Chapter

Modulation of Oxytocin Release by Internal Calcium Stores

Cristina Velázquez-Marrero and José R. Lemos

Abstract

This chapter elucidates the role of depolarization-induced oxytocin (OT) vs. arginine vasopressin (AVP) secretion in the absence of external calcium, and calcium release from ryanodine-sensitive internal stores as a significant physiological contributor to neuropeptide secretion from hypothalamic neurohypophysial system (HNS) terminals. This has important therapeutic implications, given that exogenous administration of OT to children with autism spectrum disorders (ASD) has shown some success in improving social behavior and lowering anxiety. However, this nonspecific treatment has side effects, including seizures, increased heart rate variability, and psychotic symptoms. Alternatively, facilitating the physiological neuronal release of OT but not AVP from the HNS via modulation of ryanodine vs. inositol triphosphate receptor (IP_3R) calcium stores would specifically facilitate central vs. peripheral OT release in ASD patients.

Keywords: oxytocin, vasopressin, neurohypophysis, ryanodine receptor, inositol trisphosphate receptor, autism spectrum disorders, ADP-ribosyl cyclase/CD38

1. Introduction

1.1 Hypothalamic neurohypophysial system (HNS)

The main mechanism for neuropeptide release from neurohypophysial terminals (NHT) acts via depolarization-secretion coupling. This refers to the relationship between neuronal depolarization and the subsequent release of hormones, specifically oxytocin (OT) and arginine vasopressin (AVP), from the posterior pituitary gland (**Figure 1**). This hypothalamic-neurohypophysial system (HNS) plays a critical role in regulating various physiological processes, including social behavior, reproduction, and water balance. Within the HNS, specialized neurons located in the hypothalamus synthesize and package OT and AVP into vesicles. These magnocellular neurons (MCNs) extend their axons through the pituitary stalk and terminate in the posterior pituitary (also known as (aka) neurohypophysis) gland, where the hormones are stored and released into the capillary bed for systemic delivery.

The classic understanding of neuropeptide release involves depolarization of the hypothalamic neurons which receive excitatory input which activates action potentials to their terminals. This depolarization leads to the opening of voltage-gated

