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Research report

The artificial sweetener Splenda intake promotes changes in expression of *c*-Fos and NeuN in hypothalamus and hippocampus of rats

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HIGHLIGHTS

- Splenda provoked *c*-Fos expression in hypothalamus.
- Splenda induced *c*-Fos staining in hippocampus.
- NeuN expression was enhanced in hypothalamus in Splenda-treated rats.
- Hippocampus showed NeuN expression in Splenda-treated rats.

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ABSTRACT

Background: Obesity is the result of the interaction of multiple variables, including the excessive increase of sugar-sweetened beverages consumption. Diets aimed to treat obesity have suggested the use of artificial sweeteners. However, recent evidence has shown several health deficits after intake of artificial sweeteners, including effects in neuronal activity. Therefore, the influence of artificial sweeteners consumption such as Splenda, on the expression of *c*-Fos and neuronal nuclear protein (NeuN) in hypothalamus and hippocampus remains to be determined.

Objectives: We investigated the effects on *c*-Fos or NeuN expression in hypothalamus and hippocampus of Splenda-treated rats.

Methods: Splenda was diluted in water (25, 75 or 250 mg/100 mL) and orally given to rats during 2 weeks *ad libitum*. Next, animals were sacrificed by decapitation and brains were collected for analysis of *c*-Fos or NeuN immunoreactivity.

Results: Consumption of Splenda provoked an inverted U-shaped dose-effect in *c*-Fos expression in ventromedial hypothalamic nucleus while similar findings were observed in dentate gyrus of hippocampus. In addition, NeuN immunoreactivity was enhanced in ventromedial hypothalamic nucleus at 25 or 75 mg/100 mL of Splenda intake whereas an opposite effect was observed at 250 mg/100 mL of artificial sweetener consumption. Lastly, NeuN positive neurons were increased in CA2/CA3 fields of hippocampus from Splenda-treated rats (25, 75 or 250 mg/100 mL).

Conclusion: Consuming Splenda induced effects in neuronal biomarkers expression. To our knowledge, this study is the first description of the impact of intake Splenda on *c*-Fos and NeuN immunoreactivity in hypothalamus and hippocampus in rats.

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1. Introduction

Obesity is considered as a pandemic disease since it is present in several countries, including the United States of America (Samaras and Elrick, 2005; Hruby and Hu, 2015; Smith and Ryckman, 2015; World Health Organization, 2017). The genesis of obesity is the result of complex interaction of several elements such as diet, socioeconomic status, family influence, cultural aspects, life style, etc. (Kimbro et al., 2017; McPherson et al., 2017). Moreover, obesity has been linked with the appearance and develop of multiple health problems, including cancer, heart diseases, sleep disorders, among many others (Crönlein, 2016; Kachur et al., 2017). In the midst of a plethora of therapeutic intervention for obesity, structured diets promote favorable changes in weight loss (Kreider et al., 2011; Zivkovic, 2012). In this regard, since consuming sugar-sweetened beverages have been suggested as part of the weight gaining issue (Qi et al., 2012; Malik et al., 2013), then, diets have substituted sugar-sweetened beverages to high-intensity artificial sweeteners like aspartame, sucralose, and saccharin (Phelan et al., 2009; Swithers et al., 2010). In turn, these chemicals have increased markedly their consumption among population (Sylvetsky et al., 2017b). However, in an apparent contradiction, current evidence suggests that frequent consumption of sugar substitutes induce health complications, including type 2 diabetes, cardiovascular dieses, weight gain, metabolic syndrome, etc. (Mann et al., 2000; Swithers et al., 2012, 2013, Swithers, 2013; Schiffman and Rother, 2013; Murray et al., 2016; Nettleton et al., 2016; Chan et al., 2017; Rebholz et al., 2017). Molecularly, it has been demonstrated that sugars consumption activate Fos-like immunoreactivity (Retzbach et al., 2014; De la Cruz et al., 2015) whereas reduction in neuronal proliferation has been also reported as well (van der Borght et al., 2011). Although experiments have shown the effects of consumption of artificial sweeteners in health aspects (Santos et al., 2018a,b; Rother et al., 2018), including Splenda (Chassaing and Gewirtz, 2018; Hernández García et al., 2018; Qin, 2018), no study has yet addressed the question of what disturbances are caused in neuronal biomarker expression such as *c*-Fos or NeuN in brain areas after Splenda consumption. Since previous findings have suggested that Fos protein expression is a reliable molecular marker of neuronal activity whereas NeuN has been linked with neurogenesis activity (Duan et al., 2016; Kovacs, 2017; Sadeghi et al., 2018) both approaches may determine whereas Splenda promotes neuronal changes. Furthermore, determining the putative changes after Splenda intake in *c*-Fos and NeuN expression in the hypothalamus (as key element in flavor preference) and hippocampus (related with learning and memory in food choice) would provide further insights of the neurobiological effects of the consumption of artificial sweeteners in these critical brain areas (Kohno, 2017; Bodnar, 2018). Thus, to address this hypothesis, we used immunohistochemical approaches to investigate the effects Splenda intake on *c*-Fos and NeuN expression in hypothalamus and hippocampus of rats.

