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Research report

Binge-like consumption of caloric and non-caloric palatable substances in *ad libitum*-fed C57BL/6J mice: Pharmacological and molecular evidence of orexin involvement

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HIGHLIGHTS

- Orexins (OX) contribute to palatable feeding in organisms with caloric needs.
- We explored OX involvement in sucrose/saccharin binge-drinking in non-deprived mice.
- OXR1 antagonism blunted sucrose/saccharin binge consumption in a DID procedure.
- Four episodes of sucrose/saccharin binge drinking reduced OX mRNA expression in the LH.
- OX might be engaged during the initial stages of binge-eating disorders.

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ABSTRACT

The orexin (OX) system has been implicated in food-reinforced behavior, food-seeking and food overconsumption. Recent evidence suggests that OX signaling might influence consumption of palatable foods with high reinforcing value depending upon the caloric status of the animal. The present study evaluates from a pharmacological and a molecular approach the contribution of OX to excessive binge-like consumption of highly preferred palatable substances (sucrose and saccharin) in *ad libitum-fed* C57BL/6J mice. The main findings of this study are: (1) intraperitoneal (ip) injection of SB-334867 (10, 20 or 30 mg/kg), a selective OXR1 antagonist, significantly decreased binge-like consumption of sucrose (10%, w/v) and saccharin (0.15%, w/v) during the test day in a *Drinking in the Dark* procedure in *ad libitum*-fed animals, without evidence of any significant alteration of locomotor activity. (2) Four repetitive, 2-h daily episodes of sucrose and saccharin (but not water) binge-like drinking significantly dampened OX mRNA expression in the LH. Present findings show for the first time a role for OXR1 signaling in binge-like consumption of palatable substances in animals under no caloric needs. Targeting OXR1 could represent a novel pharmacological approach to treat binge-eating episodes.

1. Introduction

In 1998, two groups independently identified a new class of hypothalamic neuropeptides cleaved from the protein, prepro-orexin [1,2]. These peptides, called orexin-A (OX-A) and orexin-B (OX-B) (also denoted as hypocretin 1 and hypocretin 2), are produced by a very small population of neurons in the hypothalamus which send widespread projections to brain regions [3–9]. Their actions are mediated by two individual membrane receptors, orexin type 1 and type 2 receptors (OXR1 and OXR2, respectively); while orexin A (OX-A) acts on both OXR1 and OXR2, orexin B (OX-B) acts only on OXR2 [9].

The OX peptides have been involved in a variety of regulatory physiological functions including arousal [7–10], sleep-wakefulness [11,12] or homeostatic aspects of feeding. Regarding OX contribution to food intake, OX knockout mice exhibit a hypophagic phenotype compared to aged-matched littermates [13]; central administration of OX-A stimulates regular chow

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consumption [1], while pretreatment with the OXR1 antagonist SB-334867 (SB) block this effect [14,15]; central infusion of the OXR1 antagonist SB blunts by itself food consumption [16] and fasting up-regulates prepro-orexin mRNA [1]. Subsequent reports conducted in animals under no caloric needs have extended previous findings pointing to a specific role for OXR signaling in food reward. Thus, central administration of OX-A increases the consumption of highly palatable food [17] and systemic administration of the OXR1 antagonist SB reduces palatable food intake [18].

It has been recently reported that OXR1 signaling is additionally involved in food-reinforced and food-seeking behaviors. A significant reduction in variable ratio and progressive ratio (PR) responding for standard pellets has been observed in response to OXR1 antagonism in C57BL/6J mice and the same effect was reported in OX knockdown mice as well [19]. Moreover, studies aimed at comparing food seeking behaviors in *ad libitum* vs food-restricted animals support the idea that OX signaling might influence consumption of palatable foods with high reinforcing value. Thus, while central administration of the OXR1 antagonist SB did not alter responding for a sucrose solution in *ad libitum*-fed animals [20], it was able to reduce FR responding for high-fat food and sucrose [20–23] and PR responding for chocolate [24] in food-restricted rats. In the same direction, infusion of the OX antagonist SB equally decreased active lever responding and the number of saccharin reinforcers earned in *ad libitum*-fed and food-restricted rats [22].

