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Oxytocin action in the ventral tegmental area affects sucrose intake

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Abstract

Brain oxytocin is known to play a role in the control of food intake, and recent studies suggest that stimulation of central oxytocin receptors selectively suppresses carbohydrate intake. The specific oxytocin projection sites and receptor populations involved in this response are as yet unidentified. We hypothesized that oxytocin receptors in the ventral tegmental area (VTA) may play a role in limiting sucrose intake, because the VTA is known to influence palatable food intake. We first performed a dose response study in which we observed that intra-VTA oxytocin injection significantly suppressed intake of a 10% sucrose solution during a 30-min test session by 13.35% - 20.5% relative to vehicle treatment. Doses of intra-VTA oxytocin that suppressed sucrose intake had no effect on water intake. Next we examined the effects of two oxytocin receptor antagonists, (d(CH₂)₅¹,Tyr(Me)²,Orn⁸)-Oxytocin (OVT) and L-368,899. Each of these antagonists significantly increased 10% sucrose intake by 17% - 20.5% relative to vehicle when delivered directly into the VTA, at doses subthreshold for effect if injected into the cerebral ventricles. Finally, we observed that the effect of intra-VTA oxytocin to suppress 10% sucrose intake was significantly attenuated by pretreatment with L-368,899, supporting the suggestion that the VTA oxytocin treatment suppresses intake through action at oxytocin receptors. These findings support the suggestion that endogenous oxytocin action within the VTA suppresses sucrose intake. We conclude that oxytocin receptors in the VTA play a physiologic role in the control of sucrose ingestion.

Keywords

Oxytocin; Ingestive behavior; Ventral tegmental area

1. Introduction

Oxytocin (OT)*, a hypothalamic neuropeptide, is most well known for its endocrine effects on reproductive behaviors, labor, and lactation (Gimpl and Fahrenholz, 2001).

Magnocellular neurons of the paraventricular nucleus of the hypothalamus (PVN) release OT into the neurohypophysis; however, parvocellular PVN neurons containing OT project to other sites in the brain, including the nucleus of the solitary tract (NTS) and dorsal motor

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*Abbreviations: CCK, cholecystokinin; DA, dopamine; icv, intracerebroventricular; NTS, nucleus of the solitary tract; OT, oxytocin; OVT, (d(CH₂)₅¹,Tyr(Me)²,Orn⁸)-Oxytocin; PVN, paraventricular nucleus of the hypothalamus.

nucleus of the vagus nerve, and to the spinal cord (Sofroniew, 1983). Centrally-acting OT has a number of behavioral and physiologic effects, including food intake suppression (Olszewski et al., 2010a). Recent studies suggest that these neurons may be especially involved in the control of carbohydrate intake. In rats, peripheral injection of OT inhibits intake of sucrose, whereas rats injected with an OT receptor antagonist increased sucrose consumption (Olszewski et al., 2009; Olszewski et al., 2010b). Similarly, OT deficient (OT^{-/-}) mice consume significantly more than wild-type (WT) mice when given access to a 10% sucrose solution (Amico et al., 2005). Other studies found that OT^{-/-} mice have elevated daily intake of sweet and non-sweet carbohydrate containing solutions relative to WT controls, but there are no sustained differences between OT^{-/-} and WT mice when they are consuming Intralipid (Miedlar et al., 2007; Sclafani et al., 2007). In rats, a significantly greater percentage of PVN OT neurons are activated by a sucrose meal than an Intralipid meal (Olszewski et al., 2010b), supporting the suggestion that acute consumption of sugar recruits OT neurons to promote satiation. Chronic sugar consumption impairs this OT neuronal response to meals (Mitra et al., 2010), so it is possible that dysregulation in this system contributes to overeating of palatable high-sugar food.

