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Effects of 17β-Estradiol on Dopamine D2 Receptors in Thiamine-Deficient Female Rats: Consequences on Sucrose, Alcohol, Water Intakes and Body Weight

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Abstract

Our previous studies showed that 17β-estradiol (E2) modulated dopamine D2 receptor in regulating body weight set-point. The aim of this study was to understand whether thiamine deficiency influenced the E2 modulation on dopamine D2 receptors, using bromocriptine mesylate (BR) and sulpiride (SUL) as selective central dopamine-D2 receptors agonist and antagonist respectively. We studied the E2-dopamine D2 receptors interferences in a 10-day thiamine-deficient female rats for which consumptions of water, sugar, alcohol and food were daily-recorded and their consequences on body weights assessed. Our results showed that the volume of water daily ingested doubled in thiamine-deficient female rats (OXT), while sugar and alcohol consumptions collapsed with decreased weight and food consumption. On the one hand, thiamine potentiated D2/BR activity (bromocriptine-activated D2 receptors) to induce sugar intake and inhibited the same D2/BR receptors to induce water intake. On the other hand, thiamine promoted D2/SUL receptors (sulpiride-inhibited D2 receptors) for enhanced alcohol intake, increased food consumption and weight gain. Taking together, thiamine modulated the actions of 17β-estradiol on both D2/BR and D2/SUL receptors activities.

Keywords

Thiamine Deficiency, 17β-Estradiol, D2 Receptors, Sucrose, Alcohol Intakes, Body Weight

1. Introduction

Thiamine is an essential cofactor for several important enzymes involved in

carbohydrate metabolism, such as the alpha-ketoglutarate dehydrogenase complex (KGDHC), pyruvate-dehydrogenase complex, and transketolase [1]. Particularly, thiamine is involved in the process of glycolysis through the acid cycle for ATP production and cellular energy supply [2] [3]. Those metabolic aspects show up the coenzymatic function of the vitamin [4].

However, several aspects of experimental data on the non-coenzymatic function of thiamine focused on its relationships with hormonal factors [4] [5]. On the one hand, thiamine and dopamine showed a close relationship in biosynthesis processes [5]. Thiamine biosynthesis involved two independent pathways: synthesis of the thiazole and production of the pyrimidine moieties of thiamine, which are then coupled to form thiamine [6] [7]. L-tyrosine acts as an indispensable factor in the biosynthesis of the thiazole moiety of thiamine [8]. L-tyrosine is the common precursor of the biosyntheses of both dopamine and thiamine. Tyrosine hydroxylase is the rate-limiting enzyme of catecholamine biosynthesis: It converts the L-tyrosine into L-dopa, a precursor of dopamine [9]. Thiamine deficiency decreased tyrosine hydroxylase in the brain [10]. These observations suggest that thiamine influences dopamine synthesis through its interactions with both L-tyrosine and tyrosine hydroxylase. Indeed, thiamine deficiency reduced synthesis of dopamine and decreased catecholamine turnover (dopamine + noradrenaline) in the hypothalamus and other brain regions [11]. Moreover, cerebrospinal fluid (CSF) levels of thiamine decreased in patients with Parkinson's disease. Parkinson's disease patients under levodopa therapy had significantly higher CSF levels of thiamine diphosphate and total thiamine than those not treated with this drug [1]. In addition, Trovero *et al.* [12] reported a modulatory effect of sulbutiamine, a synthetic thiamine, on dopaminergic cortical transmissions. Together, these observations showed multiple facets of dopamine-thiamine interactions.

On the other hand, thiamine and 17β-estradiol (E2) interact with signaling processes. Recent discovery of thiaminylated adenine nucleotides suggests thiamine involvement in intracellular signaling pathways [13]. Thus, thiamine may modulate cAMP/Ca^{2+}-dependent estradiol-triggered responses which in turn control dopamine synthesis [5]. In addition, E2 induced the biosynthesis of thiamine carrier protein (TCP), while tamoxifen inhibited by 70% TCP secretion [14].

Furthermore, E2 is an estrogen that affects catecholaminergic system *in vivo* [15]. Thus, E2 can rapidly reduce the potency of gamma-aminobutyric acid, modifies in hypothalamic neurons the inwardly rectifying potassium channel, G protein-coupled (GIRK), thereby increases firing activity of dopamine neurons [16]. Among the various regulators of tyrosine hydroxylase transcription are cAMP and estrogens. Thus, tyrosine hydroxylase is transcriptionally regulated by 17β-estradiol in opposite directions depending on estradiol receptor subtypes alpha and beta [17]. Estradiol induces tyrosine hydroxylase gene transcription with estradiol receptor-alpha (ER-alpha), [9], requiring cAMP/calcium responses and protein kinase pathways [15], thereby increases activation of dopamine neurons [16]. For instance, the 17β-estradiol regulated through its slow

genomic actions, the transcription and synthesis of both D2L and D2S dopamine receptors isoforms [18] [19].

Recently we reported regulatory effects of E2 on D2 receptors for homeostasis needs. Our results indicated that D2S was a specific inducer of alcohol and food intakes, and increased body weight. D2S met the slow genomic actions induced by 17β-estradiol. Conversely, D2L inhibited alcohol and food intakes, but induced specifically sugar consumption, thereby regulating blood glucose levels. D2L mediated the rapid metabolic effects of E2 [20]. Our studies indicated that 17β-estradiol acted on two types of D2 receptors showing opposite functions to equilibrate energy intake vs. expenditure for weight set point regulation [20].

Together, these observations suggest that E2, thiamine and D2 receptors would be involved in a common regulatory system related to energy intake and expenditure for weight set point regulation. However, the mechanisms underlying their interactions are now being unveiled. Understanding the interactions between thiamine and E2 on D2 receptors could contribute to elucidate the mechanisms underlying overconsumption and weight gain in obese patients. To achieve this, we study the E2-dopamine D2 receptors interferences in a 10-day thiamine-deficient female rats for which consumptions of water, sugar, alcohol and food were daily-recorded. The aim of the study was to understand how these parameters interact to control body weight when thiamine is lacking.

2. Materials and Methods

These experiments were undertaken for further understanding of the role of thiamine (vitamin B1) on the E2-D2 receptors regulatory system whose disorder has been showed to be involved in mechanisms triggering woman obesity [20]. Since E2 is a female hormone, the present experiments are carried out exclusively on female rats.

