Journal of Chemical Neuroanatomy 82 (2017) 65–75

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu

Metabolic syndrome causes recognition impairments and reduced hippocampal neuronal plasticity in rats

Samuel Treviño[a], Rubén A. Vázquez-Roque[b], Gustavo López-López[a], Claudia Perez-Cruz[c], Carolina Moran[d], Anabella Handal-Silva[d], Enrique González-Vergara[e], Gonzalo Flores[b], Jorge Guevara[f], Alfonso Díaz[a,*]

[a] Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Puebla, Pue., Mexico
[b] Instituto de Fisiología, Benemérita Universidad Autónoma de Puebla, Puebla, Pue., Mexico
[c] Departamento de Farmacología, Centro de Investigaciones y Estudios Avanzados, CINVESTAV, Ciudad de México, Mexico
[d] Departamento de Biología y Toxicología de la Reproducción, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Puebla, Pue., Mexico
[e] Centro de Química, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Puebla, Pue., Mexico
[f] Departamento de Bioquímica, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, Mexico

ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form 17 January 2017
Accepted 15 February 2017
Available online 17 February 2017

Keywords:
High-calorie diet
Synaptophysin
Spines
Dentate gyrus
CA1-CA3
Dendrites

ABSTRACT

Metabolic syndrome (MS) is a serious public health problem, which can promote neuronal alterations in cognitive regions related to learning and memory processes, such as the hippocampus. However, up to now there has been information of a regional segregation of this damage. In this study, we evaluate the MS effect on the neuronal morphology of the hippocampus. Our results demonstrate that 90 days of a high-calorie diet alters the metabolic energy markers causing the MS and causes memory impairments, evaluated by the recognition of novel objects test (NORT). In addition, MS animals showed significant differences in dendritic order, total dendritic length and density of dendritic spines in CA1, CA3 and the dentate gyrus (DG) of the hippocampal area, compared with rats fed with a normocaloric diet (vehicle group). Furthermore, the immunoreactivity to synaptophysin (Syp) decreased in the hippocampus of the MS animals compared to the vehicle group. These results indicate that metabolic alterations induced by the MS affect hippocampal plasticity and hippocampal dependent memory processes.

1. Introduction

Metabolic syndrome (MS) is defined as a group of risk factors including insulin resistance (hyperinsulinemias) and high triglyceride and cholesterol levels, mainly caused by alterations in the metabolism of carbohydrates (dysglycemia) and lipids during obesity (Kassi et al., 2011; Alberti et al., 2009). Therefore, MS is considered a precursor of type 2 diabetes mellitus (T2DM) and vascular brain diseases, which are the major causes of mortality worldwide (Lin et al., 2014). Alarmingly, the onset of this metabolic condition has been increasing in young people (under 30-years). This is due to the fact that in the early stages of life, there is a tendency to consume foods rich in carbohydrates and saturated lipids, and for low physical activity (Gupta and Gupta, 2010; Padwal and Sharma, 2010).

Recent publications suggest that MS can promote the development of neurological disorders, so it is currently considered is a risk factor for cognitive decline and memory disorders in aging, such as Alzheimer's disease (Yaffe et al., 2004; Panza et al., 2010; Solfrizzi et al., 2011; Baker et al., 2010; Kidd, 2008). The evidence indicates that in similar conditions to dementia, from mild cognitive impairment to the onset of Alzheimer's disease, there is a decrease in the synaptic markers. This indicates the presence of a synaptic dysfunction followed by a clear neuronal degeneration, which becomes evident during the evolutionary course of the disease (Selkoe, 2002; Coleman et al., 2004). Similarly, in several animal models of memory impairment, an early synaptic dysfunction is observed, which progresses until neuronal loss is evident (Cunha and Agostinho, 2010).

Abbreviations: FFA, Free Fatty Acid; HCD, Hypercaloric diet; LTM, long-term memory; MS, Metabolic syndrome; NCD, Normocaloric diet; NORT, Novel object recognition task; STM, Short-term memory; Syp, Synaptophysin; T2DM, Type 2 diabetes mellitus.

* Corresponding author at: Departamento de Farmacia, Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla. 14 Sur 6301, CP. 72570, Puebla, Mexico.
E-mail addresses: alfonso.diaz@correo.buap.mx, dan_alf2005@yahoo.com.mx (A. Díaz).

http://dx.doi.org/10.1016/j.jchemneu.2017.02.007
0891-0618/

According to animal models with impaired glucose metabolism (hyperglycemic conditions), studies indicate that in animals' brains, there is a synaptic degeneration, because different synaptic markers are decreased. In addition to the synaptic loss, there also co-exists a reactive astrogliosis. Altogether, this drastically affects the structure and viability of neurons which is linked to the deterioration of memory performance (Duarte et al., 2009, 2012; Soares et al., 2013; Lemos et al., 2016). This could be the result of an alteration in neuronal communication, mainly depending on the energetic metabolism, particularly glucose. Therefore, when there is an imbalance in glucose metabolism, it causes a synaptic modification that could affect the plasticity processes, leading, consequently to neuronal death (Elenkov, 2008; Soares et al., 2013; Lemos et al., 2016; Adamopoulos et al., 2016).

