Neuroscience Letters 564 (2014) 94–98

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Evaluation of saccharin intake and expression of fructose-conditioned flavor preferences following opioid receptor antagonism in the medial prefrontal cortex, amygdala or lateral hypothalamus in rats

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HIGHLIGHTS

- Amygdala naltrexone reduced fructose-conditioned flavor preferences (CFP) expression.
- Lateral hypothalamic naltrexone failed to affect fructose-CFP expression.
- Medial prefrontal cortex naltrexone failed to affect fructose-CFP expression.
- Saccharin intake was reduced by lateral hypothalamic and amygdala naltrexone.
- Saccharin intake was unaffected by medial prefrontal cortex naltrexone.

ARTICLE INFO

Article history:
Received 18 December 2013
Received in revised form 4 February 2014
Accepted 8 February 2014

Keywords:
Naltrexone
Sweet taste
Saccharin
Conditioning

ABSTRACT

In prior studies, systemic opioid receptor antagonism with naltrexone (NTX) failed to block flavor preference conditioning by the sweet taste or post-oral actions of sugar despite reducing overall flavored saccharin intake. Further, NTX microinjections into the nucleus accumbens (NAc) shell or core failed to alter the expression of preferences conditioned by the sweet taste or post-oral actions of sugars. In contrast, fructose-conditioned flavor preferences (CFP) were reduced or eliminated by systemic or intracerebral administration of dopamine (DA) D1 or D2 antagonists in the NAc, medial prefrontal cortex (mPFC), amygdala (AMY) or lateral hypothalamus (LH). The present study examined whether NTX microinjections into the mPFC, AMY or LH would alter expression of fructose-CFP and total flavored saccharin intake. Food-restricted rats with bilateral cannulae aimed at the mPFC, AMY or LH were trained to drink a fructose (8%) + saccharin (0.2%) solution mixed with one flavor (CS+, e.g., cherry) and a 0.2% saccharin solution mixed with another flavor (CS−, e.g., grape) during 10 one-bottle sessions. Two-bottle tests with the cherry and grape flavors in 0.2% saccharin solutions occurred 10 min following total bilateral NTX doses of 0, 1, 25 and 50 μg administered into the mPFC, AMY or LH. Rats preferred the CS+ over CS− flavor following vehicle and all NTX doses administered into either the mPFC or LH. CS+ intake was significantly greater than CS− intake following vehicle and the low NTX dose in the AMY; however, at the 25 and 50 μg AMY NTX doses, CS+ intakes did not significantly exceed CS− intakes. Total flavored saccharin intake was significantly reduced by all three LH NTX doses (20–35%), by the 25 (14%) and 50 (22%) μg AMY NTX doses, but not by mPFC NTX. Thus, opioid antagonism in the AMY, but not the mPFC or LH attenuated, but did not block the expression of fructose-CFP, and LH and AMY, but not mPFC, NTX significantly reduced total saccharin intake. Therefore, whereas opioid antagonism in the LH and AMY reduces sweet intake, they appear less effective in altering fructose-CFP.

1. Introduction

Central opioid systems mediate palatable ingestive responses (see reviews: [1–3]). Opioid receptor antagonists suppress intake

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http://dx.doi.org/10.1016/j.neulet.2014.02.020
0304-3940/

of sweet solutions more than plain water (e.g., [4,5]), block that portion of feeding driven by sweet taste in food-restricted animals by reducing sucrose's positive hedonic taste reactivity responses (e.g., [6,7]), and reduce sucrose intake in sham-feeding tests (e.g., [8,9]). Although an early study implicated the opioid system in flavor preference conditioning by sugar [10], our laboratory did not support this view. Systemic NTX administration failed to alter either the acquisition (learning) or expression (maintenance) of flavor preferences conditioned by either the sweet taste (flavor-taste conditioning) of sucrose in sham-feeding rats or fructose in real-feeding rats (e.g., [11]) as well as failing to affect post-oral nutritive effects (flavor-nutrient conditioning) of sucrose [12–14]. Thus, these data appear to conflict with the concept that central opioid signaling modulates avidity for preferred foods and fluids (see review: [2]). However, systemic drug treatment may potentially obscure important contributions of specific central opioid-sensitive circuits on sugar-conditioned food preferences (CFP). In this regard, route of drug administration influenced NTX effects on sweet food-related responses [15]. Rats, given choice tests with chocolate-flavored and banana-flavored food pellets, displayed a mild preference for the chocolate flavor. Systemic NTX failed to alter the preference, but reduced intakes of both flavored foods. In contrast, NTX microinfusions into the nucleus accumbens (NAc) selectively reduced intake of the preferred chocolate-flavored food. However, NAc core or shell NTX microinfusions only minimally reduced expression of the flavor-taste fructose-CFP at the highest dose without affecting the expression of flavor-nutrient IG glucose-CFP.

In contrast to the opioid system, systemic administration of dopamine (DA) D1 and D2 receptor antagonists blocked the acquisition and expression of flavor-taste fructose-CFP, whereas DA D1 receptor antagonism selectively blocked the acquisition, but not expression of flavor-nutrient IG sucrose-CFP [17–20]. Moreover, DA D1 antagonists administered into the NAc, amygdala (AMY), medial prefrontal cortex (mPFC) or lateral hypothalamus (LH) blocked the acquisition, but not expression of flavor-nutrient IG glucose [21–24]. Further, DA D1 and D2 antagonists administered into the mPFC eliminated the acquisition, but not expression of flavor-taste fructose-CFP [25], and DA D1 and D2 antagonists administered into the Nac or AMY significantly reduced the expression of fructose-CFP [26,27]. In contrast, only DA D1 antagonists administered into the LH reduced the expression of fructose-CFP [28].

