Pharmacology, Biochemistry and Behavior 150–151 (2016) 14–21

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Muscarinic, nicotinic and GABAergic receptor signaling differentially mediate fat-conditioned flavor preferences in rats

Francis M. Rotella [a], Kerstin Olsson [b], Nancy Martinez [b], Alexandra Mordo [b], Ilanna Kohen [b], Alon Aminov [b], Jeremy Pagirsky [b], Alice Yu [b], Vishal Vig [b], Richard J. Bodnar [a,b,*]

[a] CUNY Neuroscience Collaborative, Graduate Center, City University of New York, New York, NY, USA
[b] Department of Psychology, Queens College, City University of New York, New York, NY, USA

ARTICLE INFO

Article history:
Received 19 May 2016
Received in revised form 9 August 2016
Accepted 2 September 2016
Available online 4 September 2016

Keywords:
Scopolamine
Mecamylamine
Baclofen
Learning

ABSTRACT

Rats display conditioned flavor preferences (CFP) for fats. Previous studies demonstrated that whereas expression of an already-acquired corn oil (CO)-CFP was mildly reduced by dopamine (DA) D1, DA D2, NMDA or opioid receptor antagonists, the acquisition or learning of CO-CFP was eliminated by NMDA antagonists, and significantly reduced by DA D1 and D2, but not opioid antagonists. Previous studies of fructose-CFP demonstrated that muscarinic (scopolamine) and nicotinic (mecamylamine) cholinergic receptor antagonists and $GABA_B$ (baclofen) receptor agonism reduced the expression of this acquired response, and that scopolamine, but not mecamylamine or baclofen eliminated the acquisition or learning of this response. The present study examined scopolamine, mecamylamine or baclofen effects upon expression or acquisition of CO-CFP. For expression, rats were trained over 10 sessions with CS+ (3.5% CO) and CS− (0.9% CO) flavored solutions without drugs. Two-bottle choice tests with CS+ and CS− flavors in 0.9% CO examined preferences following vehicle, scopolamine (1–10 mg/kg), mecamylamine (1–8 mg/kg) and baclofen (1.5–5 mg/kg). In acquisition, eight groups of rats received vehicle, scopolamine (1, 2.5 mg/kg), mecamylamine (4, 6 mg/kg), baclofen (3, 5 mg/kg) or a limited intake vehicle control 0.5 h prior to all 10 CS+ and CS− training sessions followed by six 2-bottle CS+ and CS− choice tests in 0.9% CO. CO-CFP expression (percent CS+ intake) was significantly but marginally reduced by scopolamine (70%), mecamylamine (85%) and baclofen (74%) relative to vehicle (98%). CO-CFP acquisition was eliminated (41%) by scopolamine relative to vehicle (88%) and limited control (98%) conditions. Neither mecamylamine nor baclofen altered CO-CFP acquisition. Thus, the muscarinic cholinergic receptor system is essential for acquisition (learning) of both fat-induced and sugar (fructose)-induced preferences. In contrast, muscarinic, nicotinic and $GABA_B$ receptors were minimally involved in the expression (maintenance) of fat- and fructose-CFP.

1. Introduction

Fats and sugars contribute to the palatability of foods through both their inherent hedonic properties and the learning of preferences (Sclafani, 1999). Conditioned flavor preference (CFP) learning is a form of classical conditioning in which a particular flavor (the conditioned stimulus, CS+) is associated with the oral and/or post-oral properties of nutrients (the unconditioned stimulus, US). Flavor–flavor CFP refers to a developed preference for a target flavor (e.g., cherry flavor) mixed in a preferred solution (e.g., sweet taste of sucrose). Flavor–nutrient CFP refers to a developed preference for a target flavor paired with the nutrient's post-oral actions (e.g., sucrose). Important differences in conditioning effects are observed among sugars such that whereas glucose and sucrose are capable of conditioning flavor preferences (CFP) through flavor-flavor and flavor-nutrient processes, fructose-CFP acts through flavor-flavor, but not flavor-nutrient processes in short-term tests (Sclafani and Ackroff, 1994; Sclafani et al., 1993, 1999). Further, both the post-ingestive actions and orosensory properties of fat (e.g., corn oil (CO)) condition a flavor preference (Ackroff and Sclafani, 2009). In assessing the mechanisms by which sugar- and fat-CFP are controlled, one must examine manipulations that affect the initial learning (acquisition) of the preference in which case the manipulation is conducted during one-bottle training. One must also examine manipulations that affect the continued maintenance (expression) of the preference in which case the manipulation is conducted during two-bottle choice preference tests after the preference has already been acquired. Measures of one-bottle training and two-bottle testing intakes serve as dependent measures as well as percent CS+ intake in the two-bottle tests which is calculated as (CS+ intake / total intake) × 100.