Although OT's role in the control of food intake has been studied for several decades, there has been relatively little investigation of which central OT projection sites mediate its feeding effects. Most studies on the feeding effects of central OT have delivered OT or OT receptor antagonists into the cerebral ventricles, an approach that lacks site-specificity. One region that has been implicated is the NTS in the caudal brainstem. The OT projection to the NTS has been proposed to mediate some of the effects of the adiposity hormone leptin and gastrointestinal satiation signals such as cholecystokinin (CCK). Both leptin and CCK induce c-Fos expression in PVN OT neurons that project to the NTS, and central blockade of OT receptors impairs the anorexic responses to leptin and CCK (Olson et al., 1991a; Blevins et al., 2003; Blevins et al., 2004). However, the NTS is not the only feeding-relevant areas of the brain that contains OT terminals and receptors (Olszewski et al., 2010a). Here, we focus on the OT neuronal projection to the ventral tegmental area (VTA). The VTA is known to play a role in reward and motivated behavior in general, and targeted manipulations of VTA neurons affect palatable food intake (Lutter and Nestler, 2009). For example, peripheral or intra-VTA injection of leptin decreases the firing rate of VTA dopamine (DA) neurons, and also decreases food intake in rats (Hommel et al., 2006). Conversely, leptin receptor knockdown in the VTA of rats causes an increase in the consumption of highly palatable sucrose solution (Hommel et al., 2006; Davis et al., 2011).

The presence of OT fibers and receptors within the VTA is well documented (Sofroniew, 1983; Vaccari et al., 1998), and the effects of OT on several behaviors have been examined. Intra-VTA OT treatment promotes grooming behavior in rats, an effect which appears to be mediated by DA receptors at other sites (Stivers, et al., 1988). VTA injection of OT also induces penile erection in male rats (Melis et al., 2007), and influences lordosis as well as maternal behavior in female rats (Pederson et al., 2007). Recent studies have reported that OT fibers are observed in close apposition to DA neurons within the caudal part of the VTA (Melis et al., 2007), and intra-VTA OT treatment increases DA release in the nucleus accumbens (NAc), a major projection site for VTA DA neurons (Melis et al., 2007; Shakaroh et al., 2010). Here, we investigated the possibility that VTA OT receptors also play a role in food intake control, and specifically hypothesized that VTA OT limits sugar intake. We present data from a series of behavioral studies demonstrating that intra-VTA OT suppresses intake of a sucrose solution whereas blockade of OT receptors in VTA increases sucrose intake.

2. Results

2.1. VTA OT effect on sucrose intake

Naïve rats ($n = 10$) received injections of sterile 0.9% saline vehicle or 0.3, 1, or 3 μg OT into the VTA prior to a 30-min 10% sucrose intake test. OT significantly reduced sucrose intake ($F(3, 27) = 6.09$, $p < 0.01$) at both 1 and 3 μg relative to saline (p 's < 0.05), whereas the 0.3 μg dose was not effective (Figure 1A). Experimenters observed increased grooming during the sucrose test sessions after OT injection, but this was not measured quantitatively. OT had no significant effects on overnight chow intake or body weight.

2.2. VTA OT effect on water intake

Icv injection of OT can suppress water intake (Arletti et al., 1990), so it is possible that the observed effect of VTA OT on 10% sucrose intake was due to suppression of fluid intake in general. To test this possibility, we examined the ability of intra-VTA OT to suppress water intake at doses that significantly suppressed sucrose intake. Naïve rats ($n = 8$) were water-deprived overnight in order to stimulate water intake independent of feeding. They received injection of sterile 0.9% saline vehicle, 1 or 3 μg OT into the VTA 15 min before the start of a 30-min water intake test. OT had no effect on water intake during these test sessions (Figure 1B). As was the case in the OT dose response for sucrose intake, OT injection appeared to stimulate grooming in this study, but this was not quantified. Overnight chow intake was not affected, nor was body weight.

2.3. VTA OVT effect on sucrose intake

We next tested the effect of OT receptor blockade with the OT receptor antagonist ($\text{d}(\text{CH}_2)_5^1, \text{Tyr}(\text{Me})^2, \text{Orn}^8$)-Oxytocin (OVT) (Bachem, Torrance, CA), delivered to naïve rats ($n = 6$) at doses of 0.1, 3, or 10 μg . Intra-VTA OVT significantly increased 10% sucrose intake during the test session ($F(3, 15) = 4.58$, $p < 0.05$) at all 3 doses relative to saline (p 's < 0.05) (Figure 2A). Overnight chow intake and body weight were not affected by VTA OVT treatment.