2.1. Animals

Nulliparous females of Wistar rats, bred in our colony, which were 12 weeks old and weighing 200 - 205 grams, were used in our experiments. They were maintained under standard laboratory conditions at an ambient temperature of 30˚C ± 2˚C, with light/dark cycles of 12 hours each and relative humidity reaching 85% ± 3%. Rats were individually housed in polypropylene cages (27 × 37 × 18 cm) with the floor covered by wood shavings and fed with pellet chow diet and water ad libitum. One week prior to the onset of the tests, they were acclimated to the experimental conditions. All experiments were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals, and the study received institutional approbation of the experiments.

2.2. Drugs and Chemicals

Drugs and Chemicals used in these experiments were: 17β-estradiol (estradiol or E2), bromocriptine mesylate, sulpiride and dimethyl sulfoxide (DMSO) manufactured by Sigma-Aldrich Chemie GmbH (Eschenstrasse 582,024 Taufkirchen,

Germany). DMSO was the solvent used for all drug dilution [20]. Bromocriptine mesylate (BR) and sulpiride (SUL) were used as selective agonist and antagonist respectively, targeting central dopamine-D2 receptors [21] [22].

2.3. Procedures

The use of E2, BR and SUL in the present experiments was related to our previous studies indicating that 17β-estradiol acted on two types of D2 receptors showing opposite functions to equilibrate energy intake vs. expenditure for weight set point regulation [20]. However, thiamine is the rotating wheel for body energy supply [4]. The following experiments aimed to understand how the E2-D2 receptors regulatory system behaved in vitamin B1-deficient female rats for further elucidation of the mechanisms of obesity.

2.3.1. Experiment 1: Control

Female rats were treated for 10 days respectively with 17β-estradiol: 5 µg/kg [23], bromocriptine mesylate: 0.1 mg/kg [24], sulpiride: 20 mg/kg [25] and a concomitant administration of "17β-estradiol + bromocriptine" or "17β-estradiol + sulpiride", and DMSO vehicle: 0.7% [26]. Precisely, thirty-six (36) nulliparous female rats, individually housed, were divided into 6 treatment groups (6 rats/treatment group) and were administered for 10 consecutive days as follows [20]:

- Group C (control): Six females non-drug-treated, injected with DMSO vehicle.
- Group C + E2 (17β-estradiol): Six females subcutaneously injected (s.c.) with 17β-estradiol (5 µg/kg body wt).
- Group C + BR (bromocriptine): Six females intraperitoneally injected (i.p.) with bromocriptine mesylate (0.1 mg/kg body wt), a D2 agonist.
- Group C + SUL (sulpiride): Six females treated with intraperitoneal injection (i.p.) of sulpiride (20 mg/kg body wt), a D2 antagonist.
- Group C + E2 + BR (17β-estradiol + bromocriptine): Six females treated with concomitant administration of 17β-estradiol (5 µg/kg body wt, s.c.) and bromocriptine (0.1 mg/kg body wt, i.p.).
- Group C + E2 + SUL (17β-estradiol + sulpiride): Six females treated with concomitant administration of 17β-estradiol (5 µg/kg body wt, s.c.) and sulpiride (20 mg/kg body wt, i.p.).

Within each experimental group, drinking solutions (water, 10% sucrose and 10% alcohol) were measured daily during an experimental period of 10 days. Food consumption and body weight were also daily measured in each cage. Drugs injections and the various measurements of weight or food and solutions intakes were performed every day at the same hour, 17:00 pm, corresponding to the beginning of activities, as the rats were nocturnal animals. Solution intake was measured by direct reading of the volume absorbed on graduated bottles, every 24 h. Fluids intake were measured in a four-bottle preference condition. After daily drug injection, the remaining content of the vial (one drug for a non-

interchangeable 5 ml vial), as well as drinking solution contained in a 150 ml bottle were refreshed every day [20].

2.3.2. Experiment 2: Thiamine Deficiency

These experiments were undertaken to show whether the interactions between 17β-estradiol and dopamine D2 receptors were disrupted by thiamine deficiency. Thirty-six (36) nulliparous Wistar female rats, bred in our colony, which were 12 weeks old and weighing 200 - 205 grams, were individually housed and divided into 6 treatment groups (6 rats/treatment group). In each group, each rat received oxythiamine (OXT) treatment at a dose of 20 mg/kg sc./rat/day for 10 consecutive days, to induce thiamine deficiency in rats [27]. The treatment groups were: OXT group; OXT + E2; OXT + BR; OXT + SUL; OXT + E2 + BR and OXT + E2 + SUL, for 10 consecutive days. Drugs injections procedures and doses used were identical to experiment 1.

- Group OXT (oxythiamine): Six females subcutaneously injected (s.c.) with oxythiamine 20 mg/kg to induce thiamine deficiency.
- Group OXT + E2 (17β-estradiol): Six females OXT-treated (s.c.) and co-treated with subcutaneously injected 17β-estradiol (5 µg/kg body wt).
- Group OXT + BR (bromocriptine): Six females OXT-treated (s.c.) and intraperitoneally injected (i.p.) with bromocriptine mesylate (0.1 mg/kg body wt), a D2 agonist.
- Group OXT + SUL (sulpiride): Six females OXT-treated with intraperitoneal injection (i.p.) of sulpiride (20 mg/kg body wt), a D2 antagonist.
- Group OXT + E2 + BR (17β-estradiol + bromocriptine): Six females OXT-treated with concomitant administration of 17β-estradiol (5 µg/kg body wt, s.c.) and bromocriptine (0.1 mg/kg body wt, i.p.).
- Group OXT + E2 + SUL (17β-estradiol + sulpiride): Six females OXT-treated with concomitant administration of 17β-estradiol (5 µg/kg body wt, s.c.) and sulpiride (20 mg/kg body wt, i.p.).

The various measurements of weight or food and solutions (10% alcohol, 10% sucrose and tap water) intakes were performed as previously.

2.4. Statistical Analysis

The two-way ANOVA was used to compare the effects of treatments on thiamine-deficient females (6 factors) and controls (6 factors) × 10-day treatment period (10 factors). Post hoc testing (p's = 0.05) was carried out using the Protected Least Significant Difference (PLSD) for means comparison [28]. However, to facilitate graphic reading, figures showed only the consecutive 10-day average values for each variable within each treatment group (N = 6 female rats).