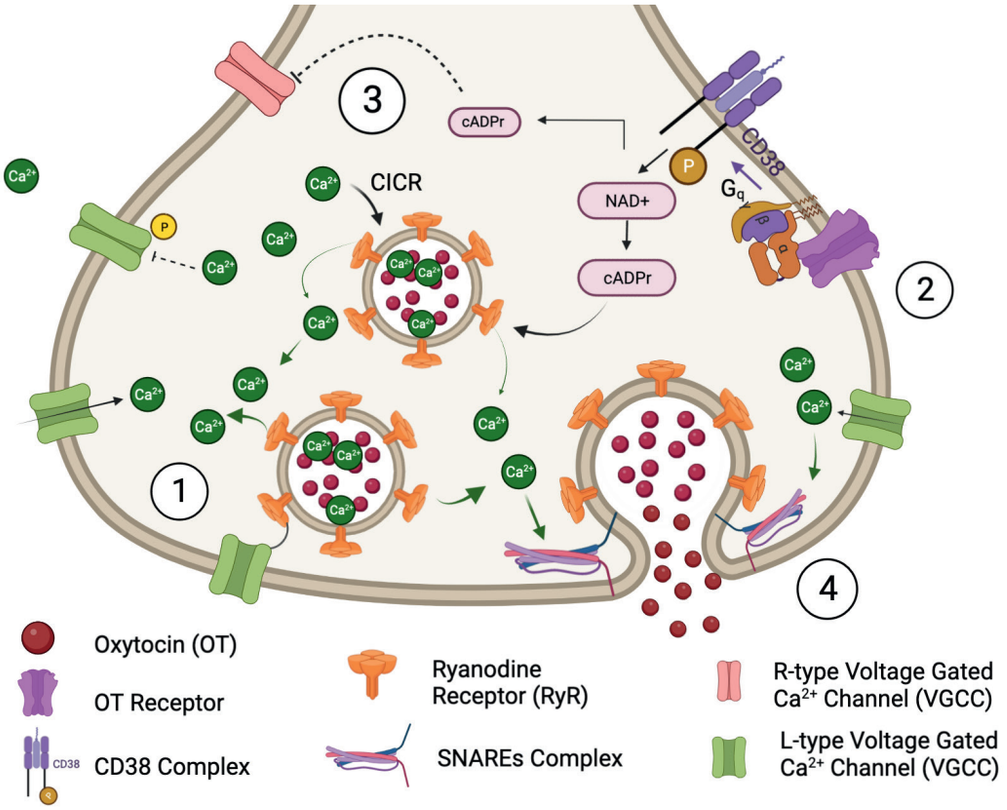

Figure 1.
Mechanisms of $[Ca^{2+}]_i$ affecting oxytocin (OT) release. 1) depolarization of L-type voltage-gated calcium channel (VGCC) mechanically opens the ryanodine receptor (RyR), which leads to the release of calcium from ryanodine-sensitive stores. 2) activation of the OT receptor (OTR) initiates cyclic adenosine diphosphate ribose (cADPr) signaling, presumably via Gq activation of ADP-ribosyl cyclase/CD38 (cluster of differentiation 38) complex catalyzing the conversion of NAD^+ into cADPr. Cyclic ADPr (cADPr) subsequently leads to the activation of ryanodine receptors (RyRs) on neurosecretory granules (NSGs) in the terminals. 3) inhibition of R-type VGCC. The release of diffusible second messenger Ca^{2+} from ryanodine-sensitive stores subsequently results in 4) OT release via SNARE complex activation. Notably, CD38, found only in OT terminals, facilitates hormonal secretion by releasing intraterminal Ca^{2+} and Ca^{2+}-induced Ca^{2+} release (CICR), other RyR types, and/or other stores [1]. This would traffic more vesicles to release sites, thus, facilitating release.

calcium channels on the terminal membrane, allowing calcium ions (Ca^{2+}) to enter (**Figure 1**). The influx of calcium into the neurons initiates a cascade of events that ultimately lead to the exocytosis of OT- and AVP-containing vesicles into the bloodstream. The increase in intracellular calcium concentration serves as a key signal for the fusion of vesicles with the neuronal membrane, resulting in the secretion of hormones into the extracellular space. The coupling between depolarization and hormone secretion involves several important steps. When calcium enters the neurons, it binds to proteins such as synaptotagmin, which triggers the fusion of hormone-containing vesicles with the plasma membrane. This fusion process allows the release of OT and AVP. Additionally, calcium influx also activates calcium-dependent enzymes, such as protein kinases, which can modulate the secretion process. These enzymes can regulate the activity of proteins involved in vesicle fusion and neurotransmitter release, thereby fine-tuning the coupling of depolarization and hormone secretion.

In this chapter, we highlight the coupling of depolarization and secretion in the HNS via the release of calcium (Ca^{2+}) from intraterminal Ca^{2+}, specifically voltage-and ryanodine-sensitive, stores as opposed to Ca^{2+} influx via voltage-gated calcium channels (VGCCs). In the absence of external Ca^{2+}, depolarization-induced secretion of oxytocin

(OT) and arginine vasopressin (AVP) can still occur, albeit with certain limitations. Recent evidence supports the hypothesis that Ca^{2+} from internal stores plays a crucial role in triggering the exocytosis of hormone-containing vesicles from nerve terminals. These alternative mechanisms can contribute to the release of OT and AVP in the absence of external calcium, thereby helping to shape both the amount and frequency of neuropeptide release differentially from OT vs. AVP terminals.

1.2 Calcium stores

These internal calcium stores within the HNS have originally referred to the endoplasmic reticulum (ER), which is a specialized organelle within cells involved in calcium storage and release. When an action potential reaches the neurohypophysial terminal, voltage-gated calcium channels on the plasma membrane open, allowing calcium ions (Ca^{2+}) to enter the terminal from the extracellular space. This calcium influx triggers the release of calcium from the internal stores, specifically the ER and the granules containing OT and AVP themselves, through a process known as calcium-induced calcium release (CICR). The released calcium from intraterminal stores amplifies the calcium signal and contributes to the overall calcium concentration in the cytoplasm. This increased calcium concentration is crucial for the fusion of neurotransmitter-containing vesicles with the plasma membrane, facilitating the release of oxytocin and arginine vasopressin into the bloodstream.