The pharmacological substrates of the acquisition and expression of sugar- and fat-CFP have been examined for dopamine (DA), opioid and *N*-methyl-D-aspartate (NMDA) receptor systems. Systemic administration of DA D1 (SCH23390) or D2 (raclopride), but not opioid (naltrexone), receptor antagonists eliminated the acquisition and expression

* Corresponding author at: Office of Research and Graduate Studies, Queens College, CUNY, 65-30 Kissena Blvd., Flushing, NY 11367, USA.
E-mail address: richard.bodnar@qc.cuny.edu (R.J. Bodnar).

http://dx.doi.org/10.1016/j.pbb.2016.09.001
0091-3057/

of flavor-flavor conditioning studies elicited by sucrose in sham-feeding rats (Yu et al., 1999, 2000a, 2000b) or fructose in real-feeding rats (Baker et al., 2003, 2004), implicating both DA receptor families, but not opioids in both the learning and maintenance of these responses. Yet DA D1, but not D2 or opioid receptor antagonists eliminated acquisition, and to a lesser degree, reduced expression of flavor-nutrient conditioning elicited by intragastric (IG) sucrose infusions (Azzara et al., 2000, 2001), implicating only the DA D1 receptor in the learning of this response. In contrast, fat (CO)-CFP acquisition and expression was only attenuated by DA D2, but not D1 or opioid receptor antagonists (Dela Cruz et al., 2012a, 2012b), indicating that combined flavor-flavor and flavor-nutrient mechanisms reduce the effectiveness of DA antagonism to affect the response. The non-competitive NMDA receptor antagonist, MK-801 eliminated the acquisition, but not expression of CFP induced by fructose (Golden and Houpt, 2007) or CO (Dela Cruz et al., 2012a), implicating glutamatergic signaling in the learning of this response. The more limited pharmacological effects on CO-CFP is similar to that observed following glucose that also activates both flavor-flavor and flavor-nutrient processes. Thus, oral glucose-CFP was significantly though marginally attenuated in expression studies by DA D1, DA D2 or NMDA receptor antagonism (Dela Cruz et al., 2014).

Cholinergic muscarinic and nicotinic receptor signaling and $GABA_B$ receptor signaling have been recently implicated in the mediation of fructose-CFP (Rotella et al., 2015, 2016). Fructose-CFP expression was significantly reduced by systemic administration of muscarinic (scopolamine (SCOP: 2.5–10 mg/kg: 65–68%)) and nicotinic (mecamylamine (MEC: 4–8 mg/kg: 67–73%)) cholinergic receptor antagonists, but only at doses that reduced total saccharin intake. However, this occurred despite the fact that CS+ preference intakes were significantly higher than CS− preference intakes, indicating minimal actions of cholinergic signaling on expression of this response. Further, fructose-CFP acquisition was eliminated by SCOP at doses of 1 (40–54%) and 2.5 (45–58%) mg/kg, and was accompanied by a failure to observe CS+ and CS− intake differences. On the other hand, MEC failed to alter fructose-CFP acquisition, indicating a critical role for muscarinic cholinergic receptors in the learning of this response. In contrast, MEC, but not SCOP enhanced the magnitude and persistence of quinine-induced conditioned flavor avoidance for a fructose solution (Rotella et al., 2015), indicating different cholinergic receptor mechanisms in preference and avoidance responses. Whereas systemic administration of the $GABA_B$ receptor agonist, baclofen (BAC) minimally reduced the expression, but not the acquisition of fructose-CFP, the magnitude and persistence of quinine-induced conditioned flavor avoidance for a fructose solution was enhanced (Rotella et al., 2016), indicating a limited role for $GABA_B$ signaling in expression of this response.

