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EVALUATION OF D4 DOPAMINE RECEPTOR BLOCKADE IN THE NUCLEUS ACCUMBENS ON PALATABLE FOOD MOTIVATION

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— Abstract—

At the central level, regulation of information processing of food's motivational and rewarding properties depends principally on brain structures such as the Nucleus Accumbens Shell (NAcS), where dopaminergic transmission plays a preponderant role in activating dopamine receptors related to reward. Anatomical and neurochemical studies indicate that D4 dopamine receptors (D4R) are presynaptically expressed and regulate the release of glutamate to GABAergic neurons in the NAcS. The purpose of this study was to evaluate the effects of the pharmacological blockade of D4R in the NAcS on the regulation of motivation for palatable food (MPF) in male Sprague Dawley rats using a progressive ratio (PR) schedule of reinforcement. According to our results, blockade of the D4R with the antagonist, L-745,870 (1 µg), in the NAcS decreases the number of lever presses, the number of obtained reinforcers and the breakpoints. Accordingly, the blockade of D4R in the NAcS decreases the MPF, consequently it could be a pharmacological target in the treatment of feeding pathology by decreasing the reinforcing properties of palatable food.

Keywords

Nucleus Accumbens Shell; Dopamine D4 receptor; L-745,870; Motivation; palatable food.

Palatable food consumption is associated with the increase of dopamine release in the nucleus accumbens (NA) (Hajnal *et al.*, 2004), a region of the brain that is part of the reward circuitry and the main site of action of some drugs of abuse such as cocaine, methamphetamine and alcohol (Kelley *et al.*, 2005). Dopamine can bind to five receptor subtypes (D1, D2, D3, D4, or D5), and the signaling pathways activated by these receptors require either Gs (D1 and D5) or Gi (D2, D3, D4) proteins (Missale *et al.*, 1998). Particularly the D4 receptor subtype (D4R) has been linked to alterations in the processing of reinforcing stimuli (Ducci & Goldman, 2012), reason why this receptor is of interest in the present study.

The mRNA that encodes for D4R has been located in several brain regions such as the frontal cortex, the amygdala and the hippocampus (among other areas), nuclei that send glutamatergic projections to the NA. According to experimental evidence, in NA D4R is present in axons and presynaptic terminals that do not express tyrosine hydroxylase, suggesting that its main function is the modulation of the release of non-catecholaminergic neurotransmitters (Svingos *et al.*, 2000).

In studies of radioactively tagged neurotransmitters release in rat brain explants, it was found that activation of D4R in NA decreases the release of glutamate but not GABA or dopamine (González *et al.*, 2012). Accordingly, there is evidence that activation of D4R produces inhibition of glutamate release in other areas of the brain such as the paraventricular nucleus of the hypothalamus (Tejas-Juárez *et al.*, 2014). Also, Bonaventura *et al.* (2017) found that D4R plays a key role in modulating corticostriatal glutamatergic neurotransmission. Additionally, it has been reported that D4R knockout mice with have a higher striatal glutamate basal release than wildtype mice (Thomas *et al.*, 2009), consequently under physiological conditions, the blockade of presynaptic D4R in glutamatergic terminals could explain the increased release not only of glutamate to GABAergic neurons, but also of GABA in the areas of the brain to which the glutamatergic cells project, including the shell region of the NA shell (NAcs). The increased extracellular concentration of GABA in the NAcs induces intense short latency hyperphagia (Reynolds & Berridge, 2002). Taken these findings together, the involvement of D4R appears to be critical for the regulation of activity in the reward circuit and, consequently, in this study we hypothesize that D4R-mediated dopamine transmission is a key element in increasing palatable food consumption by enhancing its reinforcing properties. Thus, the objective of this study is to evaluate the effects of pharmacological blockade of D4R present in NAcs on motivation for palatable food (MPF, breakpoints) in rats, using an operant protocol (progressive ratio schedule of reinforcement).