3. Results

3.1. Effects of 17β-Estradiol, Bromocriptine and Sulpiride on Water Consumption in Thiamine Deficient Rats

The two-way ANOVA analysis on water intake (**Figure 1**) indicated a main

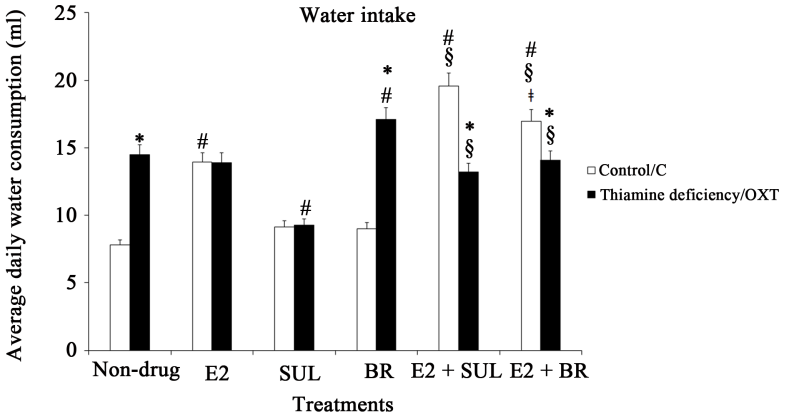

Figure 1. Effects of thiamine deficiency on 17β-estradiol (E2), bromocriptine (BR) and sulpiride (SUL) regulating water intake. The average volume of water absorbed/rat/day is represented in control (C) and OXT-treated rats (OXT). The effects of 17β-estradiol (E2), D2 (SUL and BR) receptors and their respective interactions on water intake are compared between control and thiamin-deficient rats. The consecutive 10-days average values (ml/rat/day ± SEM) of water intake within each experimental group (N = 6 female rats for one group), were represented in control (C = 6 groups) and thiamine-deficient rats (OXT = 6 groups); non-drug-treated (DMSO vehicle), 17β-estradiol (E2), sulpiride (SUL), bromocriptine (BR), E2 + SUL or E2 + BR, designating the 6 constitutive groups of control and OXT respectively. "*" denoted a significant difference between control (C) and oxythiamine-treated (OXT) female rats subjected to the same treatments, $p < 0.01$; "#" significantly different from their corresponding control group C or control group OXT, non-drug-treated rats, $p < 0.01$; "§" denoted a significant difference between hormone-D2 receptors association effects (E2 + SUL and E2 + BR) vs individual D2 receptors stimulation effects (SUL and BR) in both control and oxythiamine-treated rats $p < 0.01$; "ǂ" significantly different from E2 + SUL ($p < 0.01$).

difference among treatment groups [$F(11, 600) = 51.254$, $p < 0.0001$], with significant changes over days [$F(9, 600) = 3.503$, $p = 0.003$] and no reliable treatment × day interactions [$F(99, 600) = 0.232$, $p = 0.99$]. During the 10-day treatment period, water intake was moderated in control (C) animals (7.78 ± 0.25 ml/rat/day). Post-hoc comparisons using Fisher's PLSD test ($p = 0.05$) showed that B1 vitamin-deficient animals (OXT) doubled their daily volume of water ingested (14.48 ± 0.7 ml/rat/day) compared to C [$p < 0.0001$], indicating that in healthy animals, thiamine exerted a tonic inhibition on water consumption. Water consumption did not vary significantly when the C + E2 group (13.93 ± 0.38 ml/rat/day) was compared to the OXT + E2 group (13.91 ± 0.67 ml/rat/day), [$p = 0.97$], showing that thiamine did not influence directly 17β-estradiol-activated water consumption in control rats. There was also no significant difference in water consumption [$p = 0.817$] when the C + SUL group (9.11 ± 0.33 ml/rat/day) was compared to the OXT + SUL group (9.28 ± 0.31). These observations indicated that thiamine did not influence directly either E2 or sulpiride to regulate water consumption in the control. However, water consumption was significantly increased in the OXT + BR group (17.11 ± 0.70 ml/rat/day), compared to the C + BR group (9 ± 0.29 ml/rat/day), [$p < 0.0001$], indicating that thiamine exerted a tonic inhibitory action on BR activity under

physiological conditions which was released when thiamine is lacking as in the OXT rats. The last observations suggest that under physiological conditions, the tonic inhibition of thiamine on water intake must be transmitted by the BR-activated dopamine D2 receptors (D2/BR).

Moreover, water consumption was exacerbated in the C + E2 + SUL group (19.55 ± 0.51 ml/rat/day), while it was reduced in the OXT + E2 + SUL group (13.20 ± 0.52 ml/rat/day), [$p < 0.0001$]. Similarly, OXT + E2 + BR group (14.07 ± 0.73 ml/rat/day), compared to C + E2 + BR group (16.97 ± 0.47 ml/rat/day), showed a significant reduction in water consumption [$p < 0.0001$]. These observations indicate that thiamine lack reduced E2 activation on both BR and SUL receptors, thereby decreasing water intake. It appears that under physiological conditions, thiamine increased E2 activation on both BR and SUL receptors to control water intake.

3.2. Effects of 17β-Estradiol, Bromocriptine and Sulpiride on a 10% Sucrose Solution Intake in Thiamine-Deficient Rats

The two-way ANOVA on sucrose intake (Figure 2) yielded the main treatment effects [$F(11, 600) = 569.466$, $p < 0.0001$], with no significant changes over days

