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Review

Biochemical and endocrine aspects of oxytocin production by the mammalian corpus luteum

Fredrick Stormshak*

Address: Departments of Biochemistry/Biophysics and Animal Sciences, Oregon State University, Corvallis, OR, USA

Email: Fredrick Stormshak* - fred.stormshak@orst.edu

* Corresponding author

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Abstract

A review of the current state of knowledge of oxytocin production by the preovulatory follicle and corpus luteum is presented. Corpora lutea of a number of mammalian species have been found to synthesize oxytocin. However, the synthesis and secretion of this nanopeptide by the corpus luteum of the ruminant has been most extensively studied because of the potential role of this peptide in facilitating luteal regression. While much information exists relative to various biochemical and endocrine factors that impact on oxytocin gene expression, this aspect about luteal synthesis of this peptide hormone remains enigmatic. Prostaglandin F-2α (PGF-2α) has been shown to be a primary endogenous hormone responsible for triggering luteal secretion of oxytocin. Details are provided regarding the PGF-2α-induced intracellular signal transduction pathway that ultimately results in exocytosis of luteal oxytocin. Evidence is also presented for potential autocrine/paracrine actions of oxytocin in regulating progesterone production by luteal and granulosa cells. Concluding remarks highlight aspects about luteal oxytocin production that require further research.

Ovarian Oxytocin

Ott and Scott [1] are believed to be the first investigators to unknowingly demonstrate that the corpus luteum is a rich source of oxytocin. These researchers reported that an aqueous extract of the corpus luteum when injected into a goat, stimulated immediate milk flow.

Forty-three years elapsed before Du Vigneaud et al. [2] reported the amino acid sequence of oxytocin and nearly another 30 years passed before Wathes and Swann [3] demonstrated by radioimmunoassay and chromatography that the ovine and human corpus luteum contained oxytocin. In subsequent years, presence of luteal oxytocin was reported for the cow [4], cynomolgus monkey [5], goat [6], baboon [7] and sow [8]. Although corpora lutea of the sow have been shown to contain oxytocin it is the uterus of this species that produces the majority of oxytocin of reproductive tract origin [9,10]. Similarly, in the rat [11] and apparently the mare [12] the uterus, and not the ovary, is the primary source of oxytocin.

Although oxytocin has been found to be synthesized by the corpus luteum of a number of mammalian species it is the presence of this nanopeptide in the corpora lutea of ruminants that has received considerable study. Focused interest on luteal oxytocin in these animals for the most part reflects research conducted to elucidate its role in processes of luteal regression. Therefore, the remaining aspects of this review on luteal oxytocin will encompass primarily research conducted on the ruminant.

To appreciate the unique facets of luteal oxytocin biosynthesis it is essential to recognize that initial expression of the oxytocin gene begins in the preovulatory follicle. Evidence for the existence of oxytocin in the preovulatory follicles of the cow and ewe was first reported by Wathes et al. [13,14]. Subsequently, Voss and Fortune [15] measured *in vitro* oxytocin production by granulosa cells isolated from bovine preovulatory follicles during the early, mid- and late follicular phase. Granulosa cells isolated from the late stage preovulatory follicle, approximately 20 h after the onset of estrus, were found to produce maximal quantities of oxytocin as compared to granulosa cells recovered during the early and mid-follicular phase. These authors suggested that exposure of the granulosa cells to the surges of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) may have either directly or indirectly stimulated synthesis of oxytocin. And indeed, when granulosa cells of preovulatory follicles were exposed to LH or FSH *in vitro*, a marked increase in oxytocin secretion occurred during the culture period [15,16]. Similarly, incubation of granulosa cells isolated from an early preovulatory follicle with LH for 3 days induced transcription of the gene encoding oxytocin-neurophysin-I [17]. Based upon the results of these studies, one might conclude that cells of the developing corpus luteum would respond to enhanced systemic concentrations of LH with an increase in oxytocin production. However, as described below, this does not occur.

It should be noted that there is an apparent asynchrony that characterizes the relationship between concentrations of oxytocin mRNA and the nanopeptide in luteal cells whereas the accumulation of mRNA and synthesis of oxytocin in granulosa cells is positively correlated. In the bovine and ovine corpus luteum it is the large luteal cells, believed to be derived from granulosa cells [18] that contain the secretory granules of oxytocin [19,20]. In cows and ewes, the luteal concentration of oxytocin-neurophysin-I mRNA increases early after luteinization of granulosa cells to attain maximal levels by approximately day 3 of the estrous cycle, after which concentrations gradually decrease to low levels for the duration of the cycle [21,22]. Presence of an embryo does not appear to alter the steady decline in luteal concentration of oxytocin mRNA that characteristically occurs in the cow during the estrous cycle [23]. Luteal concentrations of oxytocin in cows and ewes are not highly correlated with the oxytocin mRNA, and actually do not reach maximal concentrations until near midcycle [24–26]. Thereafter, luteal oxytocin, like its mRNA, declines to lowest levels during proestrus of the ensuing cycle. The luteal tissue content of oxytocin is reduced markedly by the time of luteolysis [27]. The lag period that characterizes the difference in luteal concentrations of oxytocin mRNA and the peptide during the estrous cycle may be attributed to the slow increase in activity of peptidyl glycine α-amidating mono-oxygenase, the terminal enzyme in the pathway of oxytocin synthesis [28]. The increase in activity of this enzyme coincides with the gradual increase in luteal concentrations of oxytocin in the ewe, becoming maximal on day 8 of the estrous cycle. Ascorbic acid is a requisite cofactor of this enzyme but endogenous levels do not limit the activity of this enzyme. However, Luck and Jungclas [29] showed that ascorbate could stimulate oxytocin secretion by bovine luteinized granulosa cells *in vitro*.