Thus, given the roles of the AMY, mPFC and LH in DA-related mediation of flavor-taste and flavor-nutrient CFP, these sites could be candidates to mediate opioid systems in controlling CFP. Indeed, opioid receptor agonists and antagonists respectively increased and decreased food intake following infusion into the AMY [29–32], LH [33,34] and mPFC [35,36]. Thus, the present study examined whether NTX administered into the AMY, mPFC or LH altered the expression of flavor-taste fructose-CFP, and also assessed changes in sweet intake induced by flavored saccharin.

2. Methods

Adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA, 260–300 g) were housed individually in wire mesh cages, maintained at 21 °C under a 12:12 h light:dark cycle and fed a Laboratory Rodent Diet 5001 (PMI Nutrition International, Brentwood, MO, USA) and tap water. The experimental protocols were approved by the Queens College Institutional Animal Care and Use Committee. Rats were pretreated with chlorpromazine (3 mg/kg, i.p.), anesthetized with Ketamine HCl (120 mg/kg, i.m.), and stainless steel guide cannulae (26-gauge, (i.d. 0.24, o.d. 0.46 mm) Plastics One, Inc., Roanoke, VA) were aimed stereotaxically (Kopf Instruments) at bilateral placements in the mPFC (incisor bar (0 mm), 3.0–3.2 mm anterior to the bregma suture, 1.5 mm lateral and angled 10° towards the midline of the sagittal suture, and 3.5 mm below the surface of the skull), AMY (incisor bar (−3.3 mm), 2.8 mm anterior to the bregma suture, 4.1–4.5 mm lateral to the sagittal suture, and 8.0–8.4 mm from the top of the skull), or LH (incisor bar (0 mm), 3.0–3.3 mm posterior to the bregma suture, 3.3 mm lateral to and angled 10° towards the sagittal suture, and 8.8 mm from the top of the skull). These coordinates were identical to those evaluating central DA D1 and D2 receptor antagonists in previous fructose-CFP studies [25,27,28]. Cannulae were secured to the skull by four anchor screws with dental acrylic, and animals were allowed two weeks to recover from surgery before behavioral testing began.

The training solutions consisted of an 8% fructose (Sigma Chemical Co., St. Louis, MO) and 0.2% sodium saccharin (Sigma) mixture or a 0.2% sodium saccharin solution; the solutions were flavored with 0.05% unsweetened grape or cherry Kool-Aid (Kraft Foods, White Plains, NY). Half of the rats in each group had the cherry flavor added to the fructose + saccharin solution, and the grape flavor added to the saccharin only solution with flavors reversed for the remaining rats. In two-bottle preference tests, cherry and grape flavors were each presented in a 0.2% saccharin solution. The fructose + saccharin-paired flavor is referred to as the CS+, and the saccharin-paired flavor as the CS− because 8% fructose is preferred to 0.2% saccharin [37]. CS+/F refers to the fructose-containing solution used in training, and CS+ and CS− refer to the flavored solutions used during two-bottle testing. Intakes were measured to the nearest 0.1 g.

All testing took place in the rats' home cage during the mid-light phase of the light:dark cycle. Two weeks before testing began, rats were food-restricted to maintain body weights at 85–90% of their ad libitum level. Rats were initially trained with an unflavored 0.2% saccharin solution during daily 1 h sessions to produce approach to the sipper tubes with short (<1 min) latency. The limited food rations were provided 1 h after each training session.

All rats were given ten one-bottle training sessions (16 ml, 30 min/day) with the CS+/F solution presented on odd-numbered days, and the CS− solution presented on even-numbered days. On days 9 and 10, rats had access to a second sipper tube containing water to familiarize them with two sipper tubes used during subsequent choice tests; water intake was negligible. The position of the CS and water sipper tubes was reversed across days. Following training, the rats were given eight two-bottle choice test sessions (30 min/day) with unlimited (50 ml) access to the CS+ and CS- saccharin solutions with injections occurring 10 min prior to the two-bottle tests. For the first two test sessions, the rats received a vehicle (0.9% saline) microinfusion. Over the next six sessions, the rats were given NTX microinfusions at total doses of 1, 25 and 50 μg (0.5, 12.5, 25 μg/side) into the mPFC, AMY or LH. NTX (Sigma–Aldrich, St Louis, MO, USA) was dissolved in sterile isotonic saline (vehicle) and administered bilaterally at a volume of 0.5 μl/side using a 33 g stainless steel internal cannula (Plastics One) connected to a 2 μl microsyringe (Hamilton Company, Reno, NV, USA) by polyethylene tubing. During intracerebral injections, rats were held gently, the stylus was removed, and the cannula was inserted. The tip of the injection cannula protruded 1 mm beyond that of the guide. The injections were made at a rate of 0.5 μl/min, and the cannulae were left in place for an additional minute before their removal. Half of the rats in each site were tested with an ascending NTX dose order, and the remaining rats were tested in a descending NTX dose order. The rats were tested twice at each drug dose with the left-right position of the CS+ and CS− solutions counterbalanced across sessions. A one-day rest period separated each pair of drug doses for both groups in which all animals received *ad libitum* access to water and their food ration.