Commentary

Oxytocin: much more than childbirth and milk letdown

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This commentary deals with the new observations that dendritic cell (DC) oxytocin receptors play a role in the inflammatory response generated in murine animal models of colitis. The overview provides a context of the discovery of oxytocin (OT), its chemical synthesis and the cell biology of its neurohypophysial synthesis and secretion. This perspective provides insight and raises questions to be answered related to the impact of OT in the gastrointestinal tract and to further the exploration of OT as a potentially locally synthesised regulator of intestinal inflammatory pathophysiology.

Overview

As described over 125 years ago, post-pituitary extracts were observed to cause uterine contraction, milk letdown, blood pressure elevation and retention of fluid. These distinct actions are now known to be due to the peptide hormones, oxytocin (OT) and vasopressin. A main focus since that time has been on the actions of these peptides in the central nervous system (CNS: for overview, see reference [1]). More recently, it has been observed that OT and its receptor are expressed widely not only in the CNS, but also in peripheral tissues like the thymus, corpus luteum and bone. A recent publication has found that the innate immune system is also regulated by OT [2]. Specifically, in the setting of a model of murine colitis, dendritic cell (DC) OT receptors have been found to play a key role in reducing the inflammatory response. These findings represent the development of a new paradigm for the action of OT. Thus, this polypeptide can be considered not only as the childbirth-milk letdown hormone but as a pleiotropic peptide, involved in many biological processes including immune function. It is the objective of this commentary to place these observations of the function of OT receptors in DCs in the context of the discovery of OT long ago and in the perspective of the current understanding of OT synthesis, storage, and secretion to act via its receptor expressed throughout the body.

The current findings of Dou and colleagues can be placed in the context of work conducted for over a century leading to the unquestioned role for OT in the setting of childbirth [2]. For that process, OT acts on uterine and breast myometrial/myoepithelial smooth muscle. Moreover, it is now known that the G-protein-coupled receptors for OT are widely expressed not only in the CNS but also in many peripheral tissues in addition to the uterus and mammary gland. Tissues ranging from adipocytes to taste buds to bone as well as gastrointestinal enteric neurons have all been found to express receptors for OT (summarised in reference [1]). Moreover, studies of the impact of OT now range from its action on peripheral tissues to its effect on behaviour and autism-spectrum disorder (see reference [1] for a perspective). Of particular note, work stemming from the Gershon lab pointed to an action of OT on the gastrointestinal tract to regulate enteric neuronal activity, mucosal homeostasis, intestinal permeability, and intestinal inflammation [3–5]. The work by Dou and colleagues extends those observations related to intestinal inflammation [2]. It is documented that OT receptors in immune system DCs play an important role in preventing DC cell maturation, thereby diminishing the inflammatory response in a mouse experimental colitis model [2]. This new publication adds DCs to the many targets known for OT in the periphery and in the CNS.
objective of this commentary to relate the observations of Dou and colleagues [2] to OT’s discovery over a century ago and to the biochemical mechanisms of OT synthesis, storage, and secretion from both neuronal and non-neuronal cells to activate its G-protein-coupled receptor. This information can amplify the findings of Dou and colleagues and provide an extended context to conduct new studies [2].

**OT: the first peptide hormone to be synthesised**

OT can take pride of place amongst peptide hormones as the first one to be sequenced and synthesised, resulting in a 1955 Nobel Prize [6,7]. As mentioned above, the main focus for the peripheral actions of OT until recently were on its impact on uterine contraction and milk letdown. In the neurohypophysis and CNS, the focus has been on its function as a neurotransmitter and its distribution in discrete regions of the brain, as reviewed in depth by Jurek and Neuman [1] and by Sofroniow and colleagues [8]. It is only relatively recently, based on the work from the Gershon laboratory [3,4] and as emphasised by the recent work published by Dou and colleagues [2], that attention has turned to the biosynthesis of OT in the intestinal tract. Thus, the recent work of Dou and colleagues to document the impact on inflammatory DC function [2] prompts the following questions: 1. Where does the OT come from to affect the DCs?; 2. What mechanisms regulate the expression of OT and its receptor in that location?; 3. What signal pathways are triggered by OT in the DCs to affect their function? To deal with these questions, it is necessary to review the original studies dealing with the source, storage, and secretion of OT and vasopressin from the posterior pituitary gland. This perspective will enable an in-depth look at the potential impact of OT biosynthesis and action in the periphery.

