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Roles of dopamine D1 and D2 receptors in the acquisition and expression of fat-conditioned flavor preferences in rats

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ABSTRACT

Sugars and fats elicit innate and learned flavor preferences with the latter mediated by flavor–flavor (orosensory) and flavor–nutrient (post-ingestive) processes. Systemic dopamine (DA) D1 (SCH23390: SCH) and D2 (raclopride: RAC), but not opioid antagonists blocked the acquisition and expression of flavor–flavor preferences conditioned by sugars. In addition, systemic D1, but not D2 or opioid antagonists blocked the acquisition of flavor–nutrient preferences conditioned by intragastric (IG) sugar infusions. Given that DA antagonists reduce fat intake, the present study examined whether systemic D1 or D2 antagonists altered the acquisition and/or expression of conditioned flavor preferences (CFP) produced by pairing one novel flavor (CS+, e.g., cherry) with a 3.5% corn oil (CO: fat) solution relative to another flavor (CS−, e.g., grape) paired with a 0.9% CO solution. In an expression study, food-restricted rats were trained to drink either flavored 3.5% or 0.9% CO solutions on alternate days. Subsequent two-bottle tests with the CS+ and CS− flavors mixed in 0.9% CO solutions occurred 0.5 h after systemic administration of vehicle (VEH), SCH (50–800 nmol/kg) or RAC (50–800 nmol/kg). The rats displayed a robust CS+ preference following VEH treatment (87–88%) the expression of which was attenuated by treatment with moderate doses of RAC, and to a lesser degree, SCH. In an acquisition study, six groups of rats received VEH, SCH (25, 50, 200 nmol/kg) or RAC (50, 200 nmol/kg) 0.5 h prior to 1-bottle training trials with CS+ flavored 3.5% and CS− flavored 0.9% (CS−) CO solutions. A seventh Limited VEH group was trained with its training intakes limited to that of the SCH and RAC groups. Subsequent two-bottle tests were conducted with the CS+ and CS− flavors presented in 0.9% CO without injections. Significant and persistent CS+ preferences were observed in VEH (75–82%), Limited VEH (70–88%), SCH25 (75–84%), SCH50 (64–87%), SCH200 (78–91%) and RAC200 (74–91%) groups. In contrast, the group trained with RAC50 displayed a significant initial CS+ preference (76%) which declined over testing to 61%. These data indicate limited DA D1 and D2 receptor signaling involvement in the expression and acquisition of a fat-CFP relative to previous robust effects for sugar-CFP.

1. Introduction

Fats and sugars are both recognized as contributing to the palatability of foods, overeating, and diet-induced obesity. In addition to the inherent hedonic properties associated with these nutrients, learning plays an important role in the preferences for fat- and sugar-rich foods (Sclafani, 1999). Preferences are based in part on learned associations between the various flavor elements in foods, flavor–flavor conditioning, and between flavor cues and postingestive consequences, flavor–nutrient conditioning. Animal research on conditioned flavor preferences (CFP) has focused almost exclusively on sugar and sweet taste, and the putative underlying roles of dopamine (DA) and opioid systems mediating its effects (Azzara, Bodnar, Delamater, & Sclafani, 2000, 2001; Baker, Li, Lee, Sclafani, & Bodnar, 2004; Baker, Shah, Sclafani, & Bodnar, 2003; Holman, 1975; Hsiao & Smith, 1995; Yu, Sclafani, Delamater, & Bodnar, 1999; Yu, Silva, Sclafani, Delamater, & Bodnar, 2000a, 2000b). In this regard, flavor–flavor conditioning studies using either sucrose in sham-feeding rats (Yu et al., 1999; Yu et al., 2000a, 2000b) or fructose in real-feeding rats (Baker et al., 2003, 2004), identified a critical role for DA D1 and D2, but not opioid, receptor signaling in the acquisition and expression of these preferences. In contrast, flavor–nutrient conditioning studies using intragastric (IG) sucrose

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infusions (Azzara et al., 2000, 2001), identified a critical role for DA D1, but not D2 or opioid receptor signaling in the acquisition, and to a lesser degree, the expression of this preference. In addition, the acquisition of IG glucose flavor–nutrient preferences was blocked by DA D1 antagonism into the nucleus accumbens (NAc: Touzani, Bodnar, & Sclafani, 2008), amygdala (AMY: Touzani, Bodnar, & Sclafani, 2009a, 2009b) and medial prefrontal cortex (mPFC: Touzani, Bodnar, & Sclafani, 2010a), indicating the existence of a distributed brain network mediating this response (see review: Touzani, Bodnar, & Sclafani, 2010b). DA D1 and D2 antagonism in the NAc and AMY reduced, but did not eliminate the expression of fructose-conditioned flavor–flavor CFP. The acquisition of a fructose-CFP was completely blocked by DA D1 and D2 antagonism into the mPFC (Malkusz et al., 2010), but DA D1 and D2 antagonism in the NAc or AMY only hastened the extinction of these preferences (Bernal et al., 2008, 2009). In contrast, naltrexone administered into either the shell or core regions of the NAc failed to alter the expression of either fructose-conditioned flavor–flavor or IG glucose-conditioned flavor–nutrient CFP (Bernal et al., 2010).

