The Anorexigenic Neural Pathways of Oxytocin and Their Clinical Implication

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Abstract

Oxytocin was discovered in 1906 as a peptide that promotes delivery and milk ejection; however, its additional physiological functions were determined 100 years later. Many recent articles have reported newly discovered effects of oxytocin on social communication, bonding, reward-related behavior, adipose tissue, and muscle and food intake regulation. Because oxytocin neurons project to various regions in the brain that contribute to both feeding reward (hedonic feeding) and the regulation of energy balance (homeostatic feeding), the mechanisms of oxytocin on food intake regulation are complicated and largely unknown. Oxytocin neurons in the paraventricular nucleus (PVN) receive neural projections from the arcuate nucleus (ARC), which is an important center for feeding regulation. On the other hand, these neurons in the PVN and supraoptic nucleus project to the ARC. PVN oxytocin neurons also project to the brain stem and the reward-related limbic system. In addition to this, oxytocin induces lipolysis and decreases fat mass. However,

these effects in feeding and adipose tissue are known to be dependent on body weight (BW). Oxytocin treatment is more effective in food intake regulation and fat mass decline for individuals with leptin resistance and higher BW, but is known to be less effective in individuals with normal BW. In this review, we present in detail the recent findings on the physiological role of oxytocin in feeding regulation and the anorexigenic neural pathway of oxytocin neurons, as well as the advantage of oxytocin usage for anti-obesity treatment.

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Introduction

Oxytocin is a neuropeptide composed of 9 amino acids, and its sequence and structure are very similar to arginine vasopressin. It is produced in the hypothalamus and is released to other regions of the central nervous system (CNS) or the circulatory system throughout the body via the posterior pituitary gland. This neuropeptide was discovered by Sir Henry Dale in 1906 and named “oxytocin” (meaning “swift birth” in Greek), because of its ability to contract the uterus of a pregnant cat [1]. In 1953, oxytocin became the first polypeptide hormone to

Fig. 1. Proposed model for oxytocin-related neural pathways that regulate food intake. The red arrows indicate the stimulatory projections and the blue arrows indicate the inhibitory projections. Oxytocin neurons in the PVN and SON activate POMC neurons in the ARC. Oxytocin neurons in the PVN are subsequently activated by the POMC neuron-derived α -MSH. Oxytocin neurons in the SON express the K_{ATP} channel and play a role as a glucose sensor and, thus, activate POMC neurons in the ARC. NPY/AgRP neurons project to the PVN oxytocin neurons and suppress their neural activity. PVN oxytocin neurons project to the POMC neurons in the NTS. Melanocortin receptors are expressed in the

DMNV, which is the first nucleus of the vagal afferent, and receive projection from neighboring NTS POMC neurons. The DMNV regulates the gastrointestinal system, and delays gastric emptying. In reward-related feeding, oxytocin neurons in the PVN project to the dopamine- and GABA-containing neurons in the VTA, and activate VTA neurons. GABA neurons in the VTA regulate the activity of the dopamine neurons. VTA dopamine neurons project to the NAc to activate the neurons in the NAc and promote reward-related feeding. However, PVN oxytocin suppresses these neurons and decreases reward-related feeding.

be sequenced and synthesized, and Vincent du Vigneaud [2] was awarded the Nobel Prize for this achievement. From an evolutionary point of view, the origin of oxytocin is considered to date back nearly 600 million years, and oxytocin-like peptides/homologues can be found across almost the entire animal kingdom, from insects to mammals [3].

Oxytocin is synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON). The magnocellular oxytocin neurons in the PVN and SON project to the posterior pituitary gland and secrete oxytocin into the periphery circulation system [4]. On the other hand, parvocellular oxytocin neurons in the PVN project to and secrete oxytocin into the CNS, although recent data indicate that oxytocin neurons in the SON may also secrete oxytocin into the CNS [5, 6].

The physiological function of oxytocin was originally known for promoting delivery and milk ejection in mammalian females. However, recent studies have discovered

its new physiological functions, which include increased trust in humans [7, 8], change of paternal and maternal behavior toward their children [9–11], increase in pair bonding [12, 13], and an increased ability to understand other people's feelings or improvement of social communication [14–16]. In addition, there are many articles related to the role of oxytocin in psychiatric diseases [17–20].