2.4. VTA L-368,899 effect on sucrose intake

We also investigated the effect of OT receptor blockade with the non-peptide OT receptor antagonist, L-368,899 (Tocris Bioscience, Ellisville, Missouri), to provide additional confirmation of OT receptor effects on sucrose intake. Rats ($n = 8$) in this experiment had been previously used in the OT dose response for sucrose intake described in section 2.1. After the end of that study, they were given 2 weeks with no injections, and then began this experiment. Here, rats received sterile 0.9% vehicle vs. 1 or 5 μg L-368,899. Sucrose intake was significantly increased by intra-VTA injection of L-368,899 ($F(2, 14) = 4.88$, $p < 0.05$), with intake after the 5 μg dose differing significantly from vehicle ($p < 0.01$) and the 1 μg dose falling just short of significance ($p = 0.054$) (Figure 2B). As with the other intra-VTA treatments here, L-368,899 did not affect overnight chow intake or body weight.

2.5 OT receptor mediation of VTA OT-induced sucrose intake suppression

Although OT has high affinity for OT receptors, it can bind to vasopressin receptors with lower affinity (Chini and Manning, 2007). Because vasopressin receptors are present in VTA (Johnson et al., 1993; Vaccari et al., 1998), it is possible that the observed effects of intra-VTA OT injection are mediated by vasopressin receptors. To determine the role of OT receptors in the OT effect, we asked whether pre-treatment with intra-VTA L-368,899, at a dose of 0.5 μg , attenuates the sucrose-intake suppressive effect of VTA injection of 1 μg OT, delivered 15 min before the 10% sucrose intake test. Rats ($n = 7$) used in this experiment had previously been used in the water intake study described in section 2.2, and

were given 2 weeks of no treatment before beginning daily 10% sucrose intake test sessions. We observed a significant main effect of OT ($F(1, 6) = 14.16, p < 0.01$) and interaction between L-368,899 and OT ($F(1, 6) = 12.81, p < 0.05$). As shown in Figure 3A, VTA OT significantly suppressed sucrose intake whether or not rats were pre-treated with L-368,899 (p 's < 0.01). However, the magnitude of the OT effect was significantly smaller after L-368,899 pre-treatment (16%) relative to vehicle pre-treatment (28%) ($p < 0.01$) (Figure 3B). Sucrose intake after L-368,899 plus OT was significantly higher than after vehicle plus OT ($p < 0.05$).

3. Discussion

The results presented here support the hypothesis that OT receptors in the VTA contribute to the control of palatable food intake. We first showed that intra-VTA injection of OT suppresses 10% sucrose intake at doses subthreshold for effect when delivered icv, and that these same doses do not affect intake of water. We also observed that the sucrose intake-suppressive effect of OT was attenuated by pre-treatment with an OT receptor antagonist. These effects on sucrose intake demonstrate that pharmacologic stimulation of OT receptors in the VTA can reduce feeding, but tells us little about whether endogenously released OT acts in the VTA to affect feeding. Our observations that both OT receptor antagonists increase sucrose intake, also at doses below threshold for an effect if delivered icv, address this issue and provide strong support for the hypothesis that OT receptors in VTA play a physiologic role in the control of sucrose intake. Blockade of VTA OT receptors could only increase sucrose consumption if endogenous OT receptor stimulation at this site normally functions to limit sucrose intake.

VTA OT treatment also increases grooming behavior in male rats (Stivers et al., 1988), so we must consider the possibility that the sucrose intake suppression we observed after OT injection is secondary to this grooming effect. That is, perhaps intra-VTA OT reduced sucrose intake only because the rats were more compelled to groom instead, as opposed to a primary effect to reduce ingestion independent of other behaviors. The OT receptor antagonist experiments go some way toward addressing this issue, because we did not observe any impact of either antagonist on grooming behavior. Even if there were some degree of grooming suppression after intra-VTA OT antagonist injection, this would not necessarily cause increased sucrose intake. Rats could have chosen to explore the chamber or sleep instead. The finding that VTA OT receptor blockade increased sucrose intake suggests that these receptors can indeed impact feeding in addition to other behaviors, not merely as a consequence of effects on other behaviors.