A few studies have suggested the contribution of the OX system to food overconsumption [25] depending on the caloric status of the animal [26]. Thus, Piccoli et al. [27] demonstrated that the selective OX1R antagonist GSK1059865 was unable to inhibit highly palatable food consumption in animals under not restricted access to food, while it reduced binge-eating under mild stress and restricted-food access conditions, which might be indicative of a role for OXR1 signaling in compulsive eating behaviors, rather than hedonic eating [26,27]. However, the lack of effect of Almorexant in hedonic feeding in stressed animals suggests that periods of food restriction might be critical for the engagement of the OX system in promoting overeating of highly palatable substances [28].

The present study further evaluates, from a pharmacological and molecular approach, the contribution of OX to excessive binge-like consumption of highly preferred palatable substances in animals under no caloric needs. To that end, we first evaluate here, in *ad libitum-fed* animals, the effect of systemic administration of the OXR1 antagonist SB in binge-like drinking of sucrose (a caloric and natural hedonic substance) and saccharin (an artificial sweetener whose reinforcing property is due to its hedonic sweet taste as it lacks calories) in a Drinking in the Dark (DID) procedure. This mouse model of excessive binge-like drinking was originally developed by Rhodes et al. [29,30] to generate high levels of voluntary ethanol consumption over a short period of time (2–4 h) [31]. Interestingly, the DID procedure also triggers binge-like drinking of both caloric and non-caloric palatable substances, such as sucrose or saccharin [32–34] in *ad libitum*-fed animals. Thus, there is evidence that over a period of 2 or 4 h, in a DID procedure, mice consume, as average, more than 50% of the total amount they consume over 24 h [34,35], under a 2-bottle unlimited access procedure [32,34,36].

Molecular studies have added support to the role of OX on reward. Thus, it has been shown a reduction of OX mRNA in the LH following chronic treatment with morphine [37], cocaine [38] or ethanol [39] in rats. The second objective in this study is aimed at evaluating whether repetitive episodes of binge-like drinking of palatable, caloric (sucrose) and non-caloric (sucrose) substances have an impact on OX mRNA expression in the lateral hypothalamus (LH).

2. Material and methods

2.1. Animals and housing

Male adult C57BL/6J mice (Charles River Laboratories, Spain S.A.) were 8 weeks old and weighed 20–25 g on arrival. Animals were housed individually in polycarbonate cages with stainless steel wire mesh lids and sawdust covering the floor. Mice were allowed to acclimate to the housing environment for 1 week before any experimental procedures. The animal house was kept at approximately 21 ± 2 °C in a 12:12 h light/dark schedule (lights on 8 am–8 pm). Animals had *ad libitum* access to chow and water. Behavioral procedures were approved by the Bioethical Animal Care Committee at the University of Almeria, Spain. They were in agreement with the animal care guidelines established by the Spanish Royal Decree 1025/2005 for reducing animal pain and discomfort.

2.1.1. Drugs

The OXR1 antagonist SB-334867 (SB; 1-(2-methylbenzoxazol-6-yl)-3-[1,5]naphthyridin-4-yl urea hydrochloride; Tocris, Bristol, UK) was suspended in 1.5% dimethyl sulfoxide (DMSO), 20% 2-hydroxypropyl-bcyclodextrin (HBC) and sterile water; 10, 20 or 30 mg/kg were given in a volume of 10 ml/kg (ip) 30 min prior to the test session [40]. Vehicle was delivered at the same volume as the SB solution. The OXR1 receptor antagonist SB-334867 is >1000-fold selective for OXR1 over OXR2 [41]. The selected doses of SB have been reported to attenuate reward seeking [42,43], operant self-administration of saccharin [20], reward-feeding behavior [44] and operant responding for food reinforcement [19].