METHOD

Experimental animals

Ten male Sprague Dawley rats (weighting 80-120 grams at the start of the experiment) were used. Once they arrived at the laboratory, they had a week of habituation to the 12x12 hour light/dark cycle (the lights were turned off at 9:00 am). The rats had *ad-libitum* access to standard food and water (Formulab-Diet 5008®). Animals were provided by the FES-Iztacala Vivarium. The procedures used in this study were in accordance with the Technical Specifications for the Production, Care and Use of Laboratory Animals established in the Mexican Official Standard NOM-062-ZOO-1999.

Drugs

In this study we used the selective antagonist of the D4R, L-745870 (3-(4-[chlorophenyl]piperazin-1-yl)-methyl-1H-pyrrolo[2,3-B]pyridinetri-hydrochloride, Sigma-Aldrich Química, S. de R.L. de C.V., Mexico), which has a ki=0.43 nM for D4R (Patel *et al.*, 1997). A stock solution of L-745,870 dissolved in dimethyl sulfoxide (DMSO) was prepared and then aliquots were taken and diluted with 0.9% saline to obtain a final concentration of 1 µg, the injection volume was 0.5 µL infused at a rate of 0.1 µL/min, this solution was prepared just before administration.

Surgery

The rats were anaesthetized with a mixture of ketamine and xylazine (112.5 and 22.5 mg/kg body weight, respectively) and then placed in the stereotactic apparatus for implantation of a 1.5 cm guide cannula in the overlying area of the NACS. The stereotaxic coordinates, relative to bregma, were +1.5 mm anteroposterior, 0.6 mm mediolateral and 6.0 mm dorsoventral (Paxinos, Watson, Pennisi, & Topple, 1985). The guide cannula was fixed to the skull with a stainless-steel screw and acrylic cement. The animals were treated with enrofloxacin (25 mg/kg at the end of surgery and 48h after) to prevent infections and had 7 days of recovery.

Intracanular injections

The microinjections were applied with a high precision Hamilton syringe adapted to a 1.53 cm micro-injector, which remained inserted in the guide cannula for an additional minute to ensure the correct diffusion of the drug.

In all cases, the microinjections were applied 10 minutes before starting the progressive ratio program.

All rats received two intracanular injections (L-745,870 or Vehicle). The drug dose used (1 µg) was based on previous studies by our working group, where a significant effect on food intake was observed (Tejas-Juárez *et al.*, 2014).

Assessment of motivation for palatable food (MPF)

At the beginning of the dark phase (09:00 hours), food was removed from the home-cages to facilitate the emission of the operating response (lever) during training sessions. Ninety minutes later, the rats were introduced into the operant conditioning boxes (Med Associates Inc., St. Albans, VT., USA), which were equipped with general lighting (back panel) and discriminating stimuli (front panel, top of the levers), two retractable levers and in the middle of these, a 45 mg pellet dispenser. The boxes (dimensions 30 x 23 x 20 cm, lateral and top sides in transparent Plexiglas) were located in a sound attenuating cubicle with a fan to supply fresh air and white noise.

Events and contingencies inside the operating conditioner box were monitored and controlled through a Smart Control Panel SG-716B interface (Med Associates Inc., St. Albans, VT., USA) connected to a PC with Med-PC software (Med Associates Inc., St. Albans, VT., USA) and custom programs built in MedState Notation language

Training. Initially, animals were exposed to a fixed ratio schedule of reinforcement 1 (FR1) in daily sessions of 30 minutes. One press on the lever resulted in the delivery of a 45 mg pellet as a reinforcer (45 mg chocolate-flavored sucrose pellets, Bio Serv, Frenchtown, NJ, USA). The criterion for switching from FR1 to FR5 was that a stable lever response rate was achieved (variation of no more than 20% on average over 3 consecutive days, each rat was its own control). In the FR5 schedule of reinforcement, pressing the lever 5 times resulted in the delivery of 1 reinforcer, this phase of the training lasted 3 consecutive days. Subsequently, the microinjection cannula was implanted in the NAcS and after the recovery period, they were again exposed to the FR5 schedule of reinforcement. The duration of the training sessions (FR1 and FR5) was 30 minutes. When rats achieved stability of the lever press response in the FR5 program (no more than 20% average variation for 3 consecutive days, each animal was its own control), the vehicle or L-745,870 microinjections were applied and they were exposed to a progressive ratio (PR) schedule of reinforcement 10 minutes after injection to evaluate the MPF.