2. Results

2.1. Consumption of Splenda induced changes on c-Fos expression in hypothalamus in rats

To test whether Splenda promotes changes in *c*-Fos expression, we analyzed it immunohistochemical staining in animals that consumed different concentrations of Splenda in hypothalamus. We found an increase in *c*-Fos expression in ventromedial hypothalamic nucleus in animals that consumed Splenda (25 mg/100 mL) as compared to respective control. However, intake of 75 or 250 mg/100 mL of Splenda decreased *c*-Fos immunohistochemistry in ventromedial hypothalamic nucleus in comparison to corresponding control (Fig. 1; Panels A–D). No significant changes were found in other hypothalamic areas.

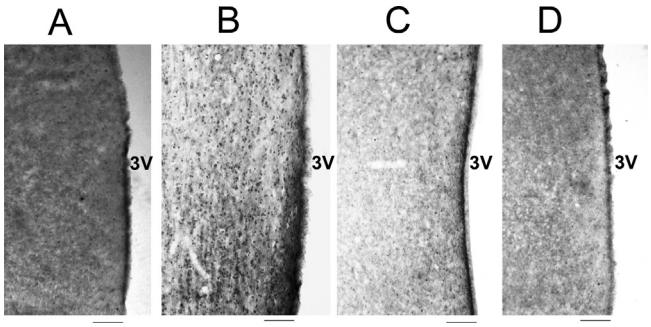

Fig. 1. Effects of *c*-Fos expression in hypothalamus of rats that consumed either tap water (Panel A) or Splenda (25, 75 or 250 mg/mL; Panels B-D, respectively). Intake of Splenda promoted changes in expression in *c*-Fos in ventromedial hypothalamic nucleus. Abbreviations: 3 V, third ventricle. Scale bar: 100 μm.

2.2. Consumption of Splenda induced changes in c-Fos expression in hippocampus in rats

The immunohistochemical staining for *c*-Fos in hippocampus from rats that consumed Splenda showed a similar pattern than observed in ventromedial hypothalamic nucleus. The intake of 25 mg/100 mL of Splenda enhanced *c*-Fos expression specifically in dentate gyrus of hippocampus as compared to control. However, consumption of Splenda (75 or 250 mg/100 mL) decreased *c*-Fos staining in dentate gyrus compared to the corresponding control (Fig. 2; Panels A–D). No significant changes were found in other hippocampal areas.

2.3. Effects of consumption of Splenda on c-Fos expression in hypothalamus and hippocampus in rats

Compared to control rats, Splenda-treated animals showed a different number of *c*-Fos positive neurons in the ventromedial hypothalamic nucleus. Concentration of 25 mg/100 mL of Splenda enhanced the number of *c*-Fos reactive neurons whereas 75 or 250 mg/100 mL in Splenda-treated rats decreased the quantity of *c*-Fos positive neurons in the ventromedial hypothalamic nucleus as compared to respective control (Fig. 3; Panel A; $P < 0.05$). Similar pattern of c-Fos expression was observed in dentate gyrus of hippocampus of animals that consumed Splenda at 25, 75 or 250 mg/100 mL, compared to corresponding control (Fig. 3; Panel B; $P < 0.05$).

2.4. Drinking Splenda modified NeuN expression in hypothalamus in rats

To investigate whether consumption of Splenda affects NeuN expression, rats were given different concentration of this artificial sweetener in powder diluted in tap water (25, 75 or 250 mg/100 mL).

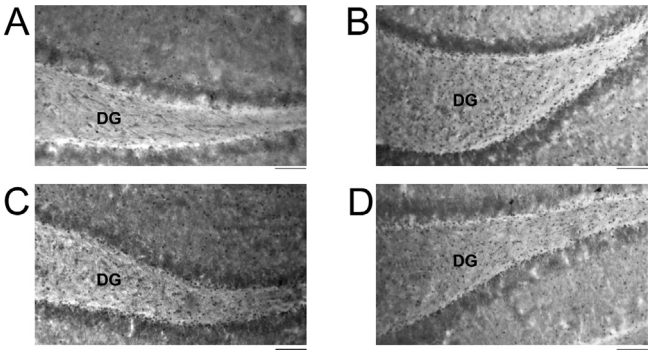

Fig. 2. Effects of *c*-Fos expression in hippocampus of rats that consumed either tap water (Panel A) or Splenda (25, 75 or 250 mg/mL; Panels B-D, respectively). As noted, Splenda intake modified *c*-Fos expression in dentate gyrus of hippocampus. Abbreviations: DG, dentate gyrus. Scale bar: 100 μm.