2.2. Experiment 1: effects of ip SB-334867 (10, 20, 30 mg/kg) on sucrose and saccharin binge-like drinking

Experiment 1 was conducted to determine whether OX signaling modulates binge-like consumption of caloric (sucrose) and non-caloric (saccharin) palatable substances in *ad libitum*-fed mice as measured in a standard 4-day *Drinking in the Dark* procedure [29–31,36]. Mice ($n = 40$) were first trained and tested for binge-like 0.15% (w/v) saccharin drinking. One week later, in which animals remained in their home cage with water and food *ad libitum* and did not receive any experimental manipulation, the same group of animals were trained and tested for binge-like 10% (w/v) sucrose drinking. DID was performed as follows: on days 1–3, beginning 3 h into the dark cycle, all home-cage water bottles were replaced with a single bottle of 0.15% (w/v) saccharin or 10% (w/v) sucrose which were weighed and placed on cages for 2 h. During the DID training (days 1–3), mice were habituated to intraperitoneal (ip) injection. To that end, they were injected daily with the appropriate volume (10 ml/kg) of vehicle 30 min prior to sucrose or saccharin presentation. On day 4 (test-day), animals were assigned to groups equated for fluid consumption during the training and given an intraperitoneal (ip) injection of SB (10, 20 or 30 mg/kg) or vehicle 30 min prior to sucrose or saccharin presentation. On the test day, bottle access was extended to 4 h.

An empty cage for each shelf was used for the placement of dummy bottles to measure lost fluid which was subtracted off the total consumption as a control for fluid spillage. Water consumption and body weight measures were recorded daily. On the test day, sucrose and saccharin intake was measured as ml/kg/4 h. Food was available *ad libitum* during the study and measured on the 4-h test period as g/kg/4 h. Total calories ingested over the same test period were included in the statistical analyses as well.

2.3. Experiment 2: effects of ip SB-334867 (30 mg/kg) on locomotor activity

Because reduced fluid and food consumption might be the result of SB-elicited altered locomotor activity, and the role of OX in sleep and arousal, a separate set of C57BL/6J mice ($n = 32$) were evaluated for locomotor activity in response to the highest dose of SB employed in the previous study (30 mg/kg). Open-field locomotor activity-monitoring chambers of $40 \times 40\,cm^2$ equipped with photobeams (Versamax Animal Locomotor Activity, Madrid, SP) were employed. The locomotor activity test consisted of two phases. In the study, all mice were transported to the locomotor testing room in their home cages to habituate for 30 min before the beginning of the test. On phase I, days 1–3, animals were habituated to ip injections by administering them with a solution of vehicle (10 ml/kg) and then placed into the activity chamber for 30 min where motor behavior was recorded. Averaged locomotor activity data during the habituation stage were calculated for each animal that served as individual baseline. During phase II, day 4, all animals received a single ip injection of the OXR1 antagonist SB (30 mg/kg) and then placed into the activity chamber and their locomotor activity recorded for 30 min. The floor of the locomotor activity chamber was carefully cleaned with water after each mouse was tested. Behavioral measures recorded included total distance traveled and total movement time. Locomotor activity data during the test day (day 4) were obtained from baseline individual scores by subtracting activity data in the test-day minus activity baseline data (Test – baseline).

2.4. Experiment 3: effect of four repetitive episodes of binge-like drinking of caloric (sucrose) and non-caloric (saccharin) palatable substances on OX mRNA expression in the lateral hypothalamus (LH)

The present experiment was carried out to evaluate the impact of four, 2-h daily episodes of sucrose or saccharin binge-drinking on OX mRNA expression in the LH. For this purpose, three new groups of animals ($n = 6$) had access to saccharin, sucrose or water in a DID procedure, as previously described. On the test day, 30 min after consumption, mice were sacrificed and brains extracted. Brain dissections were collected as follow: brains were placed in a matrix, and coronal sections were made between approximately −0.88 and −1.76 mm from Bregma (Franklin and Paxinos, 2007). Brain sections were placed on a glass slide, and the LH was removed and collected under a microscope by using the fornix and third ventricle as landmarks. Collected tissue was stored in 200 μl of RNAlater (Sigma-Aldrich Co., St Louis, MO) until processed. Total RNA was purified using TRI reagent (Sigma) following the manufacturer's instructions. One microgram of RNA was retrotranscribed using RevertAid Reverse Transcriptase (Fermentas, Thermo Scientific) according to the manufacturer's recommendations. Quantitative real-time PCR (qPCR) was performed in an Applied Biosystems 7300 real-time PCR system using a mix containing 2.5 ng of cDNA, 5 μM of each primer and PyroTaqEvaGreener Master mix (Cultek). The PCR conditions were: 1 cycle, 95 °C for 15 min; 40 cycles, 95 °C for 15 s, 60 °C for 30 s and 72 °C for 40 s. Each independent sample was assayed in duplicate and normalized using the extensively used $2^{-\Delta\Delta C_t}$ method in qPCR assays. Thus, fold change was calculated according to the differences in C_t values between the orexin and the housekeeping GAPDH genes within the same sample, and between each sample and a reference sample in the water condition. The sequences of the primers were as follows: OX: 5′-GACCACTGCACTGAAGAGATCATC-3′ and 5′-GCCCAGGGAACCTTTGTAG-3′. GAPDH: 5′-TTCACCTGGCACTGCACAA-3′ and 5′-CCACCATCCGGGTTCCTATAA-3′.

