Anandamide and sucralose change ΔFosB expression in the reward system

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Food reward has been studied with highly palatable stimuli that come from natural additives such as sucrose. The most common food additive is sucralose, a noncaloric sweetener present in many food products of daily intake. The role of anandamide [N-arachidonylethanolamide (AEA)], an endogenous cannabinoid, has been widely studied in food behavior. Studies have shown that cannabinoids, such as AEA, 2-Arachidonilglycerol, and Tetrahydrocannabinol, can provoke hyperphagia, because they enhance the preference and intake of sweet and high-fat food. Taste perception is mediated by receptors taste type 1 receptor 3 (T1R3); therefore, there could be a synergistic effect between receptors CB1 and T1R3. This could explain why cannabinoids could change sweet taste perception and therefore the activity of neural nuclei involved in taste and reward. In this study, we evaluated the activity of dopaminergic nuclei implicated in food reward after the chronic administration of AEA (0.5 mg/kg bw) and sucralose intake (0.02%). We analyzed the expression of ΔFosB by immunohistochemistry. Our results show that the chronic administration of AEA and sucralose intake induces an overexpression of ΔFosB in the infralimbic cortex (Cx), nucleus accumbens (NAc) core, shell, and central nucleus of amygdala (Amy). These results suggest that the possible interaction between receptors CB1 and T1R3 has consequences not only in taste perception but also that AEA intervenes in the activity of dopaminergic nuclei such as the NAc, and that the chronic administration AEA and sucralose intake induce long-term changes in the reward system. *NeuroReport* 31: 240–244 Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

Palatable food is first detected by the peripheral, while sweet taste is detected by taste receptors, specifically by the heterodimer of taste type 1 receptor 2 (T1R2) and taste type 1 receptor 3 (T1R3), distributed in multiple organs including the tongue, pancreas, gut, adipose tissue, and the arcuate nucleus of the hypothalamus (ARC) [1,2]. A study that evaluated the perception of sweet taste after the systemic injection of 2-AG showed an enhance in the electric response of the tympanic cord when rats drank sweet solutions, the same research found a coexpression of receptors T1R3 and CB1 in the circumvallate and foliate taste bud cells [3]. The cannabinoid system presents CB1 and CB2 receptors for endogenous ligands, among which are N-arachidonylethanolamide (AEA), 2-Arachidonilglycerol (2-AG), and Oleamide [4,5]. Research has shown that anandamide participates in reproductive behaviors, reward, and food intake by the role of CB1 receptors in the paraventricular nucleus of the hypothalamus (PVN) in the regulation of eating and energy homeostasis [6,7]. The implication of the cannabinoid system in these behaviors could be due to its interaction with other systems such as the opioid system, dopaminergic system, and hormones involved in prepandrial and postpandrial signals, such as leptin, ghrelin, insulin or y-neuropeptide [8,9]. Studies have shown that the systemic administration of cannabinoids in rodents may induce hyperphagia, increasing their preference for palatable substances; these studies support the fact that sweet taste perception is modulated by the cannabinoid system and suggest that it plays an important role in food perception as well as food selection influenced by its hedonic value [10,11]. Nowadays, food selection is based upon the hedonic value rather than its nutritional properties.

There is a wide variety of foods that contain artificial sweeteners that mimic the taste of sugar yet lack caloric content [12]. One of the most used artificial sweeteners is sucralose, which was discovered in 1976 and approved for its intake in 1991; this molecule has a high stability and is 600 times sweeter than sucrose [13–15].

Artificial sweeteners are highly consumed and yet, to date, there is a lack of evidence on their role in the reward system and the role of cannabinoids in the modulation

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of taste stimuli when the taste stimulus has no energetic value. Therefore, in this study, we evaluated the interaction of sucralose and AEA in the activity of dopaminergic nuclei involved in food reward.

Methods

Subjects

Twenty-five male *Wistar* rats were used. Animal handling and internal bioterium conditions were applied in accordance to the official Mexican norm NOM-062-ZOO-1999 standards and the guide for the care and use of laboratory animals (2011) [16,17]. Every effort was made to reduce the number of animals employed to minimize animal's discomfort. Subjects were obtained via controlled cross-breeding and were weaned and housed in groups of four males on postnatal day 21. On postnatal day 29, subjects were randomly divided into five groups of five individuals per group; control group, water intake with vehicle injection (WVI), sucralose intake with vehicle injection (SVI), water intake with AEA injection (WAI), and sucralose intake with AEA injection (SAI).