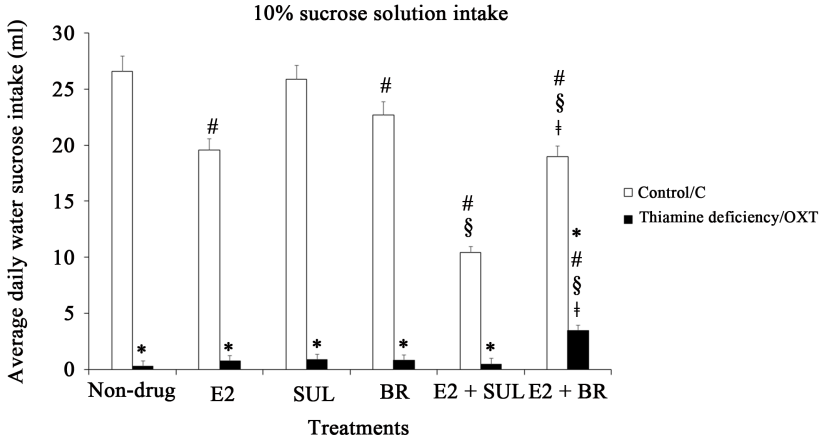

Figure 2. Effects of thiamine deficiency on 17β-estradiol (E2), bromocriptine (BR) and sulpiride (SUL) regulating sucrose intake. The average volume of a 10% sucrose solution intake/rat/day is represented in control (C) and OXT-treated rats (OXT). The effects of 17β-estradiol (E2), D2 (SUL and BR) receptors and their respective interactions on sucrose intake are compared between control and thiamin-deficient rats. The consecutive 10-days average values (ml/rat/day ± SEM) of sugar consumption within each experimental group (N = 6 female rats for one group), were represented in control (C = 6 groups) and thiamine-deficient rats (OXT = 6 groups); non-drug-treated (DMSO vehicle), 17β-estradiol (E2), sulpiride (SUL), bromocriptine (BR), E2 + SUL or E2 + BR, designating the 6 constitutive groups of control and OXT respectively. "*" denoted a significant difference between control (C) and oxythiamine-treated (OXT) female rats subjected to the same treatments, $p < 0.01$; "#" significantly different from their corresponding control group C or control group OXT, non-drug-treated rats, $p < 0.01$; "§" denoted a significant difference between hormone-D2 receptors association effects (E2 + SUL and E2 + BR) vs individual D2 receptors stimulation effects (SUL and BR) in both control and oxythiamine-treated rats $p < 0.01$; "ǂ" significantly different from E2 + SUL ($p < 0.01$).

[F (9, 600) = 0.695, p = 0.713] and no reliable treatment × day interactions [F (99, 600) = 0.290, p > 0.999], showing an abolition of sucrose intake in oxythiamine-treated rats relative to control females. Fisher's PLSD post hoc test (p's = 0.05) indicated that in the absence of thiamine, sugar consumption was totally blockaded in the OXT group (0.28 ± 0.05 ml/rat/day) compared to the C group (26.58 ± 0.69 ml/rat/day), (p < 0.0001]. During the 10-day treatment period of rats with oxythiamine, sugar consumption was strongly reduced (0.28 ± 0.04 ml/rat/day) and no other treatment of thiamine-deficient rats with either 17β-estradiol (OXT + E2: 0.73 ± 0.14 ml/rat/day), sulpiride (OXT + SUL: 0.84 ± 0.16 ml/rat/day) or bromocriptine (OXT + BR: 0.79 ± 0.14 ml/rat/day), was unable to increase sugar intake, suggesting an alteration and/or inactivation of E2 and D2 receptors in thiamine-deficient animals. Thus, the comparison of C + E2 group (19.58 ± 0.49 ml/rat/day) vs. OXT + E2 group (0.73 ± 0.14ml/rat/day), (p < 0.0001) on the one hand, and C + BR group (22.72 ± 0.62 ml/rat/day) vs. OXT + BR group (0.79 ± 0.14 ml/rat/day), (p < 0.0001) on the other hand, showed that thiamine was opposed to the inhibitory action of 17β-estradiol and D2/BR receptors on sugar consumption. Moreover, C + SUL group (25.85 ± 0.62 ml/rat/day) compared to OXT + SUL group (0.84 ± 0.16 ml/rat/day), (p < 0.0001] indicated that the lack of thiamine weakened the basic activity of D2/SUL receptors on sugar consumption. Finally, the following comparisons: C + E2 + SUL vs. OXT + E2 + SUL (p < 0.0001] and C + E2 + BR vs. OXT + E2 + BR (p < 0.0001), showed that thiamine modulated the activity of 17β-estradiol on both SUL and BR receptors to regulate sugar consumption.

3.3. Effects of 17β-Estradiol, Bromocriptine and Sulpiride on a 10% Alcohol Solution Intake in Thiamine-Deficient Rats

A two-way ANOVA on alcohol intake (**Figure 3**) indicated a main difference among treatment groups F (11, 600) = 427.077, p < 0.0001], with no significant changes over days [F (9, 600) = 0.470, p = 0.895] and no significant treatment × day interactions [F (99, 600) = 0.579, p = 0.999], showing a remarkable weakness of alcohol intake in oxythiamine-treated rats relative to control females. Fisher's PLSD post hoc comparison of the averages (p's = 0.05) showed that alcohol consumption was completely inhibited in thiamine-deficient females (0.21 ± 0.04 ml/rat/day), compared to the control females (2.87 ± 0.11 ml/rat/day), (p < 0.0001), suggesting that thiamine influenced the basic alcohol consumption. In the females treated with oxythiamine, alcohol consumption was inhibited (0.28 ± 0.04 ml/rat/day) and no further treatment of these thiamine-deficient animals with either 17β-estradiol (0.23 ± 0.06 ml/rat/day), sulpiride (0.46 ± 0.09 ml/rat/day), or bromocriptine (0.19 ± 0.06 ml/rat/day), did not increase that alcohol consumption, confirming an alteration and/or inactivation of E2 and D2 receptors. Thus, comparison of the C + SUL group (8.2 ± 0.23 ml/rat/day) with the OXT + SUL group (0.46 ± 0.09 ml/rat/day), (p < 0.0001], showed that thiamine lack blocked the sulpiride-induced alcohol intake. Similarly, the OXT + BR group significantly reduced its alcohol intake (0.197 ± 0.06 ml/rat/day)