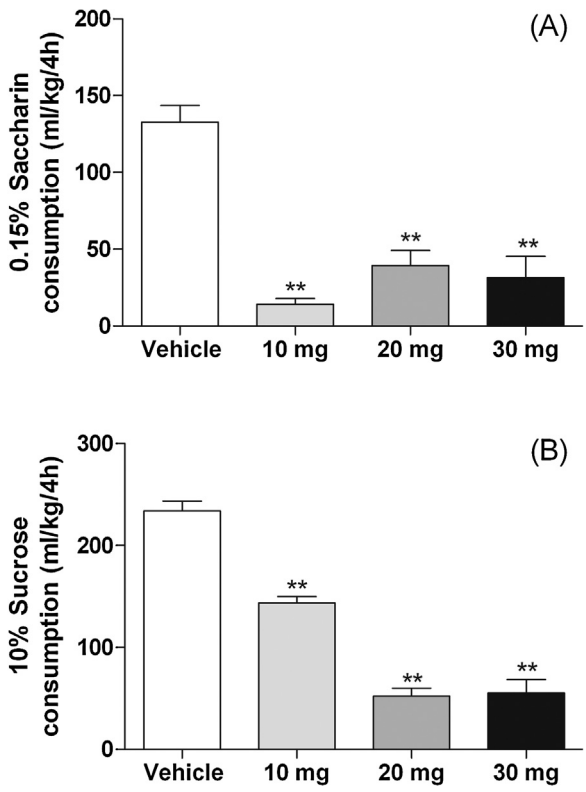

Fig. 1. Consumption (ml/kg/4 h) of 0.15% saccharin (A) or 10% sucrose (B) during the test day in the DID procedure following an intraperitoneal (ip) injection of SB (0, 10, 20 or 30 mg/kg) 30 min before access to saccharin or sucrose. The ANOVAs conducted revealed significant differences in both saccharin and sucrose binge-like consumption in OX-treated mice relative to VEH-treated mice. All values are means ± S.E.M. $^{*}p < 0.05$ and $^{**}p < 0.01$.

2.5. Data analysis

All data in this report are presented as means ± S.E.M. Independent one-way analyses of variance (ANOVAs) were used to analyze saccharin, sucrose and food intake, locomotor activity data and OX mRNA data. Outlier data were first identified by employing the Grubbs' test (standard alpha = 0.05) and then eliminated from the statistics. When significant interactions emerged, post hoc Newman–Keuls test (NK) were carried out. The accepted level of significance for all tests was $^{*}p < 0.05$ and $^{**}p < 0.01$.

3. Results

3.1. Experiment 1: effects of ip SB-334867 (10, 20 and 30 mg/kg) on sucrose and saccharin binge-like drinking in ad libitum mice

Fig. 1 shows data representing 4-h voluntary binge-like consumption of 0.15% (w/v) saccharin (Fig. 1A) and 10% (w/v) sucrose (Fig. 1B) during the test-day of the DID procedure. Independent one-way ANOVAs performed on fluid consumption data revealed that 10, 20 and 30 mg/kg doses of SB significantly reduced saccharin [$F(3, 40) = 29.56$; $p < 0.01$] and sucrose [$F(3, 40) = 87.12$; $p < 0.01$] binge-like consumption. Additional NK analysis revealed that animals treated with the OXR1 antagonist SB (10, 20 or 30 mg/kg) consumed significantly less saccharin and sucrose than did animals treated with vehicle. Additionally, NK tests showed that animals treated with 20 and 30 mg/kg of SB significantly reduced sucrose consumption relative to animals treated with 10 mg/kg of dose.