We found that OT receptor blockade prior to intra-VTA OT injection significantly attenuated the sucrose intake-suppressive effect of OT. Based on this finding, we conclude that VTA OT receptors are at least partially responsible for the effect of pharmacologic VTA OT treatment on sucrose intake. In this study, OT did still effectively reduce sucrose intake after L-368,899 pre-treatment, so it is possible that OT acts through other receptors, as well. OT can activate vasopressin receptors, which are expressed in VTA (Johnson et al., 1993; Vaccari et al., 1998). Peripheral injection of vasopressin itself suppresses food intake, and this effect could be centrally mediated (Langhans et al., 1991). However, one study has shown no significant effect of lateral cerebral ventricle injection of vasopressin on food intake in mice (Aoyagi et al., 2009), and vasopressin 1A and 1B receptor knock-out mice show reduced food intake (Nakamura et al., 2009), so the role of these receptors in the control of feeding is unclear. Alternatively, the lack of complete reversal of OT effect with L-368,899 pre-treatment observed here could be due to imperfect OT receptor blockade against OT at the doses that we used. It is notable that 1 μ g OT had a larger suppressive effect on sucrose intake in this experiment compared with the initial dose response. This

may be related to the higher baseline level of 10% sucrose intake in this group of rats, which were a bit older and larger than those in the OT dose response. It is possible that L-368,899 pre-treatment would completely block the effect of a lower dose of OT. Further questions about OT receptor specificity and whether vasopressin receptors in VTA play any role in the control of feeding are deserving of attention but are beyond the scope of the present study.

The finding that VTA OT receptors contribute to the control of sucrose intake raises a number of questions. We focused on ingestion of 10% sucrose solution because of the previous studies supporting a role for brain OT in sugar intake (e.g., Olszewski et al., 2010b). However, some studies have shown effects of OT treatment on intake of chow and low- or high-fat diets (e.g., Zhang et al., 2011; Morton et al., 2012). Other manipulations directed at the VTA, such as knockdown of leptin receptor expression, can affect intake of high-fat diet as well (Hommel et al., 2006). Thus, it is possible that OT receptors in the VTA affect feeding more generally. We did not observe any effect of intra-VTA OT or OT receptor antagonist injection on overnight chow intake after the sucrose intake test sessions, but it is possible that the effects of these drug treatments are simply short-lived. Another area for future investigation is whether VTA OT receptors affect feeding by reducing motivation for sucrose. The VTA is known to play a role in reward-motivated behavior in general, and stimulation of leptin receptors in the VTA suppresses motivation to perform operant behavior for sucrose reinforcement (Davis et al., 2011). VTA OT receptor stimulation may impact sucrose intake through this same behavioral mechanism.

The VTA-to-NAc DA neuronal projection has been implicated in reward-related behavior of many kinds, including feeding behavior. Any such effect of VTA manipulation therefore raises the question of whether the mesolimbic DA pathway plays a mediatory role. OT neurons do appear to make synaptic contact with VTA DA neurons, based on immunohistochemistry showing close apposition and overlap between OT-positive fibers and tyrosine hydroxylase-positive cell bodies in VTA (Melis et al., 2007). In addition, intra-VTA OT injection has been shown to increase DA release in the NAc (Melis et al., 2007). A large literature suggests that DA action in NAc plays a role in feeding behavior, and so it is reasonable to question whether OT's effect to increase DA release in NAc may contribute to the sucrose intake effects we observed in our experiments. We suggest that this is not likely. Sucrose ingestion increases DA release in NAc, and it is thought that this promotes, rather than inhibits, sucrose intake. This is supported by the finding that intra-NAc DA receptor antagonist treatment suppresses sucrose intake (Smith, 2004). Therefore, we speculate that OT action in VTA suppresses feeding through some other efferent pathway, perhaps a DA projection to another brain area or possibly a GABAergic projection (Fields et al, 2007).