Another important new discovery of oxytocin's function is food intake and body weight (BW) regulation. The effect of oxytocin on food intake is generally considered to be regulated mainly by the oxytocin secreted within the CNS and not through the peripherally secreted oxytocin [21]. The first report on the anorexigenic effect of oxytocin after acute intraperitoneal (IP) and intracerebroventricular (ICV) injection was reported in 1989 [22]. Oxytocin injection, either ICV, IP or subcutaneous, was found to decrease food intake in mice [23–26], rats [22–33], and monkeys [34]. Nasal treatment of oxytocin was also reported to decrease food intake in both mice and humans [24, 35, 36].

However, this reduction of BW is not only induced by food intake reduction; oxytocin also induces lipolysis in adipose tissue and decreases fat mass [29, 34, 37–40]. Recent studies have clarified the various new mechanisms of oxytocin's anorexigenic effects through the CNS and promotion of catabolic reactions on peripheral tissues. Although its precise mechanism remains to be elucidated, oxytocin's possible anorexigenic pathways are summarized in Figure 1. This review will focus on both the physiological properties of oxytocin in the CNS and the advantage of oxytocin usage for anti-obesity treatment.

Physiological Role of Oxytocin in Feeding Regulation

Oxytocin plays an important role in the termination of food intake, and oxytocin neurons are activated following food consumption or ingestion [41, 42]. Hypothalamic oxytocin mRNA expression is reduced with fasting and recovers with refeeding [43] or the administration of leptin [44]. Also, the suppression of exocytosis of oxytocin, or genetic reduction of oxytocin expression, increases food intake [25, 43], and ablation of oxytocin neurons increases BW gain by decreasing energy expenditure in male mice fed a high fat diet (HFD) [45]. The ablation of the neurons that express oxytocin receptors, or knock down of oxytocin receptor expression in the nucleus of the solitary tract (NTS) induces hyperphagia [46, 47]. In addition to NTS, oxytocin receptor activation in the arcuate nucleus (ARC) rapidly causes satiety [48]. These reports suggest that the oxytocin neurons physiologically regulate food intake responding to fasting and satiety conditions.

Furthermore, rodents generally show a circadian rhythm in feeding patterns, such as suppression of feeding in the light phase and increased feeding in the dark phase. The secretion of oxytocin also shows a circadian rhythmic pattern with a rise of circulating oxytocin level during the day [26], which was confirmed by measuring secreted oxytocin from a brain slice including the PVN where oxytocin positive neurons mainly reside [49]. In another study, ICV injection of an oxytocin antagonist increased food intake more effectively in the light phase relative to the dark phase [26]. Taking the above oxytocin effects into consideration, endogenously secreted oxytocin may play a role in suppressing food intake during the light phase in rodents. It is possible that one of the physiological roles of central oxytocin is to develop a circadian rhythmic feeding pattern.

On the other hand, there are several reports indicating that endogenous oxytocin has no effect on regulating feeding behavior. There are reports on oxytocin receptor-defi-

cient mice showing no difference in food intake [50] or specific distribution of the oxytocin neurons in the PVN revealing no change in food intake when measured on a normal chow diet [45]. However, these reports may also indicate the existence of mechanisms that can compensate for the absence of oxytocin receptor signaling. In addition, oxytocin receptor-deficient mice showed late-onset obesity and reduced thermogenesis [50], and the mice with specific ablation of oxytocin neurons exhibited increased BW under HFD conditions followed by decreased energy expenditure [45]. These reports suggest that oxytocin receptor signaling may also be involved in energy expenditure regulation, as well as feeding regulation.

The Interaction between Oxytocin Neurons, ARC Feeding-Associated Peptides and Gut Secretory Hormones

W. Bargman and E. Scharer reported in the early 1950s that oxytocin is mainly synthesized in the PVN and SON of the hypothalamus. Neurons in the PVN contain various anorexigenic neuropeptides including thyrotropin-releasing hormone (TRH), corticotrophin-releasing hormone (CRH) [51], nesfatin-1 [52, 53], and brain-derived neurotrophic factor [54], as well as oxytocin [6].