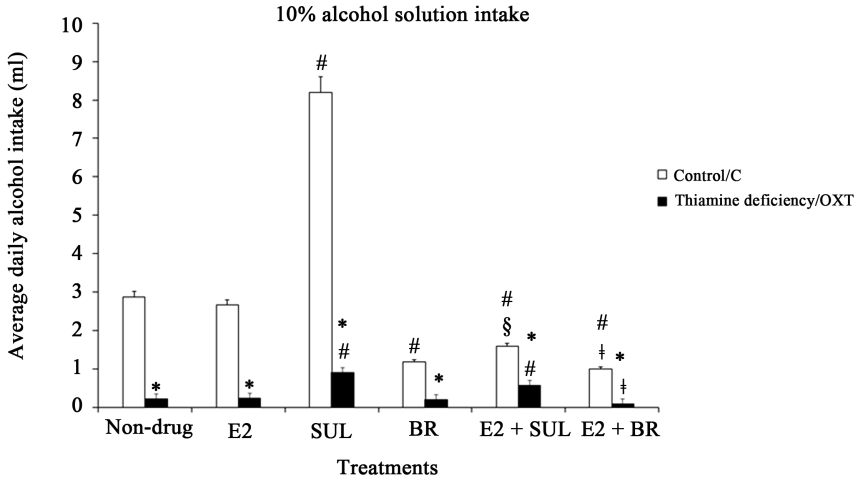

Figure 3. Effects of thiamine deficiency on 17β-estradiol (E2), bromocriptine (BR) and sulpiride (SUL) regulating alcohol intake. The average volume of a 10 % alcohol solution intake/rat/day is represented in control (C) and OXT-treated rats (OXT). The effects of 17β-estradiol (E2), D2 (SUL and BR) receptors and their respective interactions on alcohol intake are compared between control and thiamin-deficient rats. The consecutive 10-day average values (ml/rat/day ± SEM) of alcohol consumption within each experimental group (N = 6 female rats for one group), were represented in control (C = 6 groups) and thiamine-deficient rats (OXT = 6 groups); non-drug-treated (DMSO vehicle), 17β-estradiol (E2), sulpiride (SUL), bromocriptine (BR), E2 + SUL or E2 + BR, designating the 6 constitutive groups of control and OXT respectively. "*" denoted a significant difference between control (C) and oxythiamine-treated (OXT) female rats subjected to the same treatments, $p < 0.01$; "#" significantly different from their corresponding control group C or control group OXT, non-drug-treated rats, $p < 0.01$; "§" denoted a significant difference between hormone-D2 receptors association effects (E2 + SUL and E2 + BR) vs individual D2 receptors stimulation effects (SUL and BR) in both control and oxythiamine-treated rats $p < 0.01$; "ǂ" significantly different from E2 + SUL ($p < 0.01$).

compared to the C + BR group (1.18 ± 0.05 ml/rat/day), ($p < 0.0001$). These observations indicate that thiamine activates both D2 receptor isoforms to promote alcohol intake.

Furthermore, alcohol consumption was significantly reduced in the OXT + E2 group (0.23 ± 0.06 ml/rat/day), compared to the C + E2 group (2.67 ± 0.11 ml/rat/day), ($p < 0.0001$). These results suggest that thiamine opposed 17β-estradiol activities. In addition, alcohol consumption was strongly inhibited in the OXT + E2 + SUL group (0.56 ± 0.11 ml/rat/day), compared to the C + E2 + SUL group (1.58 ± 0.08 ml/rat/day), ($p < 0.0001$), and in the OXT + E2 + BR group (0.08 ± 0.04 ml/rat/day) compared to the C + E2 + BR group (1 ± 0.09 ml/rat/day), ($p < 0.0001$]. The last observations suggest that thiamine opposed the inhibitory actions of 17β-estradiol on both sulpiride (D2/SUL) and bromocriptine (D2/BR) receptors to promote alcohol intake.

3.4. Effects of 17β-Estradiol, Bromocriptine and Sulpiride on Food Consumption in Thiamine-Deficient Rats

A two-way ANOVA showed that the different treatments induced significant

variations in the average amount of food consumed in rats (Figure 4), [F (11, 600) = 100.572, p < 0.0001], with significant changes in food intake over days [F (9, 600) = 3.395, p < 0.0004], but no reliable treatment x day variations [F (99, 600) = 0.159, p > 0.999]. Post-hoc comparison of the averages, performed by the Fisher's PLSD test (p's = 0.05), showed the average amount of food consumed per day to be decreased in females treated with oxythiamine (OXT: 7.14 ± 0.33 mg/rat/day) comparatively to the controls (C: 11.8 ± 0.38 mg/rat/day), [p < 0.0001]. Thiamine lack exerted different actions on D2 receptors-induced food intake. Thus, C + SUL treatment (14.2 ± 0.38 mg/rat/day) compared to OXT + SUL treatment (3.98 ± 0.23 mg/rat/day) showed that the lack of vitamin B1 blocked the activation of sulpiride-induced food consumption [p < 0.0001]. Conversely, the C + BR group (10 ± 0.31 mg/rat/day), compared to the OXT + BR group (9.14 ± 0.38 mg/rat/day), [p = 0.091], showed that vitamin B1 deficiency did not influence the activity of receptors D2 (bromocriptine) on food consumption. However, thiamine opposed the main effects of E2 under physiological conditions. Indeed, the OXT + E2 group reduced its food consumption