Experimental session. In the PR schedule of reinforcement, the response requirement to obtain a reinforcer was increased according to the following set of values: 1, 2, 4, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268 and 328. The above values were obtained by the equation: Ratio= [5e (0.2 x test number)] - 5 (Richardson & Roberts, 1996). The PR program session ended when the rat failed to obtain at least one reinforcer within 30 minutes or in absence of responses in a 4-minute period. The breakpoint was defined as the ratio completed (last reinforcer obtained) before the end of the session. From the operating procedures, the following values were obtained: 1) number of lever pressings, 2) reinforcers obtained, and 3) breakpoints.

Histology

At the end of the experimental sessions, the rats were euthanized with a lethal dose of intraperitoneal sodium pentobarbital, the brain was removed and fixed in a 10% formaldehyde solution for 24 hours. Coronal sections of 300 µm thick were cut with a vibratome (Campden) and, based on the atlas by Paxinos and Watson (1986), the place of the drug injection was located. Only data from rats that were correctly injected into the NAcS were included in the present report.

Statistical Analysis

All values of the number of lever pressings, reinforcers obtained, and breakpoints, were expressed as the mean of the observations ± the standard error of mean (SEM) and analyzed with a repeated measures ANOVA (data obtained during training) followed by Tukey's post hoc test or a student t test when appropriate (for comparisons between 2 conditions). GraphPad Prism5® was used to calculate the significance of the differences with α of 5%.

RESULTS

In the present investigation, the effects of pharmacological blockade of D4R in the NAcS on MPF in rats were evaluated using a behavioral protocol. To achieve this objective, an operating paradigm for the MPF evaluation was established, initially by training the animals to FR1 and FR5 schedules of reinforcement, and then administering the drug directly to the NAcS and evaluating the breakpoints in a PR schedule of reinforcement. Fig. 1 shows the number of lever presses (A) and the number of reinforcers obtained (B) in the FR1 and FR5 (training) programs. In FR1 4 sessions were required to achieve 80% stability in its response rate without reaching statistically significant differences. Subsequently, they were exposed to 3 sessions in FR5

(5 responses, one reinforcer) and the following day they had the stereotactic surgery. After the rats recovered from surgery, 4 sessions of FR5 were required to achieve 80% of stability in the operant behavior. Statistically significant differences were obtained in sessions 7, 9, 10 and 11 compared to the sessions in FR1 [$F_{(7,4)}$= 8.502; p<0.001]. There were no statistically significant differences between FR1 and FR5 sessions in the reinforcers obtained [$F_{(7,4)}$= 10.83; p>0.05]. Thus, it can be established that rats adapted appropriately to the established operating paradigm. Once FR5 stability was achieved, the drug was intracanularly administered in the NAcs.