Within neurohypophysial terminals (NHT), a distinct intraterminal calcium store is characterized by local, voltage-dependent Ca^{2+} transients, known as syntillas [2, 3]. Syntillas are unaffected by the removal of extracellular Ca^{2+}, are mediated by ryanodine receptors (RyRs) within terminals, and are increased in frequency, in the absence of extracellular Ca^{2+}, by physiological levels of depolarization. The physiological role of these syntillas is under continued investigation, with recent important findings adding to the unique mosaic of regulation for OT and AVP release.

1.3 Social behavior and OT

Autism spectrum disorders (ASDs) are characterized by defects in reciprocal social interaction and communication and occur either sporadically or in a familial pattern [4–6]. The etiology of ASDs remains largely unknown and pharmacological treatments are needed. Oxytocin's role in social memory and behavior, communication, and emotional recognition has now been well established [7–10]. Although making inferences about central OT functioning from peripheral measurement is difficult, the data suggest that OT abnormalities may exist in autism and that a more direct investigation of central nervous system's (CNS's) OT function is warranted [11].

2. Intraterminal $[Ca^{2+}]i$ stores

When oxytocin neurons in the hypothalamus are activated, the depolarization of these neurons leads to the opening of voltage-gated calcium channels (VGCCs) followed by a Ca^{2+} influx through the plasma membrane. This influx of calcium ions triggers a series of events that result in the release of oxytocin both from the dendrites surrounding the cell bodies and the axonal terminals located in the neurohypophysis/posterior pituitary. However, internal calcium stores play a crucial role in maintaining

calcium homeostasis and regulating calcium signaling within the oxytocin HNS neurons. The ER is responsible for sequestering and storing calcium ions, which are released upon feedback stimulation in the cell bodies via activation of IP_3R (inositol triphosphate receptors). Less is known about the accumulation of Ca^{2+} in the neurohypophysial granules themselves, which, when released, play a significant role in modulating OT secretion from terminals. Research has shown that an increase in intracellular calcium concentration acts as a signal for the secretory granules containing oxytocin to undergo "priming" characterized by the mobilization of granules from the releasable pool to the readily releasable and subsequently, the immediately releasable pool.

Once granules are trafficked to the immediately releasable pool, calcium influx via VGCC binds to specific proteins, such as the synaptotagmin/SNARE complex, which facilitates the fusion of the secretory granules with the plasma membrane (**Figure 1**). This fusion allows the release of oxytocin into the extracellular space, where it can act on target tissues and receptors. Calcium stores within terminals are activated by three known mechanisms: calcium-induced calcium release (CICR), depolarization via a direct link of ryanodine receptor (RyR) and L-type VGCC, and ligand-mediated G-protein receptors leading to the activation of specific signaling cascades. CICR is an essential step in the amplification of the calcium signal eliciting further release from IP_3 and RyR-mediated internal stores while inactivating specific VGCC. Activation of the IP_3R and RyR via calcium or by second messengers, such as IP_3 or cyclic adenosine diphosphate ribose (cADPr), leads to the release of further calcium into the cytoplasm. Therefore, while calcium is key to triggering the process of secretion, the source of calcium necessary for fine-tuned depolarization-induced OT release comes from multiple sources that interact to optimize release.

2.1 Original findings

Previously, in experiments monitoring not only [Ca^{2+}] but also AVP release from HNS terminals in response to K^+ depolarization, it was shown that none of the classical modulators of intracellular Ca^{2+} release, such as caffeine, affected any of these measurements [12]. In contrast, several population (using dissociated NHTs) release experiments have been performed [1, 2, 13, 14], assessing the effects of both ryanodine and caffeine on basal neuropeptide release. Caffeine, a strong agonist of RyRs [15], induces an increase in neuropeptide release from these terminals, even in the absence of extracellular Ca^{2+} [1, 13, 14]. Ryanodine, at a concentration at which ryanodine is known to inactivate RyRs [15], has been shown to half-block this caffeine-induced release [14, 16]. This is similar to its effects on Ca^{2+} spark (syntilla) frequency in these NHTs [2]. Our results indicate that depolarization-induced neuropeptide secretion is present in the absence of external calcium, and calcium release from ryanodine-sensitive internal stores is a significant physiological contributor to neuropeptide secretion from HNS terminals.

