ORIGINAL

Involvement of NPY-Y1 Receptors in Periaqueductal Gray on Anxiety, and Food, Sucrose, and Alcohol Consumption in Pre-exposed Wistar Rats

Participación de los Receptores NPYY1 en la Sustancia Gris Periacueductal en la Ansiedad, el Consumo de Alimentos, Sacarosa y Alcohol en Ratas Wistar Preexpuestas

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Abstract

Periaqueductal gray (PAG) is a well-documented region on integrated defensive responses and anxiety-like behavior. However, only recently has been shown its role in drug/alcohol consumption, mainly in the relapse state and associated with anxiety. NPY is known as the major anxiolytic endogen neuropeptide, besides its well-recognized orexigenic action mediated mainly by NPY-Y_1 and NPY-Y_5 receptors respectively. Herein, we addressed the role of the NPY-Y_1 receptor in the dorsal (D)-PAG on anxiety-like behavior through a defensive burying test, as well as the food, sucrose, and alcohol consumption in male Wistar rats, with a sucrose fading paradigm at the juvenile stage as the alcohol intake initiation procedure. Present results confirmed that intra D-PAG NPY is a key modulator neurotransmitter system on anxiety-like behavior. Additionally, NPY significantly increased both the food and sucrose intake and decreased the alcohol consumption in voluntary sucrose (10%) and alcohol at different concentrations models. These results are likely mediated by NPY acting in the NPY-Y_1 receptor in the D-PAG.

Keywords: Periaqueductal gray; NPY; BIBP-3226; Anxiety; Food; Sucrose; Alcohol

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Resumen

La sustancia gris periacueductal (SGP) es una región ampliamente estudiada sobre las respuestas defensivas integradas y comportamientos de tipo ansiedad. Sin embargo, recientemente se ha demostrado el papel sobre el consumo de drogas/alcohol, principalmente en la etapa de recaída, la cual está asociada a la ansiedad. El neuropéptido Y (NPY) es conocido como el principal neuropéptido endógeno ansiolítico, además de su reconocida acción orexigénica mediada principalmente por los receptores NPY-Y1 y NPY-Y5, respectivamente. En este trabajo, abordamos el papel del receptor NPY-Y1 en la SGP-dorsal sobre el comportamiento de tipo ansiedad mediante una prueba de enterramiento defensivo, consumo de alimentos, sacarosa y alcohol en ratas Wistar macho, con un paradigma de consumo de sacarosa en la etapa juvenil como procedimiento de previo a la ingesta de alcohol. Los resultados confirmaron que la administración intra SGP-D de NPY es un sistema neurotransmisor modulador clave en el comportamiento similar a la ansiedad. Además, el NPY aumentó significativamente tanto la ingesta de alimentos como de sacarosa y disminuyó el consumo de alcohol en el modelo de consumo voluntario de sacarosa (10%) y alcohol a diferentes concentraciones. Es probable que estos resultados estén mediados por la acción del NPY en el receptor NPY-Y1 en la SGP-D.

Palabras clave: Sustancia gris periacueductal; NPY; BIBP-3226; Ansiedad; Alimentación; Sucrosa; Alcohol

Neuropeptide Y (NPY), a 36-amino acid peptide (Tatemoto et al., 1982; Tatemoto et al., 1982), is the most highly concentrated and widely expressed peptide in the mammalian brain (Allen et al., 1983; Gray and Morley, 1986). It is markedly dense in the rat central nervous system (CNS) and in the brain, specifically in the cortex, hippocampus, hypothalamus (Chronwall et al., 1985), basal ganglia particularly in nucleus accumbens, amygdala, locus coeruleus (Allen et al., 1983) and periaqueductal gray (PAG) (Kask et al., 2002). The various effects of NPY in mammals are attributed to the activation of at least four receptor subtypes designated as Y_1, Y_2, Y_4, and Y_5 (Larhammar & Salaneck, 2004). The typical signaling responses of NPY receptors are similar to those of other G_i/G_0-coupled receptors (Limbird, 1988), leading to the inhibition of adenylyl cyclase (Krause et al., 1992; Silva et al., 2002).

NPY is one of the key neuropeptide systems modulating many important physiological and behavioral functions. NPY can be considered as the major anxiolytic endogenous peptide, mainly acting in Y_1 receptors in many brain sites, as has been shown by studies using different anxiety models such as in the amygdala with a conflict test (Heilig et al., 1993), the lateral septum with the novelty-induced suppression of feeding test (Trent and Menard, 2011), the dorsal (D)-PAG (Kask et al., 1998a; Vázquez-León et al., 2017) or intracerebroventricular (Badia-Elder et al., 2003) with the elevated plus maze test (EPM), as well as in the D-PAG with a social interaction test (Kask et al., 1998b), and with defensive burying behavior test (DBB) (Vázquez-León et al., 2017, 2020).