Exposure to sucralose during adolescence

On postnatal day 30, subjects were housed in individual cages and were subjected to a two-bottle free choice test [18], giving them access to an additional bottle for 20 days. The bottle for groups control group, WVI, and WAI contained water, and for groups SVI and SAI, the water contained sucralose at a concentration of 0.02%. Subjects in groups SVI and SAI had access to 100 ml of sucralose solution and 100 ml of water daily. Sucralose solution was offered to the subjects at the first hour of their activity. Twenty-four hours later, weight and volume of the consumed liquid was measured. Food intake was measured in the same way.

Sucralose solution

Sucralose was purchased from Nutrients Scientific and prepared daily in order to avoid its change of taste, at a concentration of 0.02%. This concentration was determined by previous essays.

N-arachidonylethanolamide treatment

AEA was purchased from Sigma-Aldrich (A0580) and injected intraperitoneally at a dose of 0.5 mg/kg, with dimethyl sulfoxide (100%) as vehicle to groups WAI and SAI. AEA was administrated daily at the first hour of the dark cycle, from postnatal day 30 to postnatal day 50.

Perfusion and immunohistochemistry

After treatment, all animals were deeply anesthetized intraperitoneally with sodium pentobarbital (60 mg/kg). Transcardial perfusion was carried out with saline solution (0.9%), followed by paraformaldehyde (4%) in 0.1 M phosphate buffer (pH 7). Brains were removed and postfixed overnight in paraformaldehyde (4%), and then equilibrated to gradients of sucrose solutions (10, 20. and 30%, one day each). Coronal sections of 40 µm of the prefrontal cortex (bregma +1.18 to +0.86 mm), NAc (bregma +2.5 to +1.00 mm) and central nucleus of amygdala (bregma –2.16 to –3.36 mm) were obtained [19]. For immunohistochemistry, tissue sections were incubated in 0.5% hydrogen peroxide for 10 minutes, and then incubated in a solution containing phosphate buffer with 0.3% Triton X-100 and FosB antibody (sc-48; Santa Cruz Biotechnology, Santa Cruz, California, USA) at 1:500 dilution for 96 hours at 4°C. Following this, tissue sections were incubated in a solution containing phosphate buffer with 0.3% Triton X-100 and a biotinylated goat anti-rabbit antibody (81-6140; Invitrogen, Carlsbad, California, USA), at 1:250 dilution for 2 hours at room temperature. The reaction was visualized with a solution of 0.06% diaminobenzidine, 1% nickel sulfate, and 1% cobalt chloride.

Imagine and cell counting.

Immunoreactivity was identified as cells which had a black-purple precipitate from the DAB-nickel/cobalt reaction in the cell nucleus. Micrographs were imaged on a Motic digital compound microscope DMB3-223 with a ×40 objective. ΔFosB immunoreactive nuclei were counted in both hemispheres with a rectangular grid (101 787 μm^2) by two observers blinded to the experimental condition of the subjects. Several sections per structure were analyzed using freeware imaging analysis (ImageJ, NIH Bethesda, Maryland, USA) [20].

Statistical analysis

Data from each nucleus were analyzed by ANOVAs followed by Tukey´s post-hoc test. Sucralose intake was evaluated using Student's *t*-test. All results are expressed as mean ± SEM, and considered statistically significant with a *P* value up to 0.05. Analyzes were performed using GraphPad Prism software (v. 6.01 GraphPad software Inc., La Jolla, California, USA, 1992–2012).

Results

A weight gain was observed in all groups, but no differences were found among groups. Nevertheless, when food intake was compared between groups SAI, WAI, and control group, we found that group SAI consumed more food (SAI 20.19 ± 0.585, WAI 17.55 ± 0.497, control group 17.97 ± 0.473 g/day, $F_{4,395} = 3.935$, $P = 0.0038$).