Rodents are attracted to the flavor of fat (e.g., corn oil) and non-nutritive fat substitutes (e.g., mineral oil, sefose) from an early age which may be mediated in part by taste receptors for fatty acids (Ackroff & Sclafani, 2009; Passilly-Degrace, Gaillard, & Besnard, 2009). In addition, both the postingestive actions and orosensory properties of fat are rewarding and condition a CS+ flavor preference (Ackroff & Sclafani, 2009; Sclafani, 1999). DA mediation of the rewarding effect of fat flavor is suggested by the findings that corn oil sham-feeding promotes NAc DA release (Liang, Hajnal, & Norgren, 2006), and DA D1 and D2 antagonists suppress the sham-feeding response to corn oil and real-feeding of fats in rats (Baker, Osman, & Bodnar, 2001; Davis et al., 2006; Rao, Wojnicki, Coupland, Ghosh, & Corwin, 2008; Weatherford, Greenberg, Gibbs, & Smith, 1990; Weatherford, Smith, & Melville, 1988). In inbred mice, strain differences were observed in the ability of the D1 antagonist, SCH23390 to significantly reduce fat intake whereas the D2 antagonist, raclopride, had minimal effects on fat intake (Dym et al., 2010). Furthermore, DA D2, but not D1 receptor antagonism suppressed operant responding for corn oil in mice (Yoneda et al., 2007), whereas D1, but not D2 receptor antagonism attenuated place preference conditioning by corn oil intake (Imaizumi, Takeda, & Fushiki, 2000).

The present study examined whether systemic administration of DA D1 and D2 receptor antagonists (SCH23390 and raclopride, respectively) would attenuate flavor preference conditioning by corn oil ingestion. The first experiment revealed that a flavor (e.g., cherry) paired with a higher (3.5%) concentration of a corn oil (CO: fat) solution conditioned a flavor preference relative to a flavor (e.g., grape) paired with a lower (0.9%) concentration of a CO solution. Consequently we used this paradigm to investigate the effects of DA receptor antagonism on corn oil-CFP. To this end, SCH22390 or raclopride, at different doses, was injected systemically either prior to testing (expression) or training (acquisition) sessions. Based on the observed reductions in corn oil sham-feeding produced by both SCH23390 and raclopride (Weatherford et al., 1988, 1990), we expected that both DA D1 and D2 receptors antagonists would impair both the acquisition and expression of corn oil-CFP.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats (260–300 g, Charles River Laboratories, Wilmington, MA) were housed individually in wire mesh cages and maintained on a 12:12 h light/dark cycle with chow (5001, PMI Nutrition International, Brentwood, MO) and water available *ad libitum*, except as noted below. The experimental protocols were approved by the Queens College Institutional Animal Care and Use Committee (Protocol 69) certifying that all subjects and procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Test solutions

The training fluids consisted of 3.5% and 0.9% corn oil (CO: Sigma Chemical Co., St. Louis, MO) flavored with 0.05% unsweetened grape or cherry Kool-Aid (General Foods, White Plains, NY) and prepared as suspensions using 0.3% xanthan gum (Sigma). These concentrations are isocaloric to 8% and 2% sugar solutions used in another flavor conditioning study (Sclafani and Ackroff, unpublished). Half of the rats in each group had the cherry flavor added to the 3.5% CO and the grape flavor added to the 0.9% CO; the flavors were reversed for the remaining rats. In the two-bottle preference tests, the cherry and grape flavors were each presented in 0.9% CO. The CO + Kool-Aid + gum mixtures are hereafter referred to as solutions. The flavored training solutions are referred to as the CS+/3.5% CO and CS−/0.9% CO. The flavored 0.9% solutions used in the two bottle tests are referred to as CS+ and CS−. All testing took place in the rat's home cage during the mid-light phase of the light:dark cycle. Two weeks before testing began, the rats were placed on a food restriction schedule that maintained their body weights at 85–90% of their *ad libitum* level. The rats were initially trained to drink an unflavored 0.2% saccharin solution from sipper tubes during daily 2-h sessions. The sipper tube was mounted on the front of the cage held by a taut steel spring, and was positioned 3–6 cm above the cage floor. This training procedure was repeated daily until all rats approached the sipper tubes with short (<1 min) latency, typically within 3 days. The limited food rations were given 30 min after each training session.

2.3. Experiment 1

Eight naïve male rats were given ten 1-bottle training sessions (2 h/day) with 24 ml of the CS+/3.5% CO solution presented on odd-numbered days, and 24 ml of the CS−/0.9% CO solution presented on even-numbered days. On days 9 and 10, the rats had access to a second sipper tube containing water. This familiarized the rats to the presence of two sipper tubes used during the choice tests; water intake was negligible in these training trials. Training intakes were limited to 24 ml/session to minimize the difference between CS+/3.5% CO and CS−/0.9% CO intakes. The left–right position of the CS and water sipper tubes was counterbalanced over the 2 days. Following training, the rats were given eight two-bottle choice test sessions (2 h/day) with unlimited access to the CS+ vs. CS− solutions. Intakes during training and testing in this and all subsequent experiments were measured by weighing (0.1 g) the bottles before and after the 2 h sessions. Because some of the effects could be potentially small, care was also taken to minimize spillage. After initially weighing each bottle, it was gently shaken to insure appropriate flow of the viscous corn oil solutions. Any effluent from the bottle (~0.5–1.0 g) was collected and appropriate spillage adjustments were made to obtain an accurate pre-weight measurement. The sipper tube was occluded when the bottles were placed onto the cage and subsequently removed. The taut steel spring prevented movement of the bottles during the sessions. Visual inspection of the bottles during the study revealed minimal if any spillage because of the viscosity of the solutions. The session length of 2 h was identical to that previously used in assessing fructose-CFP (Baker et al., 2003, 2004).