NPY is a potent orexigenic peptide stimulating food intake, preferentially on carbohydrates (Jhanwar-Uniyal et al., 1993; Beck, 2006). NPY may stimulate the consumption of sweet solutions regardless of their caloric value and may potentiate sweet taste preference via an associative mechanism (Lynch et al., 1992). The NPY effects on feeding are mediated through at least two receptors, Y_1 and Y_5 receptors (Beck, 2006). The effects of specific NPY receptor agonists suggest that the Y_1 might mediate the appetitive component of ingestive behavior, whereas the Y_5 may be more involved in the consummatory component (Day et al., 2005; Beck, 2006).

NPY-Y_1 administration has been shown to reduce both anxiety-like behavior and alcohol consumption in several animal models (Bertholomey et al., 2011; Heilig et al., 1993; Wettstein et al., 1995). NPY reduces alcohol consumption in selectively bred alcohol-preferring (P) rats, and high alcohol drinking (HAD) rats given free-choice access (Badia-Elder et al., 2001; Gilpin et al., 2008; Zhang et al., 2010).

As well as, in non-selectively bred rats exposed to vapor inhalation, liquid diet, chronic intermittent access (Gilpin et al., 2008; Thorsell et al., 2005), or forced alcohol intake since juvenile age (Vázquez-León et al., 2020). However, injected NPY bilaterally into the paraventricular nucleus of the hypothalamus (PVN) of high and low alcohol drinking rat lines (HAD and LAD, respectively), feeding and ethanol drinking increased in both groups (Gilpin et al., 2004). Moreover, there is an increase in ethanol intake following NPY infusions into the PVN of male Long-Evans rats (Kelley et al., 2001). Therefore, NPY site-specific administration in brain areas associated with feeding appears to increase ethanol intake, whereas administration in brain areas associated with anxiety reduces ethanol intake (Badia-Elder et al., 2007; Vázquez-León et al., 2020). Moreover, it has been suggested that NPY may influence ethanol consumption in many ways, mainly regulating basal levels of anxiety, modulating the sedative effects of ethanol, and/or modulating ethanol's rewarding properties (Thiele et al., 2004).

The participation of the PAG in the intake of substances of abuse, that include sucrose and alcohol, might be linked to its close interaction with nuclei that are known to be involved in the reinforcement, such as the nucleus accumbens (NAc), ventral tegmental area (VTA), and hypothalamus (Koob & Volkow, 2010; Tyron & Mizumori, 2018; Vázquez-León et al., 2021a). Additionally, it has been shown that animals, including humans, with high sweet substances preference drink more alcohol than such displaying low preference (Avena et al., 2004; Kampov-Polevoy et al., 1995, 2003; Koros et al., 1998; Toalston et al., 2008).

Due to the relevance of the NPY system on anxiety, ingestive behavior, and the relationship between both, we investigated the role of the NPY-Y_1 receptor in the dorsal periaqueductal gray (D-PAG). In a previous study, we found that NPY in the D-PAG, which seems acting on NPY-Y_1 receptors, produced a significant anxiolytic effect and prominently inhibited alcohol consumption and relapse in Wistar rats (Vázquez-León et al., 2020). In the present study, we re-evaluate the function of the NPY-Y_1 receptors in D-PAG on anxiety-like behavior through the DBB. Additionally, the assessment of food intake, sucrose intake, and alcohol intake in food and fluid non-deprived (alcohol/sucrose naïve=ASN), compared to sucrose-fading model (SF) ethanol intake initiation procedure Wistar rats.

Materials and methods

Animals and housing

54 Male Wistar rats at postnatal day 21 (PND 21) were obtained from the animal care facilities in "Escuela Nacional de Ciencias Biológicas" of the "Instituto Politécnico Nacional". The experimental protocol was in accordance with the procedures established by the NIH in the Guide for the Care and Use of Laboratory Animals in the USA and by the Mexican Guidelines for Animal Care (NOM-062-ZOO-1999). Rats were kept under a light/dark cycle (lights on at 07:00) with controlled temperature (20–22 °C) and humidity (45–55%) and free access to food and water, except during sucrose-fading model (SF) and for the assessment of food, sucrose, and alcohol intake. Every effort was made to minimize the number of animals required to achieve the goals of the study and to alleviate any distress the animals might experience during the set of experiments.