2.2 Syntillas and calcium stores within NT

More conclusive evidence for Ca^{2+} stores in NHTs came from our studies demonstrating the presence of spontaneous focal Ca^{2+} transients in mouse neurohypophysial terminals [2, 17]. Since these Ca^{2+} syntillas were found in the absence of extracellular Ca^{2+}, they had to arise from intracellular stores. Additionally, the rate of syntillas is affected by agonists/antagonists of the RyR, a channel that is well known to control

the release of [Ca^{2+}]. Dihydropyridine receptors (DHPRs) function as voltage sensors within terminals of MCNs. The DHPRs appear to be linked to type-1 RyRs in a manner bearing similarities to the mechanism in skeletal muscle [17, 18]. Data from immunocytochemistry, Western blot analysis, and electrophysiology demonstrate the existence of type-1 RyRs linking neuronal activity, as signaled by depolarization of the plasma membrane, to a rise in [Ca^{2+}] in nerve terminals [12]. These results support previous findings [17] indicating the role of type-1 RyRs in response to depolarization and imply its possible physiological significance in depolarization secretion coupling (DSC) (**Figure 1**).

The cyclic ADP-ribose (cADPr) signaling pathway (**Figure 1**) initiates a signaling cascade leading to activation of the RyRs in vivo and subsequent release of [Ca^{2+}] from ryanodine-sensitive stores [19–21]. Interestingly, in NHTs, blocking cADPr signaling was shown to attenuate high [K^+] -induced rises in [Ca^{2+}] but only inhibited OT release from isolated terminals [22, 23]. This strongly suggests that the cADPr pathway is present in OT terminals [1] and linked to neuropeptide release (**Figure 1**). Cyclic ADP ribose hydrolase (CD38) is a catalyst for the formation of cADPr and nicotinic acid adenine dinucleotide phosphate (NAADP) by ADP-ribosyl cyclase from nicotinamide adenine dinucleotide (NAD) and NAD phosphate [24]. Both are known to release Ca^{2+} from intracellular ryanodine-sensitive pools as part of a second messenger-signaling pathway.

2.3 NH terminals compared to HNS cells

Facilitation of neuropeptide release from magnocellular neuron (MCN) somatodendrites can be induced by increased [Ca^{2+}] [25]. This facilitation appears to be due to the trafficking of neurosecretory granules (NSGs) toward the cell surface [26]. Such facilitation increases the somatodendritic secretory response to signals, such as OT, that mobilize intracellular Ca^{2+} (via IP_3 receptors) in ER [25]. AVP and OT normally elicit little somatodendritic secretion, but after facilitation, each neuropeptide elicits enhanced somatodendritic secretion from their respective MCNs [25, 27]. This includes the response to somatodendritic action potentials, which typically do not result in local release, but can alter facilitation by AVP or OT.

3. AVP vs. OT in terminals and HNS cell bodies

3.1 Differences in VGCC

There are subtle differences between the two types of nerve terminals concerning the distribution of the distinct types of VGCCs. The AVP terminals show the L-, N-, and P/Q types of VGCCs, while the OT terminals have the L-, N-, and R types [13]. In contrast, the [Ca^{2+}] responses induced by high K^+ (50 mM) involve all Ca^{2+}-channel subtypes in both AVP and OT somata [28]. Electrophysiological studies on the Ca^{2+} channels in the NH nerve terminals have shown that specific types (Q vs. R) of Ca^{2+} channels are uniquely important in AVP versus OT secretion (**Figure 1**).

3.2 Primed release of OT

Mechanisms involved in neurosecretion from the nerve terminals of the AVP- and OT-containing neurons of the HNS are different between this compartment and their

somatodendritic region. OT has positive and AVP has various (positive, negative, or no effect) feedback on their release from somata and dendrites, but not from NHTs. Instead, voltage is the primary regulator in terminals. Both compartments utilize intracellular release of Ca^{2+} to regulate the release of OT but not AVP, which appears regulated primarily by Ca^{2+} entry through VGCCs. However, MCN dendrites utilize IP_3Rs in ER, while NHTs utilize RyRs in NSGs to regulate OT release. Nevertheless, the trafficking of NSGs is the main mechanism for the facilitation of release in both compartments [13, 25, 29]. SNARE-mediated exocytosis is also different in somatodendritic versus NHTs. Thus, these HNS compartments are different in their regulatory mechanisms for neurosecretion. Since OT and AVP are also found in CNS axons and terminals, future experiments are required to determine if differences in the central release (see **Figure 2**) of these peptides compared to the NHTs or somatodendritic regions will have subsequent consequences on their behavioral effects.