Total calories from both sucrose (calories from chow + sucrose) and saccharin (calories from chow) were analyzed (see Table 1). Independent one-way ANOVAs performed on each set of data

Table 1
Total calories consumed (kcal/g/4 h) while drinking sucrose or saccharin during the test day after administration of the OXR1 antagonist SB (10, 20 and 30 mg/kg) or vehicle.

	Sucrose group (kcal/g)	Saccharin group (kcal/g)
VEH	3.47 ± 0.20	2.74 ± 0.42
SB (10 mg)	2.24 ± 0.14**	0.20 ± 0.09**
SB (20 mg)	1.11 ± 0.28**	1.24 ± 0.49**
SB (30 mg)	0.97 ± 0.19**	0.64 ± 0.39**

All values are means ± S.E.M.
** $p < 0.01$.

revealed that total calories ingested while drinking sucrose [$F(3, 40) = 33.38$; $p < 0.01$] and saccharin [$F(3, 39) = 8.95$; $p < 0.01$] were significantly reduced by SB. NK post hoc independent analysis performed on calorie data obtained in each study revealed that in both cases, animals treated with SB consumed significantly fewer calories than did animals treated with vehicle and groups treated with 20 and 30 mg/kg of SB significantly reduced calories consumed relative to the 10 mg/kg-dose group.

3.2. Experiment 2: effects of ip SB-334867 (30 mg/kg) on locomotor activity

Independent one-way ANOVAs conducted on each set of locomotor activity data (changes in locomotor activity from baseline) revealed that administration of the OXR1 antagonist SB did not significantly alter total distance traveled (mean ± S.E.M. vehicle: −1334.24 ± 494.31; SB (30 mg): −2968.90 ± 670.28; [$F(1, 12) = 3.85$; $p > 0.05$]) or movement time (mean + S.E.M. vehicle: −122.12 ± 43.61; SB (30 mg): −250.26 ± 58.63; [$F(1, 12) = 3.07$; $p > 0.05$]) during the 30-min test day.

3.3. Experiment 3.3: effect of binge-like drinking of caloric (sucrose) and non-caloric (saccharin) substances on OX expression in the lateral hypothalamus (LH)

The ANOVA conducted on mRNA data revealed that four repetitive 2-h daily episodes of sucrose and saccharin binge-drinking, but not water (see Table 2 for consumption data), significantly decreased OX mRNA expression in the LH [$F(2, 3) = 19{,}540$; $p < 0.05$] (Fig. 2).

4. Discussion

Two main observations emerged in the study: (1) systemic administration of SB, a selective OXR1 antagonist, significantly decreased binge-like consumption of sucrose and saccharin in a DID procedure, in *ad libitum*-fed animals, without evidence of any significant alteration of locomotor activity. (2) Four repetitive, 2-h daily episodes of sucrose and saccharin (but not water), binge-like drinking significantly dampened OX mRNA expression in the LH.

Because the highest dose of SB employed in the consumption studies did not significantly alter locomotor activity exhibited on an open arena, it is unlikely that the effect on binge-like consumption was due to drug-induced altered motor behavior. Our conclusion is consistent with previous studies which have reported non-altered locomotor activity in both rats [18,45] and mice [19,40] following administration of the same doses of SB that we have employed here. Furthermore, present data are in agreement with previous findings, showing that peripheral administration of 20–30 mg/kg of SB had no effect on inactive lever presses in sucrose [20] and cocaine [24] nor on responding during a cue-induced reinstatement test for saccharin [22].

Table 2
Consumption data (ml/kg/2 h) of water, 0.15% saccharin or 10% sucrose during the DID procedure associated to the mRNA study.

	Water consumption (ml/kg/2 h)	Sucrose consumption (ml/kg/2 h)	Saccharin consumption (ml/kg/2 h)
Day 1	34.40 ± 0.64	72.42 ± 8.78	67.70 ± 6.86
Day 2	23.42 ± 3.50	117.72 ± 10.46	94.41 ± 15.24
Day 3	22.14 ± 0.87	125.80 ± 4.16	77.88 ± 6.87
Day 4	21.12 ± 2.29	103.74 ± 8.07	86.69 ± 3.64

All values are means ± S.E.M.