Experimental design

Figure 1 shows the experimental design. The first period of SF began at 43 PND, at 60-68 PND the first imposed withdrawal period, at 69-85 PND the second SF period, and at 86-94 PND the second imposed withdrawal period. During these periods, the ASN group had free access to food and water. PND 95 both groups (ASN and SF) underwent stereotaxic surgery to implant the guide cannula in D-PAG in PND 102 drug treatments (i.e., VEH or NPY, or BIBP) were randomly microinjected to the corresponding group (ASN or SF), and the DBB test was performed. In PND 103, the results of the voluntary

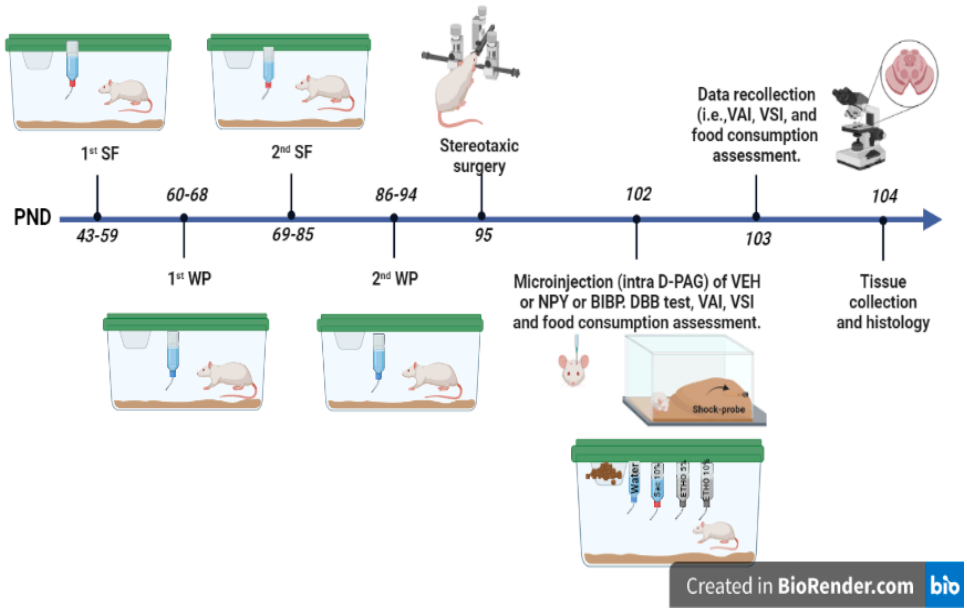

Figure 1. Diagram of the experimental design. BIBP, BIBP-3226; DBB, defensive burying behavior test; D-PAG, dorsal periaqueductal gray; NPY, neuropeptide Y; SF, Sucrose-fading model; VAI, voluntary alcohol intake; VEH, Vehicle; VSI, voluntary sucrose intake; WP, withdrawal period.

evaluation of alcohol, sucrose, and food consumption were obtained. Finally, the animals were sacrificed (in DPN 104) to obtain the brains and carry out the histological analysis (Fig. 1).

Sucrose-fading model

Sucrose a highly reinforcing sweet substance in rodents (Koob, 2008) can be gradually exchanged for alcohol. The SF consists of combining ethanol (in increasing concentrations) and sucrose (in decreasing concentrations) in a solution together with filtered water until reaching ethanol (10% v/v) with sucrose (1% w/v) (Samson, 1986; Samson et al., 1999). This model was originally used as a method to investigate appetitive or consummatory behavior elicited by a reinforcer in repeated training sessions in operant chambers (Samson, 1986). Since sucrose intake/preference assessment often occurs in the animal's cage (Koob, 2008), we adapted the model to develop subsequent alcohol or sucrose consumption.

SF for the alcohol intake initiation procedure was performed in two stages, the first from day 1 to 16 (PND 43-59) and the second from day 24 to 41 (PND 69-85) of the experiment as follows: 2 days of 20% sucrose; 3 days of sucrose 10% and ethanol 2% (v/v); 3 days of sucrose 10% and ethanol 5% (v/v); 2 days of sucrose 10% and ethanol 10% (v/v); 2 days of sucrose 5% and ethanol 10% (v/v); 2 days of sucrose 2% and ethanol 10% (v/v); and finally 2 days sucrose 1% and ethanol 10% (v/v) adapted from (Samson, 1986; Samson et al., 1999). This alcohol intake initiation procedure provides another way to investigate together sucrose and alcohol consumption in animals that have not been either food or fluid-deprived (Samson, 1986).

Stereotaxic surgery

The animals were anesthetized with ketamine (80 mg/kg, Pisa®) and xylazine (15 mg/kg, Pisa®) intraperitoneally, supplemented with additional ketamine when

necessary. A one-sided guide cannula directed from the skull to the D-PAG (AP: –7.3 mm, ML: 0.5 mm and DV: 4.0 mm) from bregma was implanted, using a stereotaxic frame (mod: 502,650, World Precision Instruments® Sarasota FL) and coordinates from the atlas of Paxinos and Watson (2014). The guide cannula was held in place to the skull with two screws and dental acrylic, and a stylet was inserted into the guide.