Cholinergic receptor signaling has also been implicated in the mediation of food and fat intake. Food increases Ach release in the amygdala and the nucleus accumbens (NAC) (Avena et al., 2006, 2008a, 2008b, 2008c; Hajnal et al., 1998; Mark et al., 1992, 1995). Consumption of a high-fat diet for one week reduced acetylcholinesterase activity in the frontal cortex, hypothalamus and midbrain, as well as increased both β2-nAChR binding in the medial prefrontal cortex and substantia nigra, in addition to α7-nAChR binding in the lateral and ventromedial hypothalamus (Morganstern et al., 2012). MEC blocked the enhancements in exploratory and novelty-seeking behaviors induced by high-fat consumption (Morganstern et al., 2012). Chronic nicotine reduced body weight in mice, particularly those maintained on a high-fat diet, an effect blocked by MEC co-treatment (Mangubat et al., 2012). Accumbal microinjections of SCOP markedly reduced fat intake elicited by accumbal administration of the mu-opioid receptor agonist, DAMGO, and also reduced food intake in food-deprived rats (Perry et al., 2009; Will et al., 2006). However, accumbal SCOP failed to affect fat intake itself (Will et al., 2006).

$GABA_B$ receptor signaling has also been implicated in the mediation of fat intake. BAC administered into limbic and hypothalamic sites increased food intake (e.g., Arnt and Scheel-Kruger, 1979; Echo et al., 2002; Stratford and Kelley, 1997; Ward et al., 2000; Wirtshafter et al., 1993), and was reported to be mediated through GABA receptor interactions between the ventral tegmental area and nucleus accumbens (Miner et al., 2010). Systemic BAC increased fat intake under normal conditions (e.g., Bains and Ebenezer, 2013; Ebenezer and Patel, 2011), but suppressed fat intake under "binge-type" conditions (Avena et al., 2014; Berner et al., 2009; Buda-Levin et al., 2005; Corwin and Wojnicki, 2009; Rao et al., 2008; Wojnicki et al., 2014; Wong et al., 2009). Therefore, the present study examined whether systemic administration of muscarinic (SCOP) and nicotinic (MEC) cholinergic receptor antagonists and a $GABA_B$ receptor agonist (BAC) would alter expression and acquisition of fat-CFP elicited ingestion of a flavor (e.g., cherry) paired with a higher (3.5%) CO concentration relative to a flavor (e.g., grape) paired with a lower (0.9%) CO concentration.

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 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) as described previously (Dela Cruz et al., 2012a, 2012b). 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 0.05% cherry and grape flavors were each presented in similar 0.9% CO + 0.3% xanthan gum suspensions. The CO + Kool-Aid + gum mixtures with the flavored training solutions are hereafter referred to as CS+/3.5% CO and CS−/0.9% CO, and the flavored 0.9% CO two-bottle test solutions 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. In the two weeks prior to testing, the rats were placed on a food restriction schedule that maintained their body weights at 85–90% of their ad libitum level to increase approach behavior to the solutions. This procedure has been consistently applied in all of our and other prior CFP studies using oral intake or intragastric infusions of sugars and fats (e.g., Azzara et al., 2000, 2001; Baker et al., 2003, 2004; Dela Cruz et al., 2012a, 2012b, 2014; Golden and Houpt, 2007; Rotella et al., 2015, 2016; Yu et al., 1999, 2000a, 2000b). It is important to note that examination of these effects in food-deprived rats not only increases motivation to drink, but also produces an energy deficit. Many prior studies have explored both taste and consumption of palatable diets under both sated and deprived conditions, and have found dissociable behavioral and neurochemical processes. In other words, distinct hedonic and homeostatic processes can govern approach and consumption, and can presumably differentially alter preference for flavored solutions (see review: Baldo et al., 2013). For instance, administration of naloxone into the basolateral nucleus of the amygdala blocked increased fat intake induced by accumbal administration of D-Ala2-NMe-Phe4-Glyol5-enkephalin (DAMGO), but not increased fat intake induced by food deprivation (Parker et al., 2010). In contrast, administration of naloxone into the central nucleus of the amygdala blocked food deprivation-induced increases in fat intake, but not fat intake induced by accumbal DAMGO (Parker et al., 2014). Further, whereas