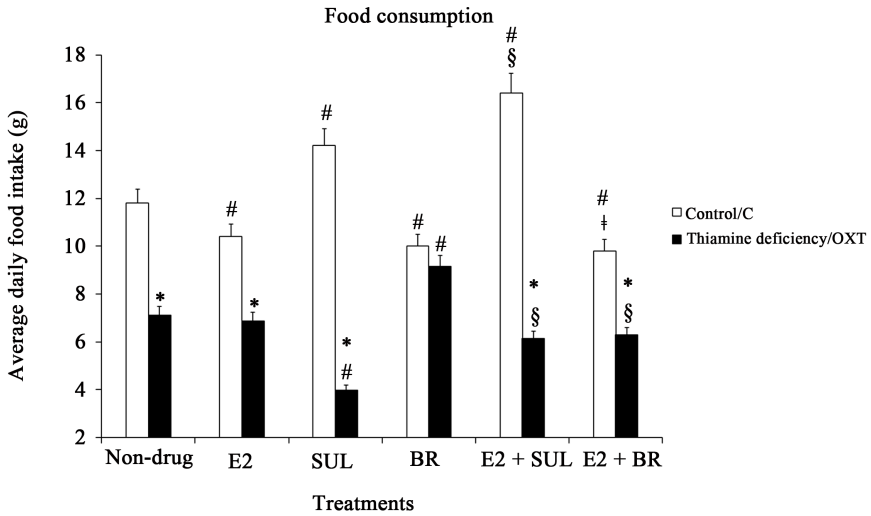

Figure 4. Effects of thiamine deficiency on 17β-estradiol (E2), bromocriptine (BR) and sulpiride (SUL) regulating food intake. The average amount of food ingested/rat/day is represented in control (C) and OXT-treated rats (OXT). The effects of 17β-estradiol (E2), D2 (SUL and BR) receptors and their respective interactions on food consumption are compared between control and thiamin-deficient rats. The consecutive 10-day average values (g/rat/day ± SEM) of food intake within each experimental group (N = 6 female rats for one group), were represented in control (C = 6 groups) and thiamine-deficient rats (OXT = 6 groups); non-drug-treated (DMSO vehicle), 17β-estradiol (E2), sulpiride (SUL), bromocriptine (BR), E2 + SUL or E2 + BR, designating the 6 constitutive groups of control and OXT respectively. "*" denoted a significant difference between control (C) and oxythiamine-treated (OXT) female rats subjected to the same treatments, p < 0.01; "#" significantly different from their corresponding control group C or control group OXT, non-drug-treated rats, p < 0.01; "§" denoted a significant difference between hormone-D2 receptors association effects (E2 + SUL and E2 + BR) vs individual D2 receptors stimulation effects (SUL and BR) in both control and oxythiamine-treated rats p < 0.01; "ǂ" significantly different from E2 + SUL (p < 0.01).

(6.88 ± 0.26 mg/rat/day), compared to the C + E2 group (10.4 ± 0.38 mg/rat/day), [$p < 0.0001$], suggesting that thiamine is opposed to the reducing action of 17β-estradiol on food consumption under physiological conditions. In addition, comparisons of the C + E2 + SUL group (16.4 ± 0.43 mg/rat/day) with the OXT + E2 + SUL group (6.14 ± 0.32 mg/rat/day), [$p < 0.0001$] and the C + E2 + BR group (9.8 ± 0.24 mg/rat/day) with the OXT + E2 + BR group (6.27 ± 0.35 mg/rat/day), [$p < 0.0001$] showed that in the absence of thiamine, there was an amplification of the inhibition of 17β-estradiol on the D2/SUL and D2/BR receptors resulting in a drastic decrease in food consumption.

3.5. Effects of 17β-Estradiol, Sulpiride and Bromocriptine on Body Weight of Thiamine-Deficient Rats

A two-way ANOVA on body weight (Figure 5) yielded the main treatment effects [$F(11, 600) = 200.529$, $p < 0.0001$], but no significant variations in body weight over days [$F(9, 600) = 0.582$, $p = 0.812$], and no reliable treatment x day interactions [$F(99, 600) = 0.730$, $p = 0.973$]. During the 10-day treatment period, the average weights of the animals were measured. A post hoc comparison

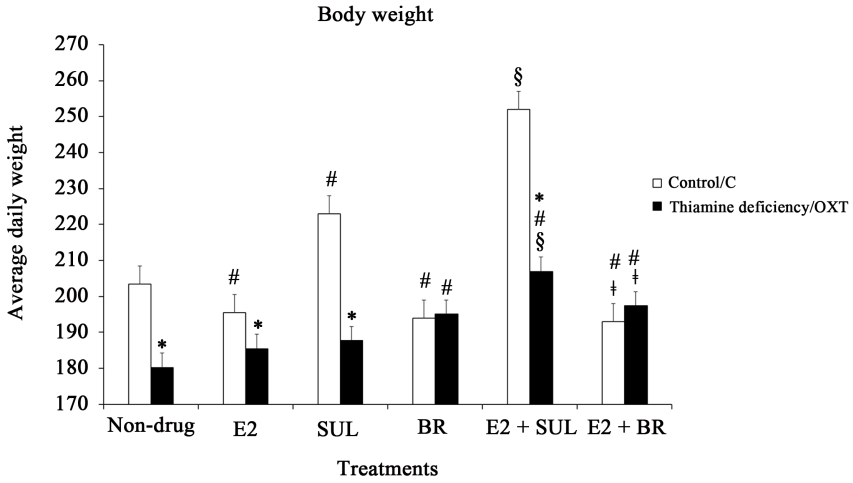

Figure 5. Effects of thiamine deficiency on 17β-estradiol (E2), bromocriptine (BR) and sulpiride (SUL) regulating body weight. The average body weight variations measured/rat/day is represented in control (C) and OXT-treated rats (OXT). The effects of 17β-estradiol (E2), D2 (SUL and BR) receptors and their respective interactions on body weight are compared between control and thiamin-deficient rats. The consecutive 10-day average values (g/rat/day ± SEM) of body weight within each experimental group (N = 6 female rats for one group), were represented in control (C = 6 groups) and thiamine-deficient rats (OXT = 6 groups); non-drug-treated (DMSO vehicle), 17β-estradiol (E2), sulpiride (SUL), bromocriptine (BR), E2 + SUL or E2 + BR, designating the 6 constitutive groups of control and OXT respectively. "*" denoted a significant difference between control (C) and oxythiamine-treated (OXT) female rats subjected to the same treatments, $p < 0.01$; "#" significantly different from their corresponding control group C or control group OXT, non-drug-treated rats, $p < 0.01$; "§" denoted a significant difference between hormone-D2 receptors association effects (E2 + SUL and E2 + BR) vs individual D2 receptors stimulation effects (SUL and BR) in both control and oxythiamine-treated rats $p < 0.01$; "ǂ" significantly different from E2 + SUL ($p < 0.01$).

using the Fisher's PLSD test (p's = 0.05) showed that thiamine deficiency decreased females body weight (180.25 ± 1.54 g/rat), compared to the controls (203.45 ± 0.80 g/rat), [$p < 0.0001$], showing that thiamine is involved in body weight gain. Indeed, the OXT + E2 treatment significantly reduced body weight (185.48 ± 1.35 g/rat), compared to the C + E2 treatment (195.53 ± 0.44 g/rat), [$p < 0.0001$], suggesting that thiamine counteracted the reducing action of E2 on body weight. On the one hand, the OXT + BR group (195.1 ± 1.08 g/rat) showed no significant increase in body weight compared to the C + BR group (194 ± 0.70 g/rat), [$p = 0.571$], indicating that thiamine did not exert any synergistic action on D2 (bromocriptine) receptors to reduce body weight. In addition, the OXT + E2 + BR group (197.34 ± 1.2 g/rat) showed no significant increase in body weight compared to the C + E2 + BR group (193 ± 0.60 g/rat), [$p = 0.12$], suggesting that thiamine has also no influence on the E2-D2/BR receptors complex to reduce body weight. On the other hand, the OXT + SUL group (187.73 ± 1.83 g/rat), compared to the C + SUL group (223 ± 0.99 g/rat), showed a significant decrease in body weight [$p < 0.0001$], suggesting that thiamine potentiates the inductive action of sulpiride on weight gain. In addition, the OXT + E2 + SUL group (206.96 ± 0.81 g/rat), compared to the C + E2 + SUL group (252 ± 2.11 g/rat), [$p < 0.0001$] showed that thiamine acts in synergy with E2 to potentiate sulpiride receptors activation (D2/SUL) and increase body weight.