The PVN receives abundant projections from the ARC [55]. There are two opposite types of neurons regulating feeding behavior distributed in the ARC [51]: neurons containing proopiomelanocortin/cocaine- and amphetamine-regulated transcript (POMC/CART); and neurons containing neuropeptide-Y/agouti-related peptide (NPY/AgRP) [51, 56].

POMC is a precursor of several peptides including α -melanocyte-stimulating hormone (α -MSH) [57]. α -MSH is a strong anorexigenic peptide, and its receptor, melanocortin 3/4 receptor (MC3/4R), is distributed in numerous nuclei including the PVN [58]. MC4R deficiency promotes hyperphagia and obesity in both mice and humans [59, 60].

On the other hand, NPY is a strong orexigenic peptide [61], and its ICV injection or specific activation of its neurons [62] promotes feeding behavior. NPY suppresses the activities of neurons containing various anorexigenic neuropeptides in the PVN, and therefore promotes food intake. However, AgRP, another neuropeptide that resides together with NPY, is the antagonist of MC3/4R and prevents α -MSH from binding to MC4R-expressing PVN neurons, which suppresses the activation of PVN neurons and promotes feeding behavior [63].

Originally, neurons in the ARC were known as the first-order neurons [51]. This is because the tight junction of the blood brain barrier in this area is looser than the other brain regions, and easily receives and senses the peripheral condition through factors in the blood stream such as leptin, ghrelin, glucose, etc. [64]. The peripheral hormonal information received in the first-order neurons in ARC is conveyed to the second-order neurons including the PVN. It has been considered that the processing from first-order neurons to second-order neurons induces orexigenic/anorexigenic behavior.

Oxytocin and ARC POMC

Concerning the relation between POMC neurons in the ARC and oxytocin neurons in the PVN, it is considered that α -MSH derived from POMC neurons activates PVN magnocellular oxytocin neurons [65] and increases secretion of oxytocin from the PVN [49]. MC4R mRNA is most abundantly distributed in the PVN in both humans and rodents [66] and MC4R expression was confirmed in some, if not all, of the oxytocin neurons in the PVN [67]. Central or local PVN administration of α -MSH or its ligands powerfully inhibits food intake [68], and induces c-fos expression in the PVN oxytocin neurons [69]. The majority of c-fos positive PVN neurons, which express c-fos after fasting and refeeding, are contacted by terminals of POMC-immunoreactive neurons [70]. The anorexic effect induced by the central administration of α -MSH can be attenuated by oxytocin antagonist pretreatment [71], indicating the involvement of oxytocin on α -MSH-mediated food intake regulation. Our study also confirmed a significant increase in oxytocin secretion when 10^{-8} M α -MSH was applied to a brain slice containing rat PVN [49]. These reports and our data indicate the physiological importance of the projection of ARC POMC neurons (first order) to the PVN oxytocin neurons (second order) in the regulation of feeding.

Oxytocin and ARC NPY/AgRP

On the other hand, the NPY/AgRP neurons in the ARC also affect activities of PVN oxytocin neurons and food intake. By using optogenetics technology, Atasoy et al. [72] demonstrated that AgRP neurons in the ARC induce feeding via inhibition of oxytocin neurons. In the report, the activation of AgRP neurons increased food intake, while the simultaneous activation of both AgRP and PVN oxytocin neurons had no effect on food intake. Furthermore, AgRP activation-induced feeding was almost blunted by local injection of the NPY Y1 receptor or GABA_A receptor antagonist into the PVN [72]. These results indi-

cate that both the NPY Y1 receptors and GABA_A receptors are essential for the anorexigenic effect in the PVN.

As for the NPY, both Y1 and Y5 receptors are distributed in the PVN [73], and NPY fibers project to the PVN, indicating that NPY Y1 and Y5 receptor subtypes mediate NPY's action in feeding regulation in the CNS. These reports also emphasize the physiological importance of the projection from POMC and NPY/AgRP neurons (first order) to the PVN oxytocin neurons (second order) in the regulation of feeding.