The hippocampus presents many afferent and efferent connections within the cerebral cortex. It has been shown that the projections of the entorhinal cortex (EC) represent the major pathway of communication with the hippocampus by means of the tri-synaptic circuit, which involves the dentate gyrus (DG) in the CA3 region. In addition, the Schaffer fibers, which communicate with neurons in the CA1region (Witter et al., 1988; Nolan et al., 2004) are involved. All these neuronal projections are involved in the process of acquisition and storage of information (Remondes and Schuman, 2004). Therefore, when there is a structural deterioration of these neuronal projections at the level of dendritic processes or dendritic spines, learning and memory can be affected progressively.

Recently it has been shown that rats with streptozotocin-induced hyperglycemia present dendritic retraction and reduced dendritic spine density in hippocampal neurons (Duarte et al., 2009, 2012; Xue et al., 2016; Lazcano et al., 2014). Hyperglycemia contributes to the deterioration of brain function because it modifies neurochemical processes and causes loss of synapses, which in turn detonates a series of neurodegenerative events that together affect plasticity processes (Sripetchwandee et al., 2016; Soares et al., 2013; Lemos et al., 2016).

Likewise, MS rats induced by chronic fructose consumption or high-fat diets, present electrophysiological alterations as a decrease in the amplitude of the long-term potentiation of hippocampal cells, thus affecting learning and memory (Soares et al., 2013; Arnold et al., 2014; Cisternas et al., 2015; Cai et al., 2016; Lemos et al., 2016). However, the impact of a hypercaloric diet at the morphological level in the CA1-CA3-DG regions in hippocampal areas of MS rats is unknown.

On the other hand, synaptophysin (Syp), a protein that is part of the pre-synaptic vesicles, is widely used as marker for neuronal activity (Gerald et al., 1993; Chen et al., 2015). It has been reported that Syp+ immunoreactivity decreases under conditions of neurodegeneration, affecting neuronal activity and consequently the proper functioning of memory tasks (see Cunha et al., 2006; Canas et al., 2009; Duarte et al., 2009, 2012; Canas et al., 2014; Díaz et al., 2014; Kaster et al., 2013). In animal models of diabetes, a decreased Syp-immunoreactivity was found in the cortex and hippocampus, mainly induced by mechanisms of neurodegeneration (see Duarte et al., 2005, 2009, 2012; Gaspar et al., 2010; Agrawal and Gomez-Pinilla, 2012; Espinosa et al., 2013). However, at present the levels of Syp+ immunoreactivity in the hippocampus under MS conditions is not clearly described.

Thus, it is necessary to describe the morphological alterations in the hippocampus of MS rats in a segregation approach in addition to the presence of Syp, and to evaluate learning and memory tasks. In the present work, we use a high-calorie diet that has been shown to induce metabolic alterations to assess the impact on dendritic arborization in different regions of the hippocampus, as well as in the execution of recognition memory in rats.

We found that the high calorie diet administered for 90 days induces metabolic changes that affected recognition memory, besides reducing the density of dendritic spines and Syp-immunoreactivity in the CA1-CA3-DG hippocampal areas. This data could help develop new therapeutic targets to overcome the damaging effects of metabolic disorders that currently affect a large part of the world's population.

2. Methodology

2.1. Animals and diets

We use one month old, male, Wistar rats (80–100 g) from the "Claude Bernard" vivarium from the Autonomous University of Puebla. The animals were housed in a temperature and humidity controlled environment on a 12 h–12 h light–dark cycle with free access to food and water. All procedures described in this study were approved by the ethics committee for animal manipulation at the University of Puebla (VIEP-BUAP/162-2015) and are in accordance with the Guide for the Care and Use of Laboratory Animals of the Mexican Council for Animal Care NOM-062-ZOO-1999. Every effort was made to minimize the number of animals used and to ensure minimal animal pain and/or discomfort.

Subjects were divided into two groups comprising 15 rats: the control group and the hypercaloric diet (HCD) group (n = 15). The control group was maintained with a normocaloric diet (NCD, n = 15). The designed HCD (Patent: MX/E/2013/047377) was comprised of 71.4% carbohydrates, as shown by bromatological analysis. The percentage composition was 80% glucose, 20% fructose, 5.8% fat, divided principally between monounsaturated and saturated fatty acids, as well as polyunsaturated fatty acids omega 3 and 6. The protein was provided by 7.3% ovalbumin. Its caloric composition is shown in Table 1. LabDiet 5001 (Laboratory rodent diet) was used as the normocaloric diet for the control group; its composition can be consulted on the manufacturer's website. The experimental diet followed specifications from *Nutrient Requirements of Laboratory Rat* as recommended by the National Academy of Sciences. Both diets were administered for 90 days "*Ad libitum*" (Treviño et al., 2015).

Table 1
Caloric composition of diets.

Composition	Caloric percentage (Kcal/g) LabDiet 5001	Caloric percentage (Kcal/g) HCD (MX/E/2013/047377)
Carbohydrates	56.36	77.82
Proteins	13.02	7.95
Fat	27.66	14.22
Fiber (Crude)	2.95	0.0
Ash	0.0	0.0
Total	**99.99**	**99.99**