3.3 Physiological relevance (bursting, behavior, etc.)

Magnocellular neurons in the supraoptic (SON) and paraventricular (PVN) nuclei (see **Figure 2**) exhibit bursting patterns leading to secretion of oxytocin in a characteristic episodic or pulsatile manner rather than in a continuous or steady stream [13, 32, 33]. The bursting pattern is regulated by various physiological and environmental factors, such as sensory stimuli, including touch, suckling, and sexual activity. These stimuli activate specific brain regions, leading to increased activity in oxytocin

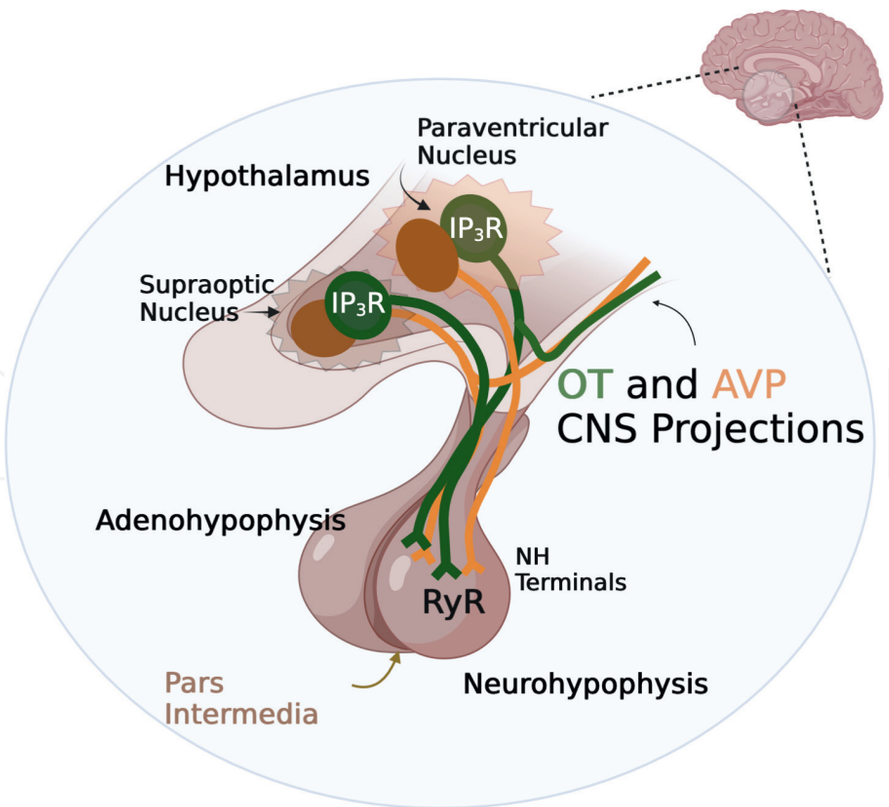

Figure 2.
The diagram of hypothalamic neurohypophysial system (HNS) showing cell bodies in hypothalamic supraoptic (SON) and paraventricular nuclei (PVN) projecting to terminals in neurohypophysis and in central nervous system (CNS) [22, 30, 31]. The priming of release takes place via IP_3R (inositol triphosphate (IP_3) receptors) in oxytocin (OT) cell bodies but via RyR (ryanodine receptors) in OT terminals.

neurons and subsequent bursts of oxytocin release [34]. The bursting pattern of oxytocin secretion is particularly relevant during reproductive processes, including childbirth, breastfeeding, and bonding between individuals [35–37].