Oxytocin and Gut-Secreted Hormone

Another important neural pathway that stimulates oxytocin neurons in the hypothalamus is the vagal afferent-NTS pathway. NTS contains key nuclei that respond to meal-related signals that enter the brain from the periphery via the vagal afferent [74]. It is known that insulin and gut-secreted hormones such as CCK and GLP-1 activate the vagal afferent pathway [75–77]. The vagal afferent senses these gut-secreted peptides and inputs this information into the NTS neurons, which receive abundant projections from PVN oxytocin neurons [78, 79]. IP injection of CCK induces c-fos expression in the brainstem and PVN, where oxytocin neurons are selectively activated [80, 81]. Chemogenetic activation of NTS neurons elicit c-fos-positive neurons in the PVN and reduce food intake and BW in mice [82]. Taking these reports into consideration, postprandial secretion of the gut hormone contributes to suppressing appetite by mediating the vagal afferent NTS-PVN oxytocin neurons.

The next question would be regarding the mechanism for the oxytocin neurons in the PVN to affect food intake. We have shown in the past that the anorexigenic effect of central oxytocin administration can be abolished by pretreatment with melanocortin receptor blockers [53]. This may indicate that oxytocin induces an anorexigenic effect via POMC neurons, which are distributed in the ARC and NTS in the CNS. In other words, PVN oxytocin neurons and POMC neurons in the ARC and NTS may reciprocally regulate one another. We will discuss the mechanism of oxytocin-induced food intake regulation through patterns of subsequent projections from PVN oxytocin neurons in the following sections.

The Effectors of Anorexigenic Oxytocin Pathway

PVN Oxytocin Neurons → NTS POMC Neurons

Although the number of POMC neurons is less in the NTS compared to the ARC, POMC neurons in the NTS

are considered to play an important role in feeding regulation. Zhan et al. [83] demonstrated that POMC neurons in the NTS regulate acute feeding suppression. In contrast, POMC neurons in the ARC regulate long-term suppression of feeding behavior. NTS POMC neurons are known to be stimulated by gut hormones via the vagal afferent pathway [84, 85].

There are several reports showing the projection of the PVN neurons to the brain stem or spinal cord [70, 86, 87]. Both oxytocin and vasopressin neurons in the PVN project to the spinal cord and/or the dorsal vagal complex. The stem contains area postrema, NTS, and the dorsal motor nucleus of the vagus (DMNV) [86]. Application of oxytocin into the 4th ventricle or microinjection into the NTS in rats reduces food intake [33, 88]. Endogenous oxytocin receptor signaling in the NTS mediates food intake-inhibitory effects [47]. In addition, oxytocin immunoreactive varicosities closely appose or contact tyrosine hydroxylase immunoreactive neurons [89] and POMC neurons [53] in the NTS. The neurons in the NTS project to the adjacent DMNV [90], and gastric motility is inhibited by the microinjection of oxytocin into the DMNV [91]. Oxytocin receptors are abundantly distributed in the NTS and DMNV [92], which regulates gastric motility via the noncholinergic inhibitory or cholinergic pathway [90]. Leptin-responsive oxytocin neurons project to the caudal brain stem and reduce food intake [87]; however, oxytocin receptors in the hindbrain are also known to react to circulating oxytocin and contribute to food intake regulation [88]. These reports suggest the importance of oxytocin in feeding regulation via dorsal vagal complex-regulated gastric motility.

PVN Oxytocin Neurons → ARC POMC Neurons

There is evidence that oxytocin decreases food intake via the hypothalamic ARC. Oxytocin receptors are distributed throughout the ARC with nearly 50% of oxytocin receptor-positive neurons being POMC neurons, and intra-ARC injection of oxytocin decreases food intake [6]. In addition, we detected the oxytocin axon terminals contacting the ARC POMC neurons [6] and the single POMC neurons isolated from the ARC were activated by the application of oxytocin [6]. According to the results obtained from experiments using a retrograde tracer, nearly 30% of oxytocin neurons in the PVN (a majority being the medial parvocellular oxytocin neurons) were found to project to the ARC [6]. Surprisingly, nearly 20% of oxytocin neurons in the SON were also found to project to the ARC [6]. The projection from the PVN to the ARC is

the reciprocal projection from the classical projection model, i.e., the anorectic pathway from oxytocin neurons in the PVN (classically second-order neurons) to POMC neurons (classically first-order neurons) in the ARC. Csiffáry et al. [93] also demonstrated using an electron microscope that the PVN oxytocin neurons project to the neurons expressing β -endorphin in ARC, which were derived from POMC.