4. Discussion

We evaluated the effects of thiamine (B1 vitamin) deficiency on both 17β-estradiol (E2) and dopamine D2 receptors activities. Bromocriptine mesylate (BR) and sulpiride (SUL) were used as selective agonist and antagonist respectively, targeting central dopamine-D2 receptors [21] [22] and their binding receptors were described as D2/BR and D2/SUL respectively. Interferences between E2 and dopamine D2 receptors were assessed on the consumption of water, sugar, alcohol, food and the weight of thiamine-deficient female rats (OXT). Our results showed that the volume of water ingested daily doubled in B1 vitamin-deficient female rats (OXT), compared to control female rats (C), showing that in healthy animals, thiamine exerted a tonic inhibition on water consumption. Thiamine did not directly influence E2 or sulpiride to modify water consumption in control rats. However, thiamine exerted in control rats a tonic inhibition on D2/BR receptors activities which was released by thiamine deficiency. On the contrary, E2 opposed that tonic inhibition of thiamine on D2/BR receptors. In addition, E2 activated D2/SUL receptors to increase water consumption through the synergistic action of thiamine. It therefore appears that thiamine exerts a tonic inhibition on water consumption that is released by 17β-estradiol.

Our studies also showed that in thiamine-deficient female rats, sugar and alcohol intakes were inhibited and no other treatment of these animals deficient in B1 vitamin alternatively with 17β-estradiol, sulpiride or bromocriptine, can increase these beverages consumptions. These results show functional impairment of estrogen and dopaminergic D2 receptors during thiamine deficiency. It also

appears that thiamine controls the individual activity of E2 as well as the D2/BR and D2/SUL receptors to regulate sugar and alcohol consumption. On the one hand, thiamine was opposed to the inhibitory actions of 17β-estradiol and D2/BR receptors on sugar consumption. In addition, thiamine lack weakened the basic activity of D2/SUL receptors activating sugar consumption. On the other hand, thiamine lack collapsed the sulpiride-induced alcohol intake, while it weakened the basic activity of D2/BR receptors activating alcohol consumption. These observations indicate that thiamine activates both D2 receptors isoforms to promote alcohol and sugar intakes. Consequently, it appears that thiamine promotes a rapid and massive supply of energy substances to the body in emergency situations. Finally, our results indicated that thiamine modulated the activity of 17β-estradiol on the D2/SUL and D2/BR receptors to regulate sugar and alcohol consumptions. These observations show that thiamine contributes to the body's energy supply by exerting a tonic inhibition on water consumption while promoting the consumption of liquids with a high caloric content. Thiamine therefore appears to be an important regulatory factor in hypoglycemia. Under normal physiological conditions, the actions of thiamine would lead to overweight if these effects were not countered by E2. What would be the central role of thiamine in glucose homeostasis?

Smith *et al.* [29] reported that anorexia nervosa was accompanied by thiamine deficiency and hypothermia. Administration of pharmacological doses of thiamine in such a patient improved voluntary food intake, followed by restoration of body weight and a sharp increase in sugar consumption with hyperthermia. These authors suggested the involvement of thiamine in the thermoregulation process. Molina *et al.* [30] showed that thiamine deficiency resulted in an increase in blood sugar (18%), lactate (3 to 4 times) and a 30% decrease in insulin. The main functions of pancreatic beta cells are biosynthesis and the release of insulin, the only hormone that can directly reduce blood sugar levels [31]. The pancreas maintains a high level of thiamine [32], and deficiency of this vitamin negatively affects its exocrine and endocrine functions [33]. As with all other mammalian cells, pancreatic acinar cells cannot synthesize thiamine and must obtain the vitamin from the circulation through a cell membrane transport process. According to Srinivasan *et al.* [34], pancreatic acinar cells obtained thiamine from the circulation through thiamine membrane transporters 1 and 2 (THTR-1 and THTR-2). Pancreatic islets isolated in thiamine-deficient rats secreted less insulin. Insulin secretion in response to glucose was also decreased during thiamine deficiency. These observations show that thiamine plays an important role in insulin secretion and thiamine deficiency leads to a decrease in insulin secretion and an increase in blood glucose. This explains why in our results thiamine deficiency exacerbated water intake, while it collapsed sugar and alcohol intakes. Permanent increase in blood glucose during thiamine deficiency promoted diabetes mellitus [35] [36]. Indeed, Bâ [37] suggested that increasing extracellular glucose concentrations activated thiamine transportation from the bloodstream into both pancreatic beta cells for insulin releasing, and diverse or-

gans tissues cells increasing mitochondrial ATP synthesis [38]. This process was expected to include thiamine membrane transporter coupled with a conventional G protein [39]. Since our study suggests modulating interferences between E2 and thiamine, it is conceivable that the G protein-coupled thiamine transportation may be regulated by the 17β-estradiol rapid signaling pathway. These observations suggest that, like circulating insulin, thiamine plays a major physiological role in peripheral glucose homeostasis [37]. How thiamine and 17β-estradiol control glucose homeostasis?