The episodic nature of oxytocin release allows for precise and selective activation of oxytocin receptors, ensuring proper physiological responses. The precision of these responses highlights the importance of linking OT release with appropriate physiological and environmental signaling. The pattern of the electrical activity of OT neurons exhibits a sustained outwardly rectifying potential, as well as a consequent depolarizing rebound potential, not found in AVP neurons [38]. OT neurons further exhibit specific voltage-gated calcium channels, R-type, which are modulated by distinct pathways not associated with AVP neurons [39, 40]. Furthermore, in OT neurons, but not in AVP, the activity and modulation of release are highly dependent on the presence of the second messenger, phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) [41], which permissively gates N-type channels that contribute to the Ca^{2+} influx during spike trains [42] and mediates positive feedback via activation of OT receptors (OTRs) (**Figure 2**) within the dendritic arborization of cell bodies in SON and PVN [43, 44].

The oxytocin receptor and inositol trisphosphate (IP_3) are interconnected in a signaling pathway that mediates the effects of oxytocin on cellular responses. When oxytocin binds to its receptor, it leads to the activation of a G protein associated with the receptor. This activation causes the G protein to exchange GDP (guanosine diphosphate) for GTP (guanosine triphosphate), leading to the dissociation of the G protein into its α, β, and γ subunits [45]. The α subunit of the G protein then activates phospholipase C (PLC) activating a cascade of intracellular events, including the generation of IP_3. The IP_3 messenger acts as a diffusible molecule that binds to IP_3 receptors (IP_3Rs) located on the endoplasmic reticulum (ER) membrane in OT neuronal cell bodies releasing calcium ions (Ca^{2+}) from the ER stores into the cytoplasm. This increase in intracellular calcium concentration triggers various cellular responses, including modulation of synaptic transmission in the brain. Notably, so far, the presence of endoplasmic reticulum and subsequent IP_3 signaling has not been observed in neurohypophysial terminals. It is worth noting that the interactions between oxytocin receptor signaling and IP_3 are just one aspect of the broader mechanisms underlying intercellular calcium stores and oxytocin's physiological effects. Having shown that DSC occurs in the absence of extracellular Ca^{2+} and is independent of VGCCs lends support to the general premise of the Ca^{2+}-voltage hypothesis of Parnas and colleagues [46]. Whether this process modulates or plays a key role in the initiation and/or the termination of physiological release during a burst of action potentials in the HNS, however, remains to be proven.

4. Potential therapeutic approaches for treating autism

4.1 Current understanding of the role of OT

Autism spectrum disorder (ASD) encompasses multiple complex and behaviorally defined disorders characterized by impairments in social interaction, memory, communication and language, repetitive behaviors, as well as in the range of interests and activities shown by autistic patients [47–52]. Genetic [53] and environmental factors [49, 53–57] have been identified in the development of ASD. For example, the use of the anticonvulsant drug valproate during pregnancy increases the risk for the

development of autism in children. Elevated OT levels within various brain networks have been associated with enhanced social cognition and emotional recognition [9]. A strong OT receptor labeling pattern has been observed in the medial prefrontal cortex, ventral-tegmental area, limbic and prelimbic areas [58], which are brain areas associated with social bonding, cognition, emotion, and reward. Conversely, low OT levels and a downregulated OTR gene are found in people diagnosed with various forms of ASD [59–61].

Conversely, Williams syndrome is characterized by abnormal social behavior (characterized by reduced social inhibition, an increased affinity toward attending to faces, and reduced sensitivity to fear-related social stimuli) and increased oxytocin functioning [62]. Furthermore, an empirical research study [63] demonstrated that the OT receptor is overexpressed in Williams syndrome.

4.2 Treatments currently in use and their prognosis

Importantly, OT and OT receptor knockout (KO) mice exhibit social impairments similar to those associated with ASD [58], and administration of OT to these KO mice can "rescue" them from ASD-like behaviors [8], showing that such therapy is possible. Similarly, a defective OT release process has been found to mediate the drop in OT levels in another autistic animal model, CD38 KOs [22, 23]. But, so far, treatments (as with OT injections or sprays) have focused on systemic OT levels. For example, Higashida et al. [22] have used agents (all-trans-retinoic acid) that target CD38 in the neurohypophysis. We hypothesize that instead treatments should target brain regions such as magnocellular somata (**Figure 2**) and their central nervous system (CNS) projections (e.g., medial prefrontal cortex, ventral-tegmental area, limbic and prelimbic areas) [58]. This could be done by agents that affect IP_3Rs instead of RyRs (**Figures 1** and **2**).