There are several reports demonstrating the importance of the reciprocal process from second-order to first-order neurons [94]. Krashes et al. [94] reported that the TRH and pituitary adenylate cyclase-activating polypeptide (PACAP) from the PVN induce a marked activation of AgRP neurons in the ARC and stimulate intense feeding in mice. The study also demonstrated marked fidelity in the reciprocal TRH/PACAP PVN glutamatergic → AgRP ARC GABAergic → satiety PVN neuron circuit that drives feeding. These reports showing the neural pathway from the PVN → ARC (i.e., the reciprocal process from second-order to first-order neurons) are a new concept in the study of feeding regulation, and the concept of one-way regulation of “first order neurons → second order neurons” must be reconsidered for further study to elucidate the central mechanism of feeding regulation.

Although the physiological relevance of these neural pathways (ARC → PVN or PVN → ARC) is unclear, it can be considered that the neural pathway of the PVN → ARC may function as a downstream relay of the vagal afferent-NTS pathway. This is supported by reports showing that NTS neurons receive abundant projections from PVN oxytocin neurons [78, 79]. On the other hand, the ARC → PVN pathway may be under the influence of peripheral humoral factors, such as leptin, which can penetrate the blood brain barrier. Further studies are required to clarify the physiological role of these neural pathways.

Our recent study also found that a single PVN neuron projects to both the medial part of the ARC and the NTS with axon collateral projection [95]. It is speculated that the PVN neurons with axon collateral projection can stimulate different brain regions (ARC and NTS) at the same time. We identified that these PVN neurons with axon collateral projection to the ARC and the NTS are composed of NUCB2/nesfatin-1-containing neurons. It is reported that nearly 80% of NUCB2/nesfatin-1 neurons also contain oxytocin [96]. Thus, we examined the relationship between oxytocin in PVN neurons and axon collateral projection to the ARC and NTS. Interestingly, these neurons with axon collateral projection also contain oxy-

tocin (data not shown). Further studies on the physiological role of the neurons with axon collateral projection to the ARC and NTS in feeding regulation are required.

PVN Oxytocin Neurons → Reward-Related Brain Regions

The limbic system is known to play a role in the regulation of instinct, emotion, and reward-related behavior. With regard to reward-related feeding behavior, projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) plays a critical role in the process [97, 98].

Numerous dopamine neurons are distributed in the VTA [99], and the neural pathway from VTA dopamine neurons to NAc is related to drug addiction [100]. It is considered that binge eating is also an addiction to palatable food [101]. Eating a palatable meal is known to activate dopamine neurons in the VTA and NAc [102]. This dopamine input induces a pleasant feeling and stimulates craving for binge eating [103].

Several recent articles reported the effect of oxytocin on reward-related function and addiction. In those studies, oxytocin treatment attenuated the consumption of fructose-sweetened beverages in monkeys [34], addictive cocaine-seeking behavior in rats [104], and intra-VTA injection of oxytocin reduced sucrose intake in rats [105, 106]. Also, oxytocin knockout mice showed an enhancement for palatable sucrose intake [107]. In addition to these reports, it was also confirmed that oxytocin reduces reward-driven food intake, such as a reduction of highly palatable chocolate cookie consumption in humans [108].

PVN oxytocin neurons project to the VTA dopamine neurons [109] and activate dopamine and GABA neurons within the VTA [109]. This therefore suggests that oxytocin modulates the balance of dopamine neurons [109]. On the other hand, oxytocin receptors are expressed in NAc, the site where dopamine neurons project from the VTA. Furthermore, oxytocin administration to NAc reduces food, palatable sucrose and saccharin intake [110], as well as attenuates methamphetamine seeking and demand [111]. In social rewards, coordinated activity of oxytocin and serotonin in NAc is required [112]. The interaction between dopamine, serotonin and oxytocin in reward-related behavior is complex and poorly understood. However, it is suggested that the action of oxytocin in NAc may play an important role in such behavior.