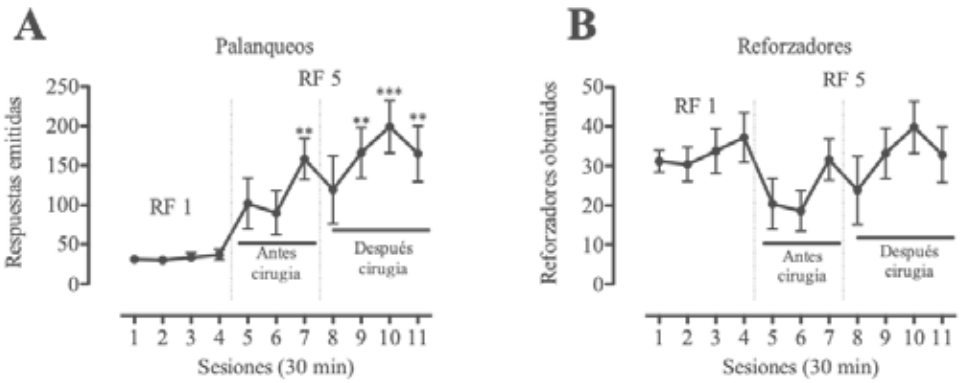

Figure 1. Responses emitted by rats exposed to the FR1 and FR5 programs before and after surgery (training). Lever presses emitted (A) and reinforcers obtained (B) in each 30-minute session in both FR1 and FR5, before and after stereotactic surgery. Data expressed in terms of means ± the EEM. There were no statistically significant differences among sessions 8, 9, 10 and 11 of the lever presses and reinforcers obtained

Figure 2 shows the effects of intracanular administration of the D4R selective antagonist, L-745,870 (1 µg, intra-NAcs) on the performance of animals in the PR program. According to our results, the local administration of L-745,870 significantly decreased the number of lever presses (t= 3.450; p<0.05) and the number of reinforcers obtained (t= 4.700; p<0.05), in the behavioral task characterized by the progressive increase of the operating demand.

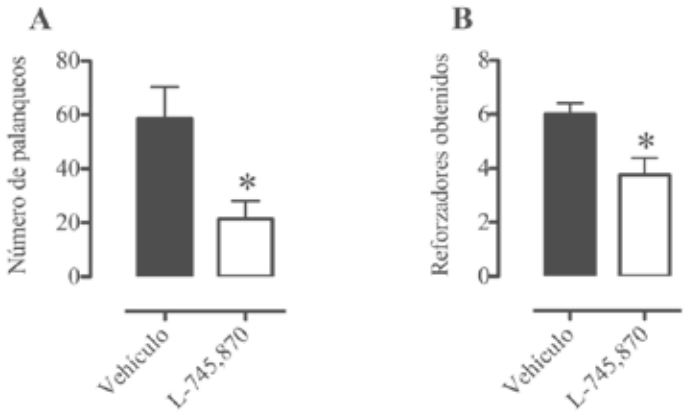

Figure 2. Number of lever presses (A) and reinforcers obtained (B) by rats exposed to the PR program that received the injection of the D4R antagonist (L-745,870, 1 µg) or vehicle in the NAcS. Data expressed in terms of means ± the MES (n= 4 per group)

To determine the effects of D4R blockade in the NAcS with L-745.870 (1 µg) on MPF, we evaluated the breakpoints in different pharmacological conditions. Breakpoints are valid values that reflect the reinforcer strength and the motivational state of the animal (Zhang *et al*., 2003). In the present study we found that the administration of L-745,870 significantly decreased the breakpoints ($t= 4.071$; $p<0.05$; $n=4$). This result indicates that by preventing D4R-mediated dopaminergic transmission, MPF decreases. Figure 3B shows the histology of correctly administered injections into the NAcS (representative photomicrograph).