**OT biosynthesis, storage, and secretion**

The first pharmacological studies of extracts of the posterior pituitary used bioassays done in vivo and in vitro that relied on vascular and smooth muscle tissue responses. This approach was in keeping with pharmacological studies of extracts of the adrenal medulla, with which the posterior pituitary extracts were compared. Thus, from the work of Oliver and Schäfer [9] and Dale [10], neurohypophyseal extracts were found to contain factors that can regulate vascular and uterine smooth muscle activity. Shortly thereafter, the ability of post-pituitary extracts to cause milk letdown was discovered [11,12]. It was not until approximately two decades later that it was realised that the biological activities of the pituitary extract are due to two distinct ‘active principles’ estimated to have molecular masses of approximately 600–1000, that account for the stimulation of uterine contractions on the one hand (oxytocic activity) and on the other, the elevation of blood pressure (pressor action) and antidiuresis [13,14]. A key procedure leading to the identification of OT and vasopressin involved ‘salting out’ the biological activity as a precipitate from aqueous pituitary extracts using ammonium sulphate or sodium chloride (1.4 M) [14,15]. Further work discovered that, like the storage of adrenaline in the adrenal gland, OT and vasopressin are present in the neurohypophysis in distinct neurosecretory granules [16]. By the mid-1960s, it was generally accepted that the main physiological roles for OT and arginine vasopressin (AVP) were, respectively, up-regulation of uterine contraction for childbirth along with stimulation of milk letdown (OT); and antidiuresis (AVP). What was not clear at the time was how the hormones were synthesised and secreted. Further, it was presumed at the time that peptide biosynthesis and secretion took place in the posterior pituitary gland and CNS.

It was controversial as to whether the active peptides were part of a ‘unitary protein’ from which they were liberated by proteolysis from the protein isolated by Van Dyke and colleagues [15], as proposed by Abel and colleagues [17,18]); or were present as small molecular weight peptides (Pitocin and Pitressin), as found by Kamm and colleagues [14]. It turned out that both claims were essentially correct. It was not known at the time that like many peptide hormones, including insulin, OT, and vasopressin are synthesised as pro-hormone precursors from three gene exons situated on human chromosome 20 [19]. The hormone precursor transcripts include the sequences of neurophysin: OT-neurophysin-I and vasopressin-neurophysin-II. Fortuitously, the complex proteolytic processing and C-terminal amidation reactions that generate active OT and vasopressin from the OT-neurophysin-I and vasopressin-neurophysin-II precursors still enables a non-covalent interaction between the peptides and the neurophysins. It is that hormone–neurophysin complex that is salted out (1.4 M NaCl) from the pituitary extracts. Using the salt precipitate procedure described by Van Dyke and colleagues [15] as a starting point to isolate the neurophysin–peptide complex, it was possible to dissociate OT and vasopressin from the neurophysins under mild acid conditions, and then isolate the two distinct neurophysins. The neurophysins were then crystallised as complexes with OT and vasopressin [20]. Subsequent to these studies it was possible to establish that the neurophysins are proteolytically processed from the neurophysin–hormone precursor within the secretory granules as they migrate from the neuronal cells of origin in the supraoptic and paraventricular nerve cell bodies to the nerve terminals
in the neurohypophysis [21]. The ‘take-home’ message from these studies is that the neurophysins are biosynthesised and co-secreted along with the hormones. Thus, the synthesis/secrecion and identification of neurophysin-I in all tissues can be used as a surrogate marker for the selective secretion of OT in a cellular microenvironment. This information is directly relevant to the impact of OT on gastrointestinal DCs, since the source of OT to mediate the anti-inflammatory action on the DCs in the GI tract was not elaborated upon by Dou and colleagues [2].

As already mentioned, the initial focus on the locale of biosynthesis of OT was on site in the CNS, where a widespread distribution of oxytocinergic, neurophysin-expressing nerves has been documented (well summarised by Jurek and Neumann [1] and by Sofroniew and colleagues [8]). The mechanism of target cell activation by OT has classically been assumed to be via either a direct neurotransmitter release-postsynaptic target cell activation mechanism or a neurohumoral transport of OT from the neurohypophysis to a peripheral target. The neurohumoral action of OT on the mammary gland to stimulate milk letdown [10, 11] was elegantly established via use of a transplanted mammary gland devoid of innervation. The denervated organ that received perfusion upon stimulation of the non-transplanted innervated mammary gland was found to respond by increasing milk secretion presumably due to the central release of OT from the pituitary [22]. A question to ask about the observations of Dou and colleagues is: Could DC-regulating OT come from the CNS-secreted peptide? Whether the peripheral concentrations of OT reached during suckling and lactation might be sufficient to affect DCs is an open question. It can be pointed out that although measurements of peripheral OT levels are subject to controversy [23], the current estimates of plasma concentrations of OT are in the 5–70 pM range. However, the $K_i$ for OT binding to its receptor is much higher, in the range of 790 nM. Our own estimate of the EC$_{50}$ values for OT stimulation of calcium signalling in cultured uterus-derived smooth muscle cells obtained from non-pregnant and pregnant human tissue is 13 and 4 nM, respectively, indicating an increased sensitivity of pregnant vs non-pregnant cells [24]. It would therefore appear unlikely that pituitary-secreted OT levels would be sufficient to activate DC OT receptors in the GI tract.