The Regulation of SON Oxytocin Neurons

There are many reports showing c-fos expression in SON neurons after feeding [41, 113, 114]. Numerous

neuropeptides are reported to reside in this nucleus, such as vasopressin, oxytocin, urocortin, NUCB2/nesfatin-1 etc. [96, 113, 115, 116]. SON is composed of magnocellular neurons that project to the posterior pituitary resulting in secretion of oxytocin into the periphery. Activity of SON oxytocin neurons is regulated by hypertonic solution [117], serotonin, noradrenalin [118], glucose [119], and α -MSH [120]. α -MSH inhibits the electrical activity of oxytocin neurons and inhibits secretion from its nerve ending but induces dendritic oxytocin release independently from its neuron activity [120]. The neurons in the SON receive the projection from the suprachiasmatic nucleus, and it may regulate daily changes in neurohypophyseal hormone secretion [121]. Also, it receives projections from the subfornical organ (SFO), and neurons in the SFO regulate excitability of both vasopressinergic and oxytocinergic neurosecretory cells for the regulation of body water balance [122]. Our histological study demonstrated the clear projection of SON oxytocin neurons to the ARC [6] suggesting that the oxytocin neurons in the SON regulate the activities of ARC neurons. The detailed relation between oxytocin neurons in the SON and ARC neurons remains unclear. Although there is little reference, the reports mentioned above suggest that SON oxytocin neurons may be involved in circadian- and osmolality-related feeding regulation.

Another factor that may contribute to food intake regulation for SON oxytocin neurons is glucose sensing. In SON oxytocin neurons, it is considered that insulin activates phosphatidylinositol 3 kinase (PI3K), which induces the translocation of glucose transporter (GLUT) 4 into the membrane [119]. Although GLUT3 is already present in the SON neuron membrane, this additional membrane translocation of GLUT4 induces further glucose uptake and glucokinase-mediated glycolysis as well as ATP production [119]. This rise in intracellular ATP promotes inhibition of ATP sensitive K^+ (K_{ATP}) channels, which express in SON oxytocin neurons (Fig. 2), and induce membrane depolarization and action potential firing, ultimately leading to oxytocin release [119]. This model could indicate that SON oxytocin neurons may function as glucose/metabolic sensors and participate in food intake regulation; however, further study is required.

Clinical Implications of Oxytocin

Currently, oxytocin is considered as a potential neuropeptide to improve social communication [123, 124], the ability to understand other people's feeling [14], autism

Fig. 2. Co-localization of oxytocin and Kir6.2 in the SON. The fluorescence image of oxytocin (**a**), Kir 6.2 (**b**), nuclear staining of DAPI (**c**), and merged images (**d**). **e–h** Enlarged images of dotted squares in **a–d**, respectively. Scale bars = 50 μ m (**a–d**) and 10 μ m (**e–h**).

[17], and schizophrenia [18] in humans. In addition, obesity prevention is expected in future through the use of oxytocin treatment.

From a clinical point of view, nasal treatment of oxytocin is attracting attention [125], as it is noninvasive [126]. However, it remains unclear how oxytocin effects the CNS [127]. Whether intranasal oxytocin acts on the

CNS through peripheral oxytocin receptors (such as through vagal afferents) or directly on CNS oxytocin receptors is controversial. We have recently reported that nasal administration of oxytocin decreases food intake without affecting locomotor activity and peripheral glucose metabolism in mice [24]. Interestingly, IP injection of oxytocin increased c-fos expression in various brain

regions including the PVN, ARC, AP, NTS and DMNV, whereas the nasal administration of oxytocin increased c-fos in limited brain regions including the PVN, AP, and DMNV [24]. These results indicate the different anorexigenic pathways of oxytocin between the nasal and IP injection, and both treatments (IP and nasal) could stimulate the CNS.

Concerning the IP and subcutaneous injection of oxytocin, its anorexigenic effect may act through the vagal afferent pathway [128]. It is known that oxytocin receptors are distributed in the gastrointestinal tract and nodose ganglion [129], and anorexigenic effects and c-fos expression in the NTS induced by the IP oxytocin injection are partly blunted by vagotomy [128]. On the other hand, there is no c-fos expression in the NTS after nasal oxytocin treatment [24]. However, it is possible that nasal treatment of oxytocin acts through a peripheral mechanism. Further study is required to clarify this mechanism.