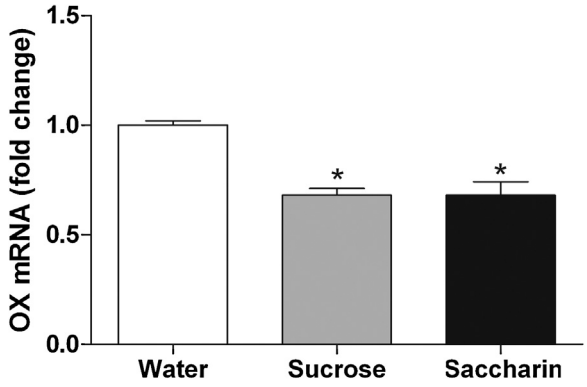

Fig. 2. OX mRNA expression in the LH after four repetitive 2-h episodes of binge-like consumption of water, 0.15% saccharin or 10% sucrose. All values are means ± S.E.M. *$p < 0.05$ and **$p < 0.01$.

An important issue to consider regarding the observed generalized reduction of fluid consumption is that drug treatment might have caused aversion, malaise and/or visceral distress, leading to unspecific effects on fluid and food intake. While this possibility cannot be completely discarded, the observation that icv administration of SB does not elicit unspecific malaise or sickness at doses effectively reducing conditioned fluid consumption of palatable substance [46] supports our conclusion that reduced binge-like drinking following OXR1 blockade are unlikely the result of generalized malaise.

The observation that SB blunted saccharin binge-like drinking (a non-caloric substance) in *ad libitum*-fed animals extends the conclusions of a recent report showing a significant decrease in active lever responding and the number of saccharin reinforces earned, both in food-restricted and non-restricted animals, pointing to a role for OX in hedonic aspects of food-seeking [22]. Noteworthy, studies aimed at testing the role of OX in hedonic eating have been conducted in food-restricted animals exposed to highly palatable, caloric substances [28,47]. Because our studies employed *ad libitum*-fed animals, present data provide evidence for the first time that OXR1 modulates excessive binge-like consumption of non-caloric substances, which support a role for OX acting on OXR1 in regulating non-homeostatic, hedonic aspects of overconsumption.

On the other hand, our finding that OX is engaged in sucrose binge-like drinking in animals under non-caloric restriction contrasts with recent reports indicating that the OX system might be differentially engaged by caloric vs non-caloric hedonic food rewards, depending on the deprivation state during consumption [22]. Thus, SB reduces FR responding for high-fat food and sucrose [4,20,21,23] and PR responding for chocolate [24] only in food-restricted animals. In the same direction, Piccoli et al. [27] demonstrated that the selective OX1R antagonist GSK1059865 is unable to inhibit highly palatable food consumption in animals under not restricted access to food, while it reduced binge-eating under mild stress and restricted food access conditions [26,27]. These findings, together with the lack of effect of Almorexant in hedonic feeding in stressed animals, have led to the hypothesis that

periods of food restriction might be critical for the engagement of the OX system in promoting overeating of highly palatable caloric substances [28].

The reason why OXR1 antagonism in the present study significantly reduced sucrose binge-drinking in animals under non-caloric restriction remains unclear but several possibilities are discussed. First, with present experimental design where the same animals were employed for the saccharin and the sucrose study, we cannot completely rule out the influence of saccharin experience on sucrose drinking. It is possible that exposure to the very sweet, but non-caloric saccharin, alters the hedonic aspects in sucrose. If so, animals might be comparing the final reward to previous experience with both rewards. In this regard, if saccharin consumption interferes with the hedonic aspects of sucrose, then, having no previous experience with saccharin consumption should trigger different rates of sucrose drinking to that achieved in our study. Arguing against this idea, previous behavioral studies where sucrose drinking is tested in a binge-like drinking procedure in the absence of previous experience with saccharin report very similar levels of sucrose intake to that achieved in our study [32]. On the other hand, it is not unusual in the literature to find a set of tastants (saccharin, sucrose, ethanol or quinine) tested on the same group of animals, either on operant, binge-like drinking or two-bottle choice, drinking tests [34,48–50].