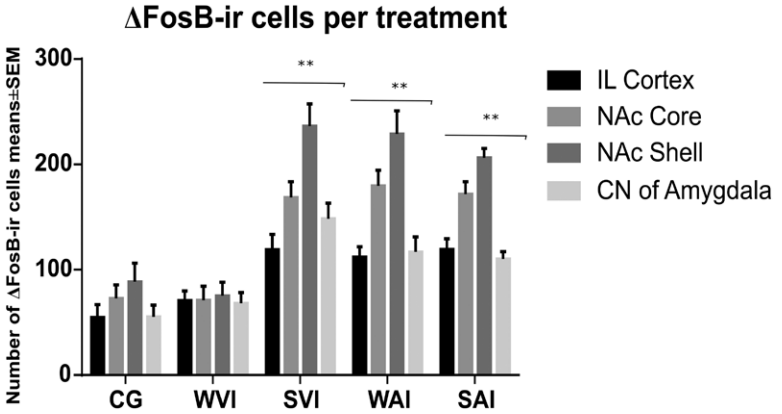
Sucralose intake

Sucralose and water intake were compared between groups SVI and SAI. This comparison was based on the average of the daily intake of water and sucralose. SAI group consumed more water compared with group SVI (23.99 ± 0.46 and 21.47 ± 0.87 ml/day, respectively, $t_{158} = 2.8934$, $P<0.05$), but no differences were found in sucralose intake (SAI 20.88 ± 0.599, SVI 21.24 ± 0.79 ml/day, $t_{158} = 0.3661$, $P = 0.7148$).

ΔFosB-ir cells in dopaminergic nuclei

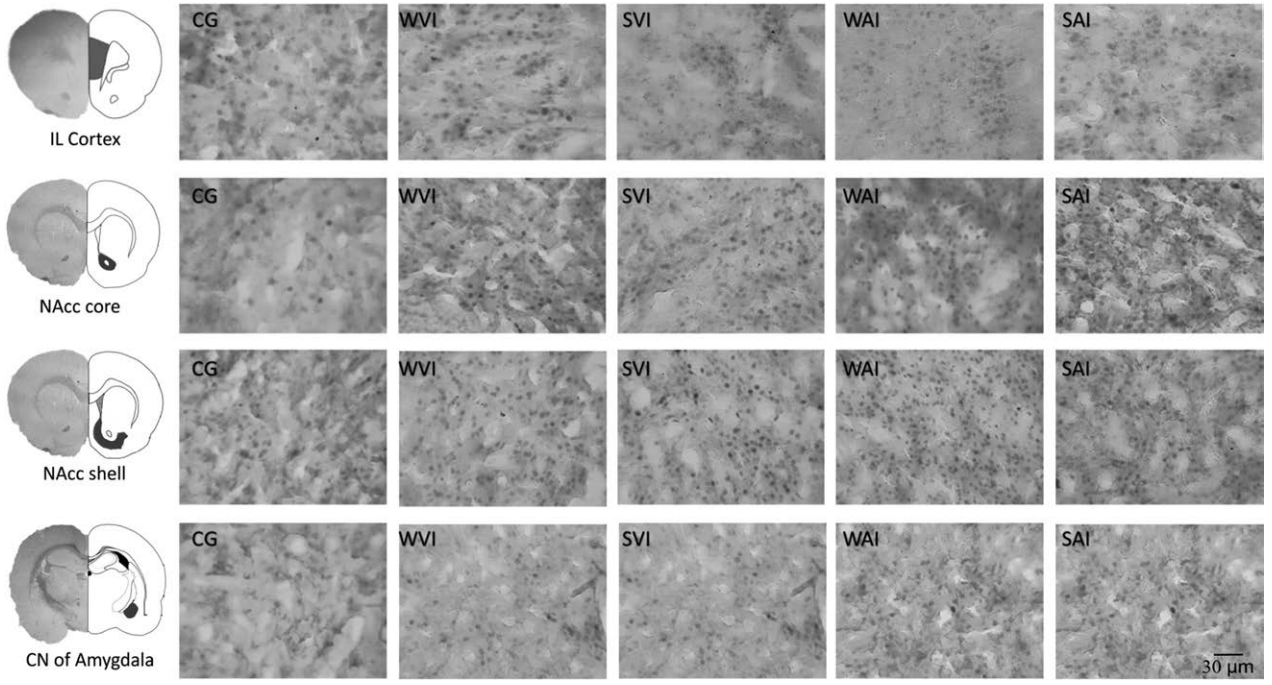
ΔFosB-ir neurons were analyzed in the Cx, NAc shell, NAc core, and Amy. A statistically significant higher number of ΔFosB-ir neurons in the NAc core in groups SVI, WAI, and SAI in comparison to other groups ($F_{4, 115}=17.02$, $P<0.0001$), in the NAc shell ($F_{4, 115}=20.97$, $P<0.0001$), Amy ($F_{4, 115}=17.02$, $P<0.0001$), and Cx ($F_{4, 115}=7.114$, $P<0.0001$) was found (Figs. 1 and 2). In the