Taken together, the results of our experiments point to VTA OT receptor involvement in the physiologic control of sugar intake. Central injection of OT was already known to suppress sucrose intake, but there has been little investigation of the specific OT receptor populations that mediate this effect. The only other site that has been investigated as a mediator of OT effects on food intake is the NTS. However, studies suggesting a role for NTS OT receptors in feeding behavior showed effects on intake of rat chow, and did not examine sucrose intake (Blevins et al., 2003; Blevins et al., 2004; Baskin et al., 2010). The results reported here are the first identification of a specific brain site that contributes to OT's effect on sucrose intake, and therefore significantly expand our knowledge about the central OT system's role in this behavior.

4. Experimental Procedures

4.1. Animals

Naïve male Wistar rats (Charles River, Wilmington, MA) were maintained individually in temperature-controlled vivariums on a 12-h-light:12-h-dark cycle in Plexiglass cages with food hoppers. Distilled water and Purina 5001 rat chow (St. Louis, MO) were available *ad libitum* except where otherwise noted. Mean body weight for rats in the single-treatment OT and L-368,899 studies examining sucrose intake was 382 g at the start, and mean body weight of rats at the start of the OVT study was 456 g. The group used in the water intake and combination L-368,899/OT studies had a mean body weight of 428 g at the start of experiments. Rats were handled daily and habituated to experimental procedures before the studies. All experimental procedures were approved by the Florida State University Institutional Animal Care and Use Committee and conform to the standards of the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

4.2. Surgery

A 26-G unilateral guide cannula (Plastics One, Roanoke, VA), was implanted 2.0 mm above the VTA under 2 to 4% isoflurane in 1 liter oxygen/minute inhaled continuously during surgery. Stereotaxic coordinates for VTA cannula placement were: 0.7 mm lateral to the midline, 5.6 mm posterior to Bregma, and then 7.5 mm below the skull. The cannula was cemented to three jeweler's screws attached to the skull, and closed with an obturator. Carprofen (5 mg/kg sc) (Butler Schein Animal Health Supply, Columbus, OH) was administered before the start of surgery and again if rats showed signs of distress (i.e., lethargy, lack of grooming, porphyrin staining around eyes or nose) over the next 2 days. Food intake and body weight were monitored while rats recovered for at least 5 days before experimental procedures began. Rats were handled daily and habituated to intra-VTA injection of saline before the experiments began.

Cannula placements were verified histologically after the end of the experiments (see Figure 4). Rats were deeply anesthetized (180 mg/kg ketamine and 30 mg/kg xylazine, i.p.) and transcardially perfused with 10mM PBS and 4% paraformaldehyde. Brains were removed and sunk in 30% sucrose in PBS and then frozen in isopentane on dry ice. Coronal microtome sections (40 μ m) through the VTA were collected into 0.02 M TBS with 0.1% sodium azide and stored at 4°C. Sections were then slide-mounted and stained with cresyl violet (Sigma Aldrich, St. Louis, MO) for examination with an Olympus BX41 microscope. Monochromatic digital images were acquired with a Retiga EXI Aqua camera and Q-Capture software (Hunt Optics, Pittsburgh, PA). Using the cannula track as a guide, we identified injection sites by injector-induced damage and appearance of gliosis, and the full series of coronal sections was examined to determine the rostro-caudal center point of injection. Injection sites within the boundaries of the VTA as drawn in the atlas of Paxinos and Watson (2007) were considered correct.

4.3. Sucrose intake test

Rats were given daily 30 min access to 10% sucrose solution in a bottle placed on their home cage, with no access to food or water during this session. After 12-15 sessions, sucrose intakes were stable (less than 10% day-to-day variation) and the experiment began. These experiments used within-subjects designs with each rat receiving all conditions in counterbalanced order. On experimental days, food and water were removed before drug delivery. 15 min before the sucrose intake test started, a 0.5 μ l volume of vehicle or drug was injected directly into the VTA with a 33G injector (Plastics One, Roanoke, VA) that extended 2 mm beyond the end of the guide cannula. In the experiment examining whether VTA L-368,899 pre-treatment could block the effect of OT, rats received 2 intra-VTA

injections. The first was either vehicle or L-368,899, delivered 30 min before the sucrose intake test began, and the second injection was either vehicle or OT, delivered 15 min before the start of the sucrose intake test.