Alternatively, a direct action of OT in a spinal microenvironment, presumed to be due to its release in the spinal cord via axons originating from neurons in the CNS, can be inferred from the effects of a spinal intrathecal administration of OT [25]. That local spinal action of OT that affects blood pressure and heart rate is in keeping with a direct autonomic effect that does not involve pituitary-secreted hormone. The source of OT that can affect DCs in a colitis setting may be either or neither of these neurohumoral or autonomic innervation possibilities that depend on the biosynthesis of OT in CNS neurons, as outlined by the scheme in Figure 1. The figure shows that apart from the neurohumoral and autonomic modes of action of OT, it is now known that OT biosynthesis occurs in a number of peripheral tissues ranging from skin, heart, and bone [26] to the testis, placenta and GI tract. Of note in this regard, oxytocic milk letdown activity had been detected in extracts of the corpus luteum and thymus in the early 1900s [12, 27]. Both of these tissues are now known to contain OT. It is therefore highly likely that the localised synthesis of OT in the intestinal tissue itself, presumably in neuronal cells as pointed out by the work of Gershon and colleagues [3], can account for the local regulation of DC function in the setting of colitis [2].

More issues to consider

The biosynthesis of OT in the periphery and its secretion to act via a paracrine mechanism poses a number of questions related to the ‘classical mechanisms’ described above for the nerve cell synthesis, storage, and secretion of OT in the CNS: 1. As discussed above, is it at all possible that a humoral mechanism can account for the OT receptor responses in the GI tract and elsewhere in peripheral tissues, like bone? 2. What are the stimuli and mechanisms that drive transcription/translation of OT-neurophysin-I in the periphery? 3. What are the transcriptional and secretory mechanisms that produce OT in the GI tract and periphery, compared with its biosynthesis and secretion from neuronal cells of the CNS? These questions merit evaluation, since the mechanisms for the cell-type-specific expression of OT and vasopressin remain to be fully clarified [28]. 4. Can neurophysin-I synthesis and secretion be used as a surrogate for OT synthesis and secretion in the periphery? 5. What signalling mechanisms are activated by OT in targets like DCs, where Gq calcium-regulated myosin contractile mechanisms are not involved? Work with intestine-derived Caco-2BB cells indicate that OT is a regulator of the PI3K/Akt/mTORC1 pathway [29]. Whether this signal pathway is involved in the DC response to OT merits evaluation. Answers to these questions are beyond the scope of this commentary and are thus left with the reader to consider.

Summing up

To sum up, the recent observation that OT receptors can regulate DC function in the setting of inflammation [2] prompts a renewed perspective on the expanded roles that this long-known peptide hormone and its receptor can play in species ranging from mammals to invertebrates [30]. The newly described impact of OT on DC function
Figure 1. Pathways for the action of OT on its target tissues

The scheme shows the many distinct pathways (arrows) whereby OT can affect its target tissues. The major sources of OT are thought to be the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus and possibly other neuronal bodies in the CNS or spinal cord (e.g. dorsal route ganglion cells (DRGs) or other). Other sources can be in the parenchymal cells of tissues like bone or in the neurons of the enteric nervous system (ENS). Upon secretion from the posterior pituitary gland, OT can act via a neurohumoral mechanism. Alternatively, a spinal cord-localised signalling by OT can affect peripheral tissues via the autonomic nervous system (ANS). Finally, OT generated on-site, e.g. by neurons in the enteric nervous system or by parenchymal cells in tissues like bone can generate OT to act in an autocrine or paracrine way.

may have a parallel in immune cell maturation in the thymus, which has long ago also been found to express this peptide. Thus, the new findings [2] provide a stimulus and open a wide door for continuing the exploration of the pathophysiology of the posterior pituitary hormone, OT, discovered over 125 years ago.

Data Availability
There are no new original data in this commentary, which refer to published manuscripts in the literature.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.
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Abbreviations
CNS, central nervous system; DC, dendritic cell; OT, oxytocin.

References
3 Welch, M.G., Tamir, H., Gross, K.J., Chen, J., Anwar, M. and Gershon, M.D. (2009) Expression and developmental regulation of oxytocin (OT) and oxytocin receptors (OTR) in the enteric nervous system (ENS) and intestinal epithelium. J. Comp. Neurol. 512, 256–270,
https://doi.org/10.1002/cne.21872
21 Kopp, M. (2020) Bachelor of Health Sciences Honours Thesis. Effect of Proteinase Activated Receptor Activation on G-Protein Coupled Signalling in Uterine Smooth Muscle Cells, University of Calgary Cumming School of Medicine BHSc programme