Our report, as well as others, has confirmed the subchronic effects of peripheral oxytocin treatment in the animal obesity model [23, 29, 34, 37, 39, 130, 131]. Although the pharmacokinetics of peripherally injected oxytocin remains unclear, oxytocin is reported to cross the blood-brain barrier [132] by 0.002% [133]. A recent study using rhesus monkeys also demonstrated that the d5-deuterated oxytocin is delivered into cerebrospinal fluid after intranasal and intravenous injection [134].

When oxytocin infusions were applied from a subcutaneously implanted osmotic mini-pump (1,600 µg/kg/day) for 10–14 days to HFD-induced obese mice, BW dramatically decreased (10% of initial BW) within 2 days, and food intake was decreased for 7 days after oxytocin treatment was initiated in both male and female mice [23, 39]. The oxytocin effect on BW was dose dependent [39], and reduced BW was maintained throughout the oxytocin treatment period. We found that mice showed increased energy expenditure with decreased respiratory quotient (RQ) during the light phase of the treatment period [23]. The RQ decrease by oxytocin treatment was also confirmed in rats, monkeys, and humans [25, 32, 34, 35, 135]. This reduction of BW by oxytocin may also be mediated by the induction of diet-induced thermogenesis [31, 47]. Other factors related to the metabolic disorder were also found to be affected by the oxytocin treatment. The lipid droplets concentrated in the hepatocyte induced by HFD intake were decreased, and the size of adipocytes in the mesenteric fat (common abdominal fat) was also decreased after oxytocin treatment [23]. In HFD-induced obese male and female mice (body fat mass: 36%), 10 days of oxytocin treatment decreased abdominal

and subcutaneous fat up to 15–20% without reducing muscle mass [39]. Similar effects of oxytocin on fat mass are found in other studies using HFD-induced obese mice/rats [25, 29, 31, 32] and genetically obese models such as db/db [131] and ob/ob mice [37]. This suggests that the cause of decreased BW induced by oxytocin treatment depends not only on the decreased food intake, but also on the stimulation of lipolysis. Oxytocin receptors are expressed in various tissues, including adipose tissue [38, 92], and previous articles reported that oxytocin increases lipolysis and fatty acid β -oxidation by acting directly on adipose tissue in rats [29, 136]. Blevins et al. [34] demonstrated that, in addition to rodents, subcutaneous oxytocin treatment reduces BW in diet-induced obesity primates (rhesus monkey) with decreased food intake, increased energy expenditure, and lipolysis. These reports emphasize the importance of oxytocin in affecting lipid metabolism in adipose tissue, as well as in food intake regulation.

Furthermore, chronic treatment of oxytocin is reported to also be effective in reducing BW in a leptin-resistant obese model such as db/db mice [128, 131], ob/ob mice [37], Koletsy rats [28], and Zucker fatty rats [137], indicating that the oxytocin effect is independent from the leptin effect. Because human obesity is based on leptin resistance, the fact that oxytocin is effective even in leptin-resistant animal models indicates a strong advantage of oxytocin as an anti-obesity agent.

A recent article reported that circulating oxytocin levels are decreased in obesity and type 2 diabetic patients [138, 139], HFD-induced obese mice [25], and Zucker fatty rats [38]. This decrease in plasma oxytocin levels may be a result of severe obesity since significant plasma oxytocin degradation was not confirmed in the studies using obese models fed HFD for 8–16 weeks [28, 39]. Moreover, the activity of oxytocinase (the main enzyme involved in oxytocin degradation) was elevated in the liver and adipose tissue in the obese Zucker fatty rats [38], whereas oxytocin receptor mRNA and/or protein levels were upregulated in the adipose tissue in the Zucker fatty rats [28] and hypothalamus in the HFD-induced obese mice [25, 140].