After the 30 min test period, sucrose bottles were removed and *ad lib* food and water was returned. 24-h chow intake and body weight were recorded. Experimental conditions were separated by at least 2 d.

4.4. Water intake test

Rats were water-deprived overnight in order to stimulate water intake during the mid-light phase, independent of food intake. They were habituated to food removal and 30-min water intake measurement on several days with no deprivation, and then received one overnight water deprivation and subsequent 30-min water intake test to establish baseline water intake under these conditions. On experimental treatment days, food was removed before drug delivery, and intra-VTA injections of saline vehicle or OT were delivered as described above, 15 min prior to the start of water access. After the 30 min test period, water bottles were removed and weighed, and then *ad lib* food and water was returned. 24-h chow intake and body weight were recorded. Experimental conditions were separated by at least 3 d to allow full recovery from the overnight water deprivation before the rats were water-deprived again.

4.5. Drugs

Doses of 0.3, 1, or 3 µg OT (Bachem, Torrance, CA) were chosen based on previous studies (Arletti et al., 1990) and preliminary data from our laboratory demonstrating that 4th -ventricular injection of 3 µg OT had no effect on 10% sucrose intake (data not shown). The OT receptor antagonist (d(CH₂)₅¹, Tyr(Me)², Orn⁸)-Oxytocin (OVT) (Bachem, Torrance, CA) was delivered at 0.1, 3, or 10 µg doses, based on a previous report that 9 nmol (10.39 µg) OVT has no effect on food intake when delivered into the lateral ventricle (Olson et al., 1991b). The OT receptor antagonist L-368,899 (Tocris Bioscience, Ellisville, MO) was given at 1 or 5 µg based on preliminary data from our laboratory demonstrating that 4th -icv delivery of 5 µg L-368,899 had no effect on sucrose intake (data not shown). We used 2 structurally different OT receptor antagonists in these experiments to increase confidence that the observed effects truly reflect endogenous OT receptor activation.

4.6. Statistical Analysis

Drug effects on sucrose or water intake in single-injection studies were evaluated by 1-way within-subjects ANOVA. Effects on sucrose intake in the L-368,899 plus OT study were assessed by 2-way within-subjects ANOVA. Differences in the magnitude of OT effect with vs. without L-368,899 pre-treatment were assessed by paired-samples Student's t-test. Holm-Bonferroni pairwise comparisons were done when significant effects were obtained in the ANOVA. $P < 0.05$ was considered significant.

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Highlights

- Ventral Tegmental Area oxytocin injection suppresses rats' intake of a 10% sucrose solution.
- Oxytocin receptor blockade in the ventral tegmental area increases rats' intake of 10% sucrose solution.
- Endogenous oxytocin receptor stimulation in the ventral tegmental area contributes to the control of sucrose intake.

Figure 1.

A) Mean $\pm$ SEM 10% sucrose intake during the 30-min test session after intra-VTA injection of vehicle or OT. B) Mean $\pm$ SEM water intake during 30-min test sessions after intra-VTA vehicle or OT injection. * $p < 0.05$ relative to vehicle.

Figure 2. Mean $\pm$ SEM 10% sucrose intake during the 30-min test session after intra-VTA injection of vehicle or OT antagonist. A) Effect of OVT on sucrose intake. B) Effect of L-368,899 on sucrose intake. * $p < 0.05$ relative to vehicle.

Figure 3.

A) Mean $\pm$ SEM 10% sucrose intake during the 30-min test session after intra-VTA injection of vehicle or OT when rats were pre-treated with either vehicle or L-368,899. Significant differences ($p < 0.05$) across conditions are represented by different letters above the bars. B) Mean $\pm$ SEM percent suppression of sucrose intake by OT after vehicle vs. L-368,899 pre-treatment. * $p < 0.05$.

Figure 4.

A) Representative image of a VTA injection site in a coronal section stained with cresyl violet. B) Diagram of representative VTA injection placements based on the atlas of Paxinos and Watson (2007), showing the VTA region at 4 different anterior-posterior levels relative to bregma.