Drugs and microinjections

One week after stereotaxic surgery, the respective pharmacological treatment was microinjected into the D-PAG region through the cannula (31 gauge × 10 mm), which extended 1 mm beyond the tip of the guide cannula. The injection cannula was connected to a 1-µL syringe (Hamilton Co., Reno, NV, USA) with polyethylene 20 tubing filled with sterile isotonic saline solution (ISS). Microinjections through the cannula were made manually over a 60 s period, and then the injection cannula was left undisturbed for 60 s to avoid back-flow of the drug. Meanwhile, any struggling movement of the rat was gently restricted, allowing only calm movements. After all the behavioral tests, the stylet was cleaned and returned to the guide cannula. All microinjections were performed during the same time window: 4 to 7 pm. ASN and SF received 0.5 µL of (i) vehicle (VEH; isotonic sterile saline solution), (ii) Neuropeptide Y human, Sigma-Aldrich® (NPY; 2.34 nmol), or (iii) R)-N-(diphenylacetyl)- N-[(4-hydroxy-phenyl)methyl]- D-arginine amide (BIBP; 0.5 nmol) (Kask et al. 1998; Vázquez-León et al., 2017, 2020).

Defensive burying behavior test

After microinjections of pharmacological treatment, the defensive burying behavior test (DBB) was performed in the shock-probe burying apparatus. It consisted of a Plexiglass chamber (40 × 30 × 40 cm) with a 5 cm layer of wood chips over the floor. Two copper wires wrapped a wooden dowel (6 × 0.5 cm outer diameter; 2000 V, 2 mA intensity) which was inserted through one hole of the chamber located 2 cm above the bedding material (Dringenberg et al., 2008; Treit et al., 1998; Vázquez-León et al., 2017, 2020, 2021b). The 15 min test began immediately after rats received the first contact induced shock from the probe. Measurements (in seconds) were latency to burying, duration of burying, and duration of immobility. Between trials, rats were returned to their home cages, the bedding material was discarded, and new bedding material was placed in the chamber for the next animal.

Food consumption

Individual cages were provided with 50g of standard rodent lab-chow food pellets (Propecua™, Mexico) in a stainless steel container located at the front, along with the four tubes as fluids diet dispensers. At 24 hours, the food container was briefly removed, and the food was weighed to determine the amount (g/day) eaten. The measurement of food intake for each rat was rectified for unavoidable spillage collected immediately under the respective cage.

Sucrose consumption

Voluntary sucrose intake (VSI) assessment by a fresh solution of sucrose (10%) was prepared by dissolving sucrose (Merck®) in filtered water. The sucrose solution was provided in a standard glass tube in the same way as the freshwater and alcohol solutions. Sucrose consumption was calculated by weighing the tube at the beginning and the end of the 24-hour period and expressed in g/day.

Alcohol consumption

Voluntary alcohol intake (VAI) was measured in all rats kept in individual cages with access to four standard glass tubes (70 mL, 25 × 200 mm) equipped with a glass mouthpiece containing a terminal hole (diameter = 1 mm) to allow fluid intake by licking

with minimum spillage. The glass tubes were previously mounted on the front of the cage, and each will be filled with different solutions: freshwater, sucrose 10%, 5% v/v ethanol, and 10% v/v ethanol. Ethanol concentrations were chosen based on those employed by Spanagel and Hölter (1999), Mendoza-Ruiz et al. (2018), and Vázquez-León et al. (2020, 2021b). Each glass tube is weighed to quantify the amount of alcohol consumed per solution. Alcohol intake is calculated in grams of absolute alcohol per kg of body weight (g/kg BW) for 24 hours.

Histology

Upon completion of all the behavioral experiments, animals overdosed with a lethal injection of sodium pentobarbital (70 mg/kg, ip). Intracardiac formaldehyde (4%) perfusion was followed by removal of the brain and then placed in formaldehyde (15%). Afterward, the brain was mounted in a 6% sucrose bath and coronally sectioned (100 µm) using a vibratome (World Precision Instruments®, Sarasota FL). The slices were dyed with cresyl violet (Sigma®, St Louis, MO) and viewed under a microscope (Nikon® SMZ-800) to identify the microinjection site in consultation with Paxinos and Watson (2014) and Swanson (2018). Only data from animals with a verified location of the cannula in the D-PAG were considered for statistical analysis.

Data analysis

Data were analyzed using Sigma Plot 12.0 software (Systat Software Inc., San Jose, CA, USA). A two-way ANOVA was performed for DBB (latency to burying (LAT), duration of burying (DB), and duration of immobility (DI)), food consumption, sucrose intake, and total ethanol intake. For all tests, the measure of central tendency was the mean while the measure of dispersion was the standard error of the mean (SEM). When a test exhibited significant differences, a Student-Newman-Keuls *post-hoc test* was applied to all pairwise comparisons. For all experiments, statistical significance was considered at $p < 0.05$.

Results

Stereotaxic surgery

A schematic of a D-PAG representative microinjection site according to Swanson (2018).