Fig. 1



Immunohistochemistry analysis. The graphs show the mean number of ΔFos-ir neurons in the different groups for each brain area ± standard error. The asterisk indicates statistically significant differences in groups SVI, WAI, and SAI compared with groups control group and WVI. SAI, sucralose intake with anandamide injection; SVI, sucralose intake with vehicle injection; WAI, water intake with anandamide injection; WVI, water intake with vehicle injection.

Fig. 2



Microphotographs of the analyzed areas for the counting of ΔFosB-ir neurons. Columns represent the different groups (control group, WVI, SVI, WAI, and SAI); rows represent the areas with a drawing that represents the region for the counting. A higher number of ΔFos-ir neurons can be clearly observed in all areas of groups SVI, WAI and SAI compared with groups control group and WVI. The scale bar represents 30 µm for all photographs. SAI, sucralose intake with anandamide injection; SVI, sucralose intake with vehicle injection; WAI, water intake with anandamide injection; WVI, water intake with vehicle injection.

NAc shell and core, the number ΔFosB-ir neurons is lower in group SAI compared with groups SVI and WAI.

Discussion

The behavioral changes induced by cannabinoids on food consumption have been related to homeostatic regulation, and in some cases with taste perception [1,10]. Our results show that subjects exposed to sucralose and anandamide increased their food intake. Similar results have been reported by the injection of anandamide into the PVN, suggesting that CB1 receptors in the PVN play an important role in the regulation of food intake [6], and according to our results, also in the periphery. This suggests the existence of a synergistic effect between anandamide and sucralose, because sucralose induces an increase in food intake as a result to energy balance [12,21].

Regarding water intake, group SAI intook more water compared with group SVI, which suggests that the anandamide could induce an increase in water intake, probably due to a change in the perception of sucralose, or to physiological changes induced by anandamide that modify water intake. In this aspect, it has also been reported that both anandamide and artificial sweeteners induce the intracellular release of Ca^{2+} in taste receptor cells causing a differential activation of the taste pathway [22,23], which could also explain the increase in water intake. This is the first report that shows an increase in water consumption in individuals treated with anandamide and sucralose, and we propose that both stimuli can allow differential activation in hypothalamic regions.

Regarding immunoreactivity, our results show that the systemic administration of sucralose and anandamide changes the expression of ΔFosB in the NAc shell and core, infralimbic cortex, and central nucleus of amygdala (Figs. 1 and 2). This means they can change the molecular structure of these areas and therefore modify their reward value and possibly their perception. A study showed that by inhibiting motivation for food, the activity of the amygdala, cortex, and striatum decreases [23]. Our results show an increase in the activity of these areas, suggesting that sucralose promotes the activity of dopaminergic nuclei. Besides, it is also known that anandamide induces the release of dopamine in the NAc [5,11]. Although not evaluated in this study, because it was not possible to collect the tissue, it is taken into account that the ventral tegmental area (VTA) plays a decisive role in the activity of NAc, which leaves open the possibility of evaluating the activity of VTA after administration of anandamide and sucralose in future studies.

A significantly lower number of ΔFosB immunoreactive cells were observed in all areas of group SAI, compared with groups SVI and WAI. This result is similar to other studies in which they analyzed the effect of environmental enrichment and substances such as nicotine or cocaine on the expression of the same transcription factor, specifically in the prefrontal cortex [20,24]. We previously mentioned that there could be a synergistic effect between sucralose and anandamide, and that it could influence food and water intake; however, this effect seems to have no effect on the expression of ΔFosB in group SAI, which suggests that there is a limit in the accumulation of the protein, which would also regulate the activity of the brain regions analyzed in this work.

In summary, our research supports the fact that anandamide and sucralose promote the overexpression of ΔFosB in dopaminergic nuclei; however, the possible synergistic effect between the receptors of both stimuli could promote another signaling pathway for anandamide, thus reducing the expression of the transcription factor and stabilizing the levels of neuronal plasticity.

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Conflicts of interest

There are no conflicts of interest.

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