mPFC also respond with structural changes to chronic stress, increasing their dendritic arborization (Gilabert-Juan et al., 2013). These changes in interneuronal structure may be mediated by the polysialylated form of the neural cell adhesion molecule (PSA-NCAM), which is exclusively expressed by interneurons in the mPFC of rodents (Gómez-Climent et al., 2011; Varea et al., 2005) and humans (Varea et al., 2007b). The expression of PSA-NCAM is regulated by chronic stress in the hippocampus (Pham et al., 2003; Sandi et al., 2001), the amygdala (Cordero et al., 2005), the piriform cortex (Nacher et al., 2004) and other cerebral regions (see (Sandi, 2004), for review). Although a recent report from our laboratory has not found changes in the number of PSA-NCAM immunoreactive neurons in the mPFC after chronic stress (Gilabert-Juan et al., 2013), changes in the expression of PSA-NCAM in this region have been reported after chronic social isolation stress (Djordjevic et al., 2012a) or chronic treatment with antidepressants (Djordjevic et al., 2012b; Guirado et al., 2012; Sairanen et al., 2007; Varea et al., 2007a). Moreover, experiments depleting PSA from or altering the expression of this molecule in the mPFC have revealed profound alterations in the inhibitory circuits of this cortical region (Castillo-Gómez et al., 2011, 2008; Guirado et al., 2014, 2012).

In order to know whether diabetes induces changes similar to those observed in depressive patients and in chronically stressed animals, particularly in cortical inhibitory networks, we have analyzed the presence of depressive-like behavior and the structure and plasticity of interneurons in the mPFC in STZ treated mice. We have evaluated the dendritic arborization of a subpopulation of interneurons and the expression of PSA-NCAM, together with those of synaptophysin, a synaptic protein considered a reliable index of synaptic density and GAD67, one of the enzymes responsible for the synthesis of GABA.

2. Material and methods

2.1. Animals

Sixteen transgenic young–adult (3 months-old) male mice, [GIN (GFP-expressing Inhibitory Neurons), Tg (GadGFP) 45,704Swn] obtained from Jackson laboratories (Bar Harbor, Maine, USA) were used in this study. In this mice strain, the expression of the enhanced green fluorescent protein (EGFP) is under the control of the glutamic acid decarboxylase (GAD) gene and thus the complete morphology of a subset of these inhibitory neurons can be observed. The animal room was maintained under controlled conditions of temperature (25 °C) and humidity (50%). We have maintained mice on a reverse light/dark cycle (lights on at 23:00 and lights off at 11:00).

Animals were housed two per cage, with free access to food and water, but were kept physically separated by a plexiglass barrier that allowed only visual, olfactory and auditory contact between them. This procedure was used to avoid intermale aggression, which is extremely frequent in this strain (Pugh et al., 2004). The animals were allowed to acclimatize to their new environment for at least 10 days prior to the start of the experiment. Two days before the experimental induction of diabetes, all mice were subjected to a light–dark test (see below) to evaluate their state of anxiety and to homogeneously distribute them in experimental groups taking this parameter into account.

All animal experimentation was conducted in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and was approved by the Committee on Bioethics of the Universitat de València. Every effort was made to minimize the number of animals used and their suffering.

2.2. Induction of diabetes mellitus

At day-0 of experiment, eight mice were rendered diabetic by a single intraperitoneal injection of streptozotocin (STZ, Sigma Chemical Co. St.Louis, MO, USA) (200 mg/kg body weight) dissolved in 0.1 N pH 4.5 citrate buffer. Eight mice were used as controls and received intraperitoneally the same volumes of citrate buffer (vehicle solution) at the same times than those injected with STZ.

To successfully induce diabetes using a STZ solution with a neutral pH, we administered the solution within 30 min of preparation, storing it on ice. In addition, the time of day in which STZ was administered has been found to be critical. Previous studies have suggested that in animals exposed to standard 12 h cycle (lights on from 06.00 to 18.00 h) the highest incidence (95%) of diabetes was seen in those injected at 16:00 h. We maintained mice on a reverse 12 h cycle (lights on from 23:00 to 11:00 h), to perform the behavioral tests in the dark phase of the cycle. Therefore, to consider the circadian rhythm of the diabetogenic effect of STZ, the drug was injected at 9:00 h. Mice were given only water for 12 h before they were injected intraperitoneally with STZ or vehicle.

Twenty-eight hours after the STZ injection, animals with glycaemia higher than 11 mM (198 mg/dL) were considered diabetic.

2.3. Body weight and glucose analysis

Body weight was measured at the beginning (day 0) and at the end of the experiment (14 days after diabetes induction), prior to the evaluation of glucose levels.

We evaluated glucose levels at day 0 (before intraperitoneal injection of STZ or vehicle), at day 2 and at day 14 of the experiment on blood drops obtained by tail tip puncture. From each animal, a drop of blood was placed directly onto a glass coverslip and then collected and analyzed with a glucometer (BREEZE^{TM} 2