Our results suggest reciprocal modulating interactions between thiamine and 17β-estradiol dependent on blood glucose levels. On the one hand, thiamine exerted a tonic inhibition on water consumption that was released by 17β-estradiol. On the other hand, 17β-estradiol exerted a tonic inhibition on sucrose intake which was released by thiamine. Under physiological conditions, D2/BR receptors mediated both actions of E2 and thiamine leading to decreased water intake and increased sucrose consumption. Indeed, bromocriptine receptor (D2L) was reported to be a specific inducer of sugar intake [20]. In addition sucrose intake collapsed in thiamine-deficient rats, showing that thiamine activated D2/BR receptors to induce sugar intake. Thiamine therefore appears to be an important regulatory factor in hypoglycemia. In conditions of high glucose demand, i.e. hypoglycemia and hyperthermia, thiamine blocked E2 inhibition on D2/BR receptors, thereby increasing sugar consumption for the body's energy supply. Literature reported 17β-estradiol to be largely involved in glucose metabolism. Nadal *et al.* [31] reported that 17β-estradiol recharged blood glucose in hypoglycemia and eliminates excess blood glucose in hyperglycemia. E2 through its rapid signaling pathway triggered via the canonic estrogen receptor β (ERβ) would initiate the synthesis and increase the expression of glucose transporter 1 (GLUT-1) sensitive to blood glucose variations [40] [41], thereby facilitate glucose transport into the cells. In addition to facilitating glucose transport, E2 promoted glycolysis through the tricarboxylic acid cycle for ATP synthesis [42]. Brinton *et al.* [43] reported that the increase in estrogen-induced ATP synthesis was induced by the ERβ isoform of the estrogen receptor [44] improving mitochondrial respiration [45]. Moreover, ERβ increased glucose stimulated insulin secretion [46] [47]. The secreted insulin regulated blood glucose levels by removing as much glucose as possible from the circulation to enter the cells and undergo glycolysis. However, recent findings involved another estrogen receptor coupled to the G protein (GPER e.g. the old GPR30) in the regulation of E2 on insulin secretion [48]. In accordance with these findings, Koricanac *et al.* [49] reported selective and inhibitory interferences between insulin and estradiol signaling pathways in the context of excessive sugar intake. Indeed, the major glucose transporter in rodent islet beta cells, the glucose transporter 2 (GLUT2) associated with glucose sensing [50], was also regulated by E2. In the nervous system, GLUT2-dependent glucose sensing controlled feeding, thermoregulation and pancreatic islet cell mass and function, as well as sympathetic and parasympathetic activities [50]. According to Bian *et al.* [51], 17β-estradiol regulated glu-

cose transporter 2 (GLUT2) and insulin secretion in rat islets β cells through G protein-coupled estrogen receptor (GPER) via Akt/mTOR pathway. Based on these results, Kumar *et al.* [52] proposed that small molecules activating GPR30 may be promising in diabetes mellitus therapy. Thiamine administration alleviated diabetes mellitus [36]. We can assume that one of the small molecules involved in the regulation of estrogen receptors coupled to G proteins GPER is thiamine. Our study suggested that one of the essential functions of 17β-estradiol on sugar metabolism may be transmitted by thiamine-modulated ERβ and/or GPER receptors to ensure blood glucose homeostasis. The focal point of that regulation may be D2/BR (long chain receptor), since thiamine opposed the inhibitory actions of 17β-estradiol on D2/BR receptors, thereby increasing sugar consumption under physiological conditions, while thiamine deficiency collapsed sugar consumption. Consequently, how thiamine lack affects food consumption and body weight?

Our studies showed that thiamine deficiency reduced body weight and food consumption. Under physiological conditions, thiamine contrasted with the reducing action of 17β-estradiol on weight and food consumption. In the absence of thiamine, there was an amplification of the inhibition of 17β-estradiol on the D2/SUL and D2/BR receptors resulting in a drastic decrease in weight and food consumption. On the contrary, thiamine potentiates the inducing action of sulpiride on food consumption and weight gain. E2 activated D2/SUL receptors to induce heavy alcohol and water consumption through the synergistic action of thiamine. For instance, thiamine lack collapsed the sulpiride-induced alcohol intake. Therefore, D2/SUL receptors and thiamine were involved in the mechanisms regulating hyperphagy and overweight. Supporting that hypothesis, thiamine did not participate in the reductive activity of D2 receptors (bromocriptine) on food consumption and body weight gain, but rather prevented the synergistic action of E2 on D2/BR receptors to reduce weight and food consumption. Ultimately, one of the important roles of thiamine was to stimulate weight gain and food consumption.

In humans, administration of neuroleptic drugs, which blocked dopamine receptors, caused hyperinsulinemia, increased weight gain and glucose intolerance. Conversely, treatment with bromocriptine improved glycemic control and glucose tolerance in obese type 2 diabetic patients as well as in non diabetic obese animals and humans [53]. Indeed, literature reported constantly increase in patients weights taking antipsychotic medication and sulpiride has been noted to induce severe weight gain [54] [55]. According to Baptista *et al.* [56] sulpiride significantly increased body weight, fat gain and food efficiency without modifying energy expenditure. Weight gain has been associated with increases in fasting glucose and lipids [57]. Selective anatgonism of D2 receptors with amisulpiride reduced largely severe weight loss and hypophagia in anorexia nervosa patients [58]. To explain the mechanisms of sulpiride-induced overweight, Parada *et al.* [59] reported that intrahypothalamic injections of sulpiride elicited feeding, even in satiated rats. Sulpiride microinjection in the ventromedial hy-

pothalamus (VMH) of obese Zucker rats downregulated D2 receptors mRNA expression within VMH and increased greatly food intake [60]. In addition, SULP-induced inactivation of the cAMP/protein kinase A/cAMP-response element-binding protein signaling pathway, down-regulating insulin and growth hormone release as prelude of metabolic diseases [61]. Consequently, overweight found its expression in dysregulated D2/SUL receptors [20]. Furthermore, Bâ [37] has shown that thiamine played a major physiological role in the homeostasis of body weight programming at birth, incrementation and set point regulation in offspring and adult female rats. The present studies also added that thiamine metabolism may lead to overweight when E2 is lacking as during the woman's menopause. Therefore, D2/SUL receptors and thiamine appeared to be potential inducers of woman obesity when E2 is lacking.

The present results reporting E2-thiamine reciprocal interactions on dopamine D2 receptors must be considered as preliminary, since further studies on biochemical and molecular aspects of thiamine-regulated G protein signaling are needed. Increasing interest was accorded to food selection based on macronutrients composition as important risk factor in the etiology of obesity [62]. Our future studies will address E2's ability in directing either D2L or D2S receptor toward specific nutrient intake.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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