We reported a negative correlation between oxytocin's effect on BW and initial BW in HFD-induced obese mice. We found that the mice with higher initial BW showed more effective reduction in BW by oxytocin treatment in both males and females [39]. Since most anorexigenic oxytocin effects have been studied in males, the details of gender difference remain unclear. However, our recent study showed that there were no gender differences in the effect of oxytocin on BW reduction in mice [39]. This is

Fig. 3. The potential of oxytocin as an anti-obesity drug. Obesity increases plasma leptin levels and promotes leptin resistance. On the other hand, obesity decreases plasma oxytocin levels and increases oxytocin receptor expression in adipose tissue and the central nervous system. Thus, exogenous oxytocin decreases fat mass and food intake in severely obese animals/humans. Since oxytocin decreases fat mass and food intake in leptin-resistant animal models, it may improve leptin resistance. In addition, oxytocin protects insulin-secreting pancreatic β -cells in obese conditions, promotes insulin secretion, and improves fatty liver.

consistent with a report by Benelli et al. [141], which showed that there is no gender difference in oxytocin-induced inhibition of feeding in rats. This BW degradation was found to be the consequence of reduced energy intake and fat mass [23, 39]. On the other hand, the effect of oxytocin on BW degradation was smaller in lean mice or rats fed a normal chow diet [32, 37, 39]. This phenomenon was also observed in Zucker lean and fatty rats [137] and Koletsky rats [28]. This is consistent with studies in humans showing oxytocin's inhibitory effect on food intake being stronger in obese men than normal-weight men [36], and the degree of oxytocin's BW degradation being greater in severe obesity (BMI >35) than mild obesity (BMI <30) [142].

As described previously, the underlying mechanism of oxytocin's anti-obesity effects being more potent in severe obesity in rodents and humans (fat mass degradation and food intake inhibition) may be due to the increment of oxytocin receptor expression in the brain and adipose tissue. In other words, severe obesity may increase the sensitivity to oxytocin in the brain and adipose tissue.

Severe obesity generally promotes insulin and leptin resistance, followed by an increase in plasma leptin and insulin levels. Thus, promoted insulin and leptin resistance exacerbates diabetes and obesity. In contrast, in obese conditions, plasma oxytocin levels are decreased, and oxytocin receptors are increased without oxytocin resistance. This is a significant advantage of oxytocin as an anti-obesity treatment, since it may decrease fat mass and therefore induce reduction of plasma leptin levels, which

ultimately leads to improvement of leptin resistance. In addition, oxytocin is known to protect insulin-secreting β -cells from chronic high-glucose conditions in mice and humans [140, 143], promote insulin secretion [24], and improve glucose tolerance in mice [23, 24, 128] (Fig. 3).

One important consideration is that peripheral administration of oxytocin may result in the release of endogenous oxytocin within the CNS, [26] through the vagal afferent and ascending projection from the NTS [26, 28, 128] in rodents. Since nasal treatment of oxytocin failed to show any effect on Prader-Willi syndrome patients, whose PVN oxytocin neurons are almost completely lost [144], the anorexigenic effect of nasal oxytocin treatment may also come from the release of endogenous oxytocin within the hypothalamus. These reports indicate that the induction of endogenous oxytocin secretion triggered by exogenous oxytocin administration is the main mechanism for its anti-obesity effect. However, neither intranasal nor intravenous injection of oxytocin led to increased endogenous oxytocin in the CSF of monkeys [134]. Further studies of the anorexigenic effect of oxytocin and exogenous oxytocin-induced endogenous oxytocin are needed in the future. Because the effect of oxytocin on the CNS has a substantial contribution to food intake and BW regulation, the discovery of an agent that may induce central oxytocin release is highly anticipated.

Furthermore, all studies using intranasal oxytocin as an experimental intervention presented in this review have limitations and controversies. Further research on its clinical application is required.

Conclusion

Considering the global increase in the number of obese and metabolic syndrome patients, oxytocin treatment has various advantages and is a promising treatment for obesity and related metabolic disorders (Fig. 3). The advantage of oxytocin treatment is further enhanced by the following facts: (1) there is no oxytocin resistance in obese patients; (2) there are no severe adverse effects reported thus far; and (3) oxytocin has already been used in the clinical setting for decades for the promotion of delivery and milk ejection after birth.

Recently, Higashida et al. [145] reported that oxytocin is transmitted into blood in the intestinal tract via the mediation of receptors for glycation end products in adult

mice. Oral oxytocin supplementation could be another potential anti-obesity treatment.

Taking these facts into consideration, oxytocin may potentially be a safe and effective anti-obesity drug.

Disclosure Statement

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