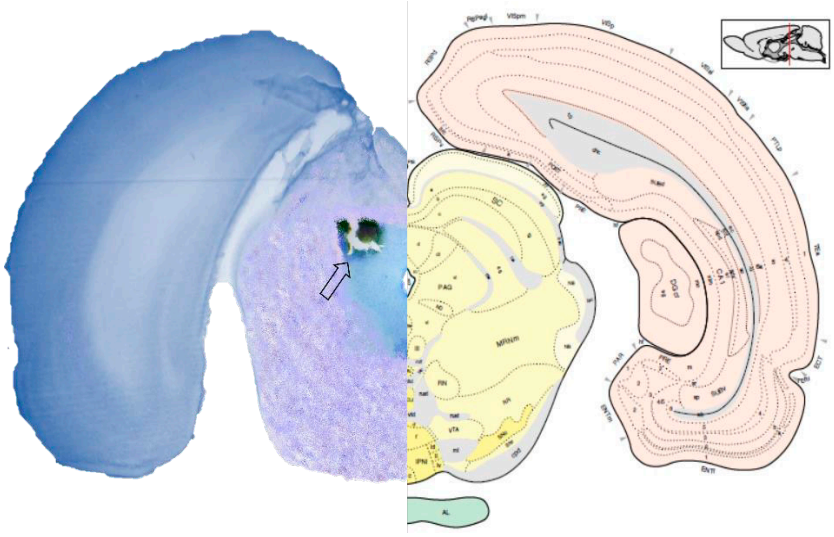

Figure 2. Histological example of the injection site in lateral D-PAG. The right image was taken from Swanson (2018).

Defensive burying behavior test

After the first shock, there were differences between pre-treatment (i.e., ASN vs. SF) both in latency for the beginning of burying behavior [$F_{(1, 48)}$ = 11.084, $p < 0.005$] and duration of burying [$F_{(1, 48)}$ = 8.83, $p < 0.01$]. In the duration of immobility, no significant differences were found between pre-treatment [$F_{(1, 48)}$ = 0.785, $p > 0.05$]. Pharmacological treatments differences [$F_{(2, 48)}$ = 67.033, $p < 0.001$] were detected with not interactions with pre-treatment [$F_{(2, 48)}$ = 1.217, $p > 0.05$]. NPY increased the latency to burying and BIBP-3226 decreased the duration of burying ($p < 0.05$) in both pre-treatment ASN and SF (Fig. 3A). Differences between pharmacological treatments [$F_{(2, 48)}$ =104.2, $p < 0.001$] were found with no interactions between pre-treatment and pharmacological treatments [$F_{(2, 48)}$ =1.326, $p > 0.05$]. The NPY produced a lower duration of burying compared with the VEH and BIBP-3226 treatment ($p < 0.05$). The rats which received BIBP-3226 showed an increase in the duration of burying compared with NPY and VEH ($p < 0.05$) (Fig. 3B). Significant differences between pharmacological treatments were found [$F_{(2, 48)}$ = 83.843, $p < 0.001$]. NPY significantly decreased the duration of immobility compared with VEH and BIBP-3226, and BIBP-3226 increased significantly immobility ($p < 0.05$) compared with VEH and NPY. The interactions between pre-treatment vs. pharmacological treatments do not differ significantly [$F_{(2, 48)}$ = 2.035, $p > 0.05$] (Fig. 3C).

Food consumption

After DBB, food consumption in 24 hours was measured. We found significant differences between pharmacological treatments [$F_{(2, 48)}$ =1.635,