Second, our data are in agreement with previous literature showing a key role for OXR1 signaling in modulating homeostatic aspects of food intake [51]. Thus, the observation that sucrose intake and total calories ingested (fluids + chow) (both in the saccharin and sucrose studies) were significantly reduced after SB administration is in accordance with previous pharmacological observation that acute systemic treatment with SB dose-dependently blocks the hyperphagic and behavioral effects of orexin-A in rats [15,17] and, when given alone at high doses (30 mg/kg), significantly inhibits food intake of both normal chow [52] and palatable food [18]. Moreover, 14-day treatment with SB reduces cumulative food intake and body weight gain in ob/ob mice [52] and OX knockout mice exhibit a hypophagic phenotype [13]. Because our study employs systemic administration of the OXR1 antagonist and given the complexity and extended physiological actions of the OX system, it will be not surprising that SB antagonized OXR1 at several anatomical brain sites modulating independent physiological functions, such as homeostatic and hedonic aspects of food intake. If this is the case, the impact of OXR1 antagonism on sucrose and saccharin binge-drinking would be caloric- and hedonic-driven, respectively. Additional pharmacological site-directed studies are needed to further explore this hypothesis.

Third, although DID is not a caloric-restricted procedure, it is worth to mention that it allows limited access (2–4 h/day) to highly preferred substances, at that circadian time point when caloric needs are higher [53]. There is the possibility that at 3 h into the dark (when palatable substances are offered in the DID test), the energy status of animals is negatively balanced which might parallel that physiological status during mild food restriction. If so, our finding showing reduced sucrose binge-drinking due to SB administration will be perfectly predicted by the hypothesis holding that the OX system might be differentially engaged by caloric vs non-caloric hedonic food rewards depending on the deprivation state during consumption [22]. Additional studies specifically manipulating the energy status during a DID test are needed to further understand the role of OXR1 signaling in binge-like consumption.

The second relevant observation in the present study show that four 2-h daily repetitive episodes of sucrose and saccharin binge-like drinking, but not water, reduced OX mRNA expression in the LH, which further supports the engagement of OX in overconsumption of highly palatable substances during DID. Furudono et al. [25] showed a significant increase in OX mRNA after saccharin overconsumption and then a gradual diminution to a basal level. Consistent with our present data, these results suggest that overconsumption and binge-like consumption of a reward, including natural caloric (sucrose) and artificial non-caloric (saccharin) sweet substances, triggers activity of the OX system, leading to a short-term adaptive, compensatory reduction of OX synthesis. There is the exciting idea that the adaptive reduction in OX synthesis might work as a protective neurochemical mechanism which prevents from overconsumption escalade; moreover, it is tempting to propose that the inability to properly blunt OX activity in response to binge-like eating episodes might turn organisms vulnerable to the development of binge-eating disorders.

An “episode of binge eating” is defined as eating in a discrete period of time (usually less than 2 h) an amount of food that is definitely larger than most individuals would eat in a similar period of time under similar circumstances [54]. Prolonged binge eating, also defined as food addiction [55], is a compulsive behavior characterized by food overconsumption without caloric need rather than craving for a specific nutrient, is a key feature of bulimia nervosa or binge-eating disorder and has been observed in obese patients as described by the DSM-V [54–56]. Given the existence of behavioral and neurobiological similarities in drug addiction and binge eating (food addiction) [55,57,58], and because DID has been proposed as a valuable experimental tool modeling pre-dependent, impulsive transitional states to alcohol dependence [59], it is tempting to speculate that DID might successfully model the initial states of binge-eating disorders. In this regard, first, given the proposal that transition to drug dependence involves sensitization of the stress system in the brain [60]; second, the role of OX in arousal and stress [61]; and third, the idea that the same neural systems involved in drug dependence are recruited during the initial pre-dependence states, there is the exciting idea that OX activity is engaged in a positive loop in vulnerable organisms where binge-eating recruit OX activity and increased OX activity further triggers binge-eating episodes and the development of a binge-eating disorder over time.

In light of the available data showing the importance of OX signaling in food-reward behaviors and food overconsumption, our present findings further support the engagement of OXR1 signaling in hedonic eating and extend the current knowledge indicating a role for OXR1 signaling in binge consumption of palatable substances in organisms under no caloric needs. Current medication (Topiramate) employed to reduce episodes of binge eating in clinical studies has been associated with a variety of side effects [62,63] and new pharmacological strategies are needed. Moreover, our findings suggest that targeting OXR1 could represent a novel pharmacological approach to control binge-eating episodes in vulnerable organisms failing to reduce OX activity with repetitive binge-eating episodes.

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