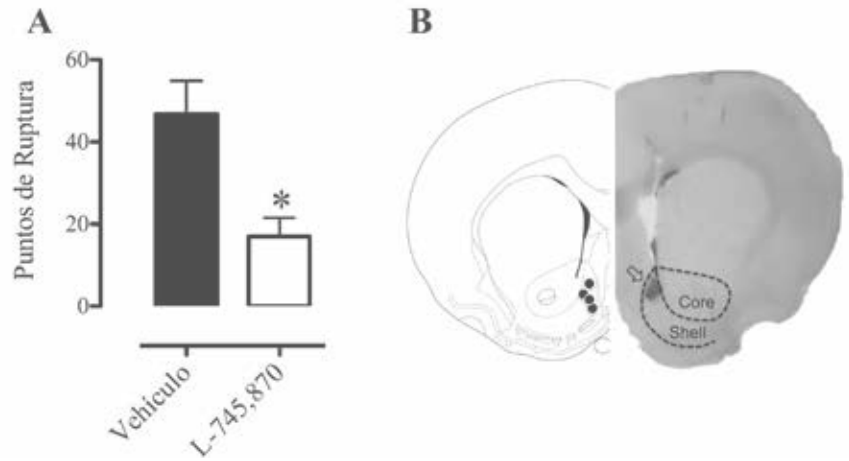

Figure 3. A) Breakpoints (last value of lever presses with reinforcer obtained in the PR program) of the rats injected with the D4R antagonist (L-745,870, 1 µg) or vehicle in the NAcS (data expressed as means ± the EEM; n= 4 per group; * p< 0.05). B) Representative photograph of a coronal section showing the injection site (right) of the drug in the NAcS (marked with an arrow) and schematic representation (left) of the injection places (black circles) in an image from the atlas by Paxinos and Watson (1998)

DISCUSSION AND CONCLUSION

The present study was aimed to obtain evidence that D4R-mediated dopaminergic transmission in the reward circuit, particularly in the nucleus accumbens shell, is an important part for the information processing of palatable food rewarding properties. Accordingly, we found that local and specific D4R blockade in NAcS with the antagonist L-745870 significantly decreased palatable food motivation.

Our results are compatible with the hypothesis that the central administration of the selective D4R antagonist in NAcS blocks the presynaptic D4 receptors located at the glutamatergic terminals (Svingos *et al.*, 2000), disinhibiting this stimulatory pathway and consequently increasing the activity of GABAergic neurons in NAcS associated with increased palatable food motivation. Accordingly, activation of presynaptic D4R has been associated with inhibition of glutamate release in other regions of the brain (due to its attachment to Gi proteins), in which the D4R blocking may increase glutamate release. In this regard, it has been shown that blocking D4R with the selective D4R antagonist (A-381393) produces increased expression of cFos immune-reactivity in the paraventricular nucleus of the hypothalamus (Bitner *et al*, 2006). Thus, increased activity of NAcS GABAergic neurons mediated by disinhibition of glutamate release via D4R blockade would reach several brain areas, including those related to food intake such as the lateral hypothalamus (LH), which expresses different orexigenic neuropeptides such as melanin concentrator hormone (MCH) and orexin (Ox) (Suyama & Yada, 2018; Stuber & Wise, 2015). The increase of the GABAergic tone in LH would have an inhibitory effect on the neurons that express MCH and Ox, which might explain the decrease in palatable food intake, since these peptides have a hypophagic effect.

On the other hand, the selective D4R antagonist (L-745,870) has been shown to produce a decrease in the severity of dyskinesias in induced Parkinson's model rats, when co-administered with L-DOPA, the drug of choice in Parkinson's disease (Huot *et al*., 2015). Additionally, other selective D4R antagonists have been studied in cancer and addictions (Lindsley & Hopkins, 2017). Currently, no information has been published regarding the utility of this drug in the regulation of food intake, so this study provides information that supports the potential use of this compound as an adjuvant in the treatment of food pathology characterized by excessive consumption of palatable food.

Although the results of the present study have implications for the understanding of the relationship between dopaminergic transmission and the regulation of palatable food motivation processes, their limitations should be considered. The main one is that it showed the effect of a single dose

of the D4R antagonist which, although it had a clear and statistically significant effect, it will be necessary to validate that such changes in behavior follow a concentration-dependent pattern, as pharmacological evidence of the specificity of the compound's action on the receptor. Additionally, it would be necessary to demonstrate that the reported effect depends on the increase of GABA concentration in the NAcS, so it is suggested that in future experiments not only GABA levels in the NAcS be measured, but also that the blocking of GABA receptors be shown to prevent the effect of L-745,870.

Finally, according to the results obtained in the present study, it is concluded that pharmacological blockade of D4R in the nucleus accumbens shell with the specific antagonist (L-745,870) decreases palatable food intake by decreasing food motivation. Future experiments should be conducted to confirm that the neurochemical mechanism by which D4R produces the above-mentioned effect via the regulation of GABA concentrations in the NAcS.

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