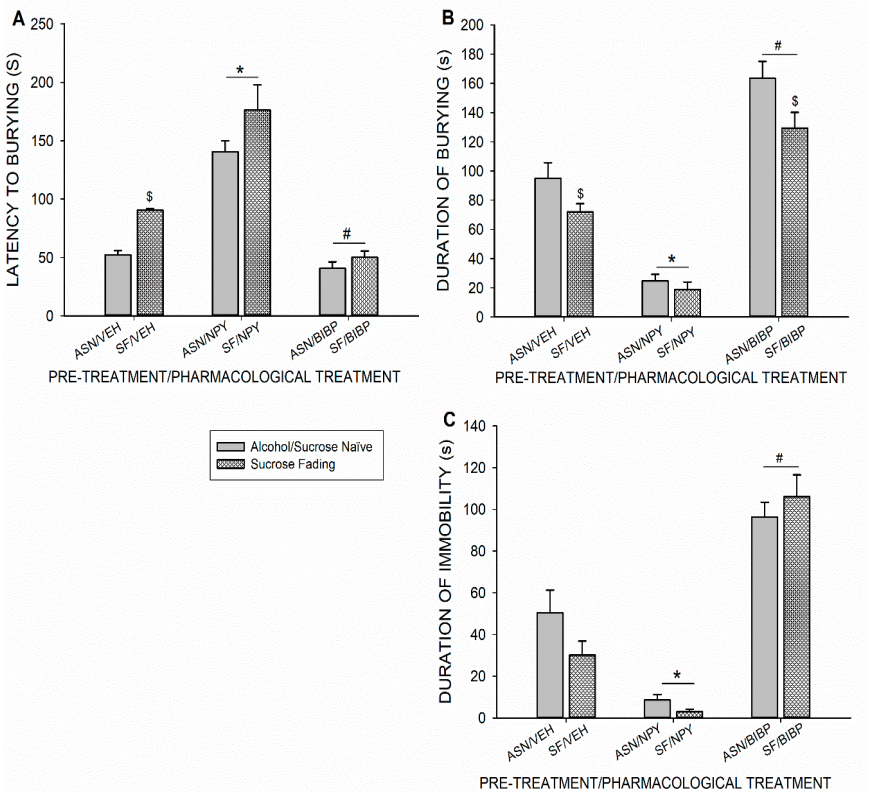

Figure 3. Effects of pre-treatment and pharmacological treatment on the latency to burying (A), duration of burying (B), and duration of immobility (C) after the first shock on the defensive burying behavior test. Comparisons were analyzed by a Student-Newman-Keuls *post-hoc* test: $^{\$}p < 0.05$ sucrose-fading vs. alcohol/sucrose naïve, $^{*}p < 0.05$ NPY vs. VEH and BIBP-3226, $^{\#}p < 0.05$ BIBP-3226 vs. VEH and NPY. Data are expressed as the mean ± SEM (n = 9 per group).

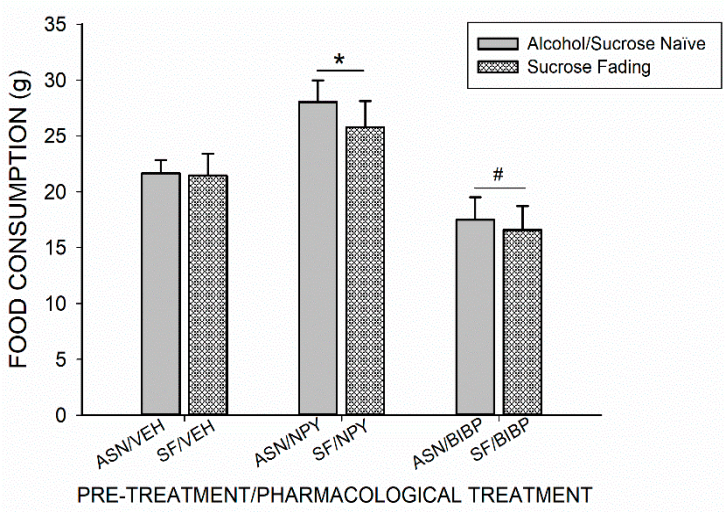

Figure 4. Effects of pre-treatment and pharmacological treatment on the food consumption in 24-hour period after the microinjection of pharmacological treatment. Comparisons were analyzed by a Student-Newman-Keuls *post-hoc* test: $^{\$}p < 0.05$ sucrose-fading vs. alcohol/sucrose naïve, $^{*}p < 0.05$ NPY vs. VEH and BIBP-3226, $^{\#}p < 0.05$ BIBP-3226 vs. VEH and NPY. Data are expressed as the mean ± SEM (n = 9 per group).

$p < 0.001$]. However, no significant differences were found between pre-treatments [$F_{(1, 48)} = 0.482$, $p > 0.05$], and interactions pre-treatments vs. pharmacological treatments [$F_{(2, 48)} = 0.141$, $p > 0.05$]. The microinjection of NPY significantly increased food consumption while the BIBP-3226 significantly decreased food consumption in both pre-treatment ASN and SF groups (Fig. 4).

Sucrose consumption

VSI assessment was performed. Significant differences between pharmacological treatments [$F_{(2, 48)} = 12.001$, $p < 0.001$] and interactions between pre-treatment vs. pharmacological treatments [$F_{(2, 48)} = 4.587$, $p > 0.05$] were detected. The SF/VEH group significantly consumes more sucrose than the ASN/VEH group. On the other hand, the ASN/NPY group significantly consumes more sucrose than both ASN/VEH and BIBP-3226 groups. Finally, the SF/BIBP-3226 group significantly consumes less sucrose than both SF/VEH and NPY groups ($p < 0.05$) (Fig. 5).

Alcohol consumption

VAI at 5% and 10% (v/v) was performed. Significant differences were found between pre-treatment [$F_{(1, 102)} = 12.605$, $p < 0.001$], pharmacological treatment [$F_{(2, 102)} = 32.009$, $p < 0.001$], and interaction pre-treatment vs. pharmacological treatments [$F_{(2, 102)} = 6.204$, $p = 0.003$]. The BIBP-3226 significantly increases total ethanol consumption compared to VEH and NPY in both pre-treatment groups (Fig. 6).

There were no significant statistical differences in water intake or interactions between pre-treatment (ASN or SF), and pharmacological treatment (VEH, NPY, and BIBP-3226).