4.3 Alternatives mediated by targeting central OT release

Release from the hypothalamic cell bodies (see **Figure 2**) is indicative of release into the CNS [9]. Such release is also thought to act hormonally to affect surrounding CNS areas [64]. Activation of multiple types of endogenous (ryanodine, cADPr, and opioid) receptors in OT-releasing rat nerve endings can specifically regulate the in vitro release of this neuropeptide [13, 65, 66]. These receptor types play a role in modulating intracellular Ca^{2+} levels, either through a Ca^{2+}-induced Ca^{2+} release (CICR)-dependent mechanism (**Figure 2**) or by regulating Ca^{2+} inflow from the extracellular environment. These receptor types are easily targeted by drugs that can cross the blood–brain barrier [67] and will facilitate novel pharmacological studies with therapeutic relevance for ASD. Furthermore, our collaborators [25, 68, 69] have already shown that the hypothalamic release of OT specifically can be potentiated by other agents that affect $[Ca^{2+}]$, e.g., IP_3 (but not ryanodine). Thus, it should be possible to specifically potentiate/inhibit endogenous levels of central OT in response to physiological stimulations. That is, IP_3R agonists would increase central, local release and electrical activity in OT cell bodies while RyR agonists would increase release from OT terminals both in NH and in central projections.

4.4 Future perspectives

CD38 knockouts (KOs) do not release OT and, consequently, have social behavioral deficits [23]. Similarly, VPA is associated with a high incidence of ASD

phenotypes in children [55, 70] and it acts similarly in rats. These KO mice and valproic acid (VPA)-injected rats are extremely useful experimental models for studying potential causes of ASD [47] and exploring new therapeutic approaches.

To target OT vs. AVP release in the relevant areas of the brain [58] for behavioral defects associated with ASD, it will be important to determine if there are differences between central and peripheral OT release using the HNS (**Figure 2**) in a compartmentalized chamber [71] or in fluorescently labeled rats [72]. Next, we have to determine central OT vs. AVP levels in the CD38 KO and VPA autistic animal models. Finally, can known regulators of OT vs. AVP release "correct" OT levels in such models (see **Figure 2**)? These receptor types are easily targeted by drugs that can cross the blood–brain barrier [67] and will facilitate novel studies with therapeutic relevance. Some, or perhaps all, of these agents, will increase OT vs. AVP release, in general, but it should be determined if any (e.g., IP_3, cADPr, mu opioid receptor (MOR) agonists) will specifically increase the central release of OT. Thus, it should make them potential therapeutic agents to test on ASD patients. This approach has been recently validated since a small molecule vasopressin V1a (V1a)-specific antagonist is sufficient to rescue normal behavior in prenatally exposed VPA rats [67]. Furthermore, these findings could eventually lead to the ability to perform clinical trials to assess the efficacy of such agents on ASD patients.

5. Conclusions

We have demonstrated that intracellular signaling pathways in particular CNS areas (see **Figure 2**) can be upregulated. Thus, it should facilitate physiologically stimulated release of OT specifically, rather than nonspecific increases (as with OT injections or sprays). Therefore, facilitating physiological neuronal release of OT but not AVP from the HNS via modulation of IP_3 vs. ryanodine calcium stores (see **Figure 2**) would specifically facilitate central vs. peripheral OT release in ASD patients. Therapeutically, this would ameliorate social behavioral deficits in such patients.

Acknowledgements

We want to thank our many colleagues who helped with this research and the funding sources NIH 1R01AA027808-01A1 (Velázquez) and NIH R01NS29470 (Lemos).

Conflict of interest

The authors declare no conflict of interest.

Appendices and Nomenclature

OT	Oxytocin
AVP	Arginine vasopressin
RyR	Ryanodine receptor
IP3R	Inositol trisphosphate receptor
ASD	Autism spectrum disorder

Gq	guanine nucleotide-binding protein (G protein, q polypeptide)
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptors
cADPr	cyclic adenosine diphosphate ribose
CD38	cyclic ADP ribose hydrolase
SON	Supraoptic nucleus
PVN	Paraventricular nucleus
CNS	Central nervous system
NH	Neurohypophysis
HNS	Hypothalamic neurohypophysial system
VGCC	Voltage-gated calcium channel
NSG	Neurosecretory granule
CICR	Calcium-induced calcium release
NHT	Neurohypophysial terminal
ER	Endoplasmic reticulum

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