Discussion

The repeated sucrose-fading as an alcohol initiation procedure from juvenile age leads to an anti-anxiety effect assessed through DBB, i.e. significantly increased latency and decreased duration of burying. Rats displaying low burying are disposed to consume more alcohol or vice versa, in an apparently reciprocal relationship between alcohol consumption and defensive strategy.

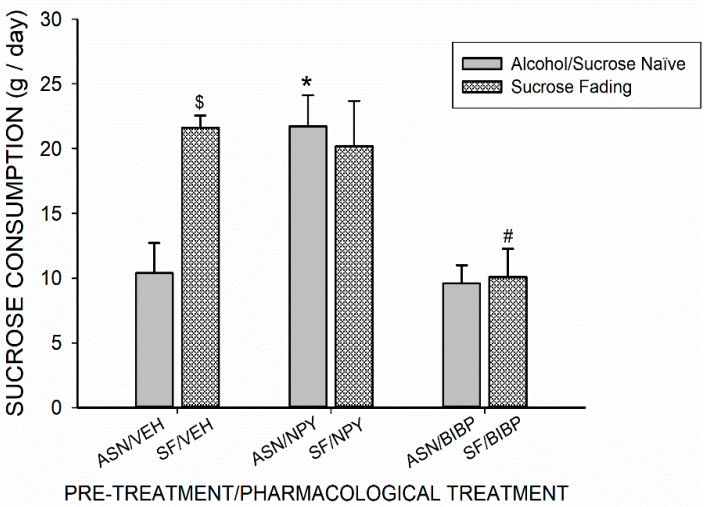

Figure 5. Effects of pre-treatment and pharmacological treatment on the sucrose intake after the microinjection of respective pharmacological treatment. Comparisons were analyzed by a Student-Newman-Keuls post-hoc test: $ $p < 0.05$ Sucrose Fading vs. Alcohol/Sucrose Naïve, * $p < 0.05$ NPY vs. VEH and BIBP-3226, # $p < 0.05$ BIBP-3226 vs. VEH and NPY. Data are expressed as the mean ± SEM (n = 9 per group).

This finding is in agreement with those by Sandbak and Murison (1996), Sandbak et al. (1998), and our previous study (Vázquez-León et al., 2020). Moreover, Koros and coworkers (1998) found that saccharine drinking, rather than the open field test parameters, may predict subsequent alcohol intake during the initial period of exposure to low ethanol concentrations.

NPY-Y_1 receptors in D-PAG participate in the anxiety-like behavior. The three parameters evaluated with the DBB clearly show an anxiolytic effect of the NPY-Y_1 receptors activation with the microinjection of NPY (2.34 nmol/0.5 µL). Such significantly increased latency and decreased both duration of burying and immobility. The opposite effect was

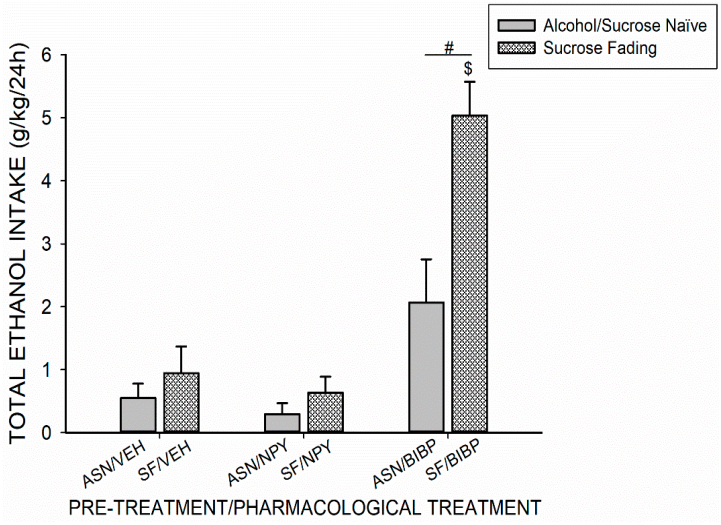

Figure 6. Effects of pre-treatment and pharmacological treatment on the total alcohol intake after the microinjection of pharmacological treatment. Comparisons were analyzed by a Student-Newman-Keuls post-hoc test: $ $p < 0.05$ sucrose fading vs. alcohol/sucrose naïve, # $p < 0.05$ BIBP-3226 vs. VEH and NPY. Data are expressed as the mean ± SEM (n = 9 per group).

observed with the NPY-Y_1 receptor antagonist BIBP-3226 (0.5 nmol/0.5 µL), i.e. significantly decreased latency and increased both burying and immobility times. Such a finding is according to the previous report from our laboratory (Vázquez-León et al., 2017) with the microinjection of [$Leu^{31}Pro^{34}$]-NPY into D-PAG through both EPM and DBB tests. Furthermore, the potent anxiolytic effect of the NPY-Y_1 activity is shown with a variety of tests and most of the brain nuclei studied mainly in rats (Kask et al., 1998a, Kask et al., 1998b, Kask et al., 2002; Heilig et al., 1993; Primaux et al., 2005; Reichmann & Holzer, 2016; Sorensen et al., 2004; Trent & Menard, 2011; Vázquez-León et al., 2020), and in mice (Deo et al., 2010).

PAG activation promotes food intake and reward processing (Luthï and Lüscher, 2014; Vázquez-León et al., 2021a). Moreover, the PAG has been reported to mediate reward information that promotes food intake, whereas PAG inhibition has anorexic effects in hungry rats (Tyron & Mizumori, 2018). NPY-Y_1 receptors stimulation in D-PAG produced an orexigenic effect, due to NPY significantly increasing the standard food rodent chow consumption, while BIBP-3226 elicited an anorexigenic effect. This observation is independent of the pre-treatment of non-deprivation or free access to food and water, which we named alcohol/sucrose naïve, and in sucrose-fading groups. Interestingly, the alcohol initiation procedure that fades sucrose from juvenile age in two periods, with withdrawal imposed between these, and at the end, elicited a subsequent increase in sucrose consumption in Wistar rats. The present result is according to that reported by Avena and coworkers (2004) in which rats with intermittent access to sugar and chow consumed the most 9% ethanol, supporting the suggestion that sugar dependence alters a rat's tendency to drink alcohol. They conclude that bingeing on either ethanol or sugar fosters intake of the other (Avena et al., 2004).

NPY-Y_1 receptors seem to have a role in such sucrose intake; due to NPY microinjected into D-PAG significantly increased its consumption, whereas the selective Y_1 receptor antagonist BIBP-3226 decreased the sucrose intake. One possible explanation is that NPY-Y_1 receptors in D-PAG elicit the appetitive behavior for food, but especially for a palatable and sweet reinforce substance such as sucrose, likely due to the critical and reciprocal connections between PAG and key brain nuclei of the reward circuit (Hasue & Shammah-Lagnado, 2002; Kelley et al., 2005; Tyron & Mizumori, 2018). To our knowledge, this is the first report that shows the participation of NPY-Y_1 receptors in the D-PAG on sucrose consumption.

Interestingly, NPY-Y_1 receptors in D-PAG prominently modulate the alcohol intake in Wistar rats, mainly in those with SF alcohol initiation procedures since juvenile age. The reached total alcohol amount intake was a mean of 5 g/kg/day in the SF/BIBP group, a quantity intake akin to that of the genetically P or HAD rat lines (Bell et al., 2006). This finding confirms that NPY-Y_1 receptors activity in D-PAG is critical in the modulation of alcohol intake, independently of the previous alcohol exposition. However, the enhanced effect was found in animals with repeated cycles of alcohol consumption and withdrawal (Vázquez-León et al., 2020). In the present study, we used the SF model in two stages, starting at the juvenile age. Likely, independently of the alcohol initiation procedure, animals administered NPY drink less ethanol than the controls, an opposite effect in the animals administered NPY-Y_1 receptors antagonist. Moreover, augmented sensitivity to the anti-alcohol dipsogenic effect of NPY is appreciated in rats with chronic ethanol exposure, imposed abstinence, and relapse (Gilpin et al., 2003). It has been argued that the inhibitory effects of administered NPY on the ethanol intake in P and HAD rats might be due to the anxiolytic actions of the peptide (Badia-Elder et al., 2001; Gilpin et al., 2003, 2004). Rats administered NPY may drink low alcohol amounts because mainly the anxiolytic actions of NPY substitute for those proper of alcohol (Gilpin et al., 2003). Conversely, NPY-Y_1 receptors antagonism in the D-PAG produced to drink high alcohol amounts, at least in part, due to its anxiogenic effects; such is confirmed with the DBB test. Furthermore, Badia-Elder et al. (2001, 2003) found that the central administration of NPY decreased ethanol consumption in rats selectively bred for high alcohol

preference and showed that equal NPY treatment also increased sucrose solution intake and reduced anxiety-like behavior in the EPM test.

Due to the complex function of the PAG in all its sub-regions, and its broad connections with key brain nuclei involved in anxiety, defensive response, and appetitive and consummatory behavior, future studies with NPY as an important pharmacological tool are needed.

Conclusions

NPY-Y_1 receptors activity in D-PAG significantly modulates the anxiety-like behavior evaluated through the DBB, either in non-deprived food and fluid or in repeated sucrose-fading and withdrawal cycles from juvenile age. Additionally, NPY-Y_1 stimulation in D-PAG with NPY produced an orexigenic effect for food and sucrose and an anti-dipsogenic effect for alcohol. The selective NPY-Y_1 receptor antagonist BIBP-3226 microinjected into D-PAG, significantly increased anxiety-like behavior, as well as produced an anorexigenic for food and sucrose, and a dipsogenic for alcohol effects in Wistar rats.

Conflict of interest statement

None.

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