Regulation of Oxytocin Gene Expression

One of the more perplexing aspects about luteal oxytocin pertains to identification of the transcription factors essential for gene expression. As described above, LH stimulates oxytocin gene transcription and mRNA translation in granulosa cells. Although small but not large bovine luteal cells are endowed with LH receptors it was anticipated that enhanced secretion of LH during the formative stages of luteal development might indirectly affect luteal concentrations of oxytocin. However, exposure of the developing bovine corpus luteum to periodic pulses of LH induced by administration of gonadotropin-releasing hormone (GnRH) from days 4 to 6 of the estrous cycle attenuated luteal concentrations of oxytocin (E.M. Jaeger, unpublished data). Thus, it does not appear that the large cells of the developing corpus luteum are affected by LH in the same manner as the granulosa cell before becoming luteinized. Voss and Fortune [30] reported that progesterone stimulated oxytocin secretion by bovine granulosa cells during the late stages of a 5 day culture. Ovine corpora lutea have been shown to possess nuclear progesterone receptors [31]. Yet, administration of the progesterone antagonist mifepristone (RU 486) to ewes from days 2 through 5 of the cycle failed to affect luteal content of oxytocin [32]. Although it is possible that the dose of RU 486 administered (175 mg/day) was insufficient, these data were interpreted to suggest that endogenous progesterone does not act in an autocrine/paracrine manner to affect oxytocin production by the developing corpus luteum of the ewe.

Multiple factors appear to be required to promote or suppress expression of the oxytocin gene. An imperfect palindromic sequence with similarity to estrogen response elements has been identified in the 5' flanking region of the human and rat oxytocin gene [33,34]. The promoter region of the oxytocin gene in the cow also contains an imperfect response element for estrogen located proximal to the transcription start site [35]. Using heterologous transfection systems the rat and human but not the bovine oxytocin gene can be stimulated by estradiol [33,34]. Thus, response of the oxytocin gene to estrogen appears to be species specific. The promoter region of the human oxytocin gene contains four pentanucleotide

repeats that bind retinoic acid [36]. All four repeats are necessary for full retinoic acid responsiveness. The first two repeats most distal to the start site overlap the estrogen response element. In the absence of the two downstream repeats, binding of the retinoic acid receptor to the two upstream repeats results in a negative transcriptional effect and antagonizes the stimulatory effect of the estrogen receptor, most likely by competing for binding to the same site on the promoter. The promoter region of both the rat and human oxytocin gene contains response elements for thyroid hormone [37]. In the case of the promoter region for the rat oxytocin gene, the two thyroid hormone response elements appear to overlap with the estrogen response element and reside further upstream of the estrogen response element. Based upon the available data, it appears that the upstream nucleotide sequence that encompasses the estrogen response element in the promoter region of the rat and human oxytocin gene may actually constitute a composite hormone response element to which different classes of nuclear hormone receptors bind.

According to Wehrenberg et al. [38] the promoter region of the bovine oxytocin gene has been shown to contain response elements for the two orphan receptors, steroidogenic factor-1 (SF-1) and chicken ovalbumin upstream promoter transcription factor (COUP-TF). Presence of SF-1 correlates well with maximal *in vivo* expression of the oxytocin gene in the developing bovine corpus luteum, i.e., with the increasing quantity of oxytocin mRNA being present immediately after ovulation. In contrast, the increased presence of COUP-TF in the bovine corpus luteum at midcycle, and thereafter, correlates with the down-regulation of the oxytocin gene. Interaction of SF-1 and (or) COUP-TF with other regulatory proteins, or specific modification of these orphan receptors, is probably needed for the up- and down-regulation that characterizes oxytocin gene expression in the corpus luteum.

In cultures of bovine granulosa cells recovered from preovulatory follicles and luteal cells of the developing corpus luteum the addition of insulin or insulin-like growth factor-1 (IGF-1) to the medium stimulated an increase in oxytocin production [39,40]. The stimulatory effects of insulin and IGF-1 on oxytocin production by granulosa and luteal cells most likely reflect their transcriptional effects on expression of the oxytocin gene.

Overall, it is evident that a number of factors are capable of regulating oxytocin gene expression in various species. However, which specific factors interact to promote oxytocin gene expression in the corpus luteum remain elusive.

Induced Secretion of Luteal Oxytocin

Flint and Sheldrick [41] were the first to report that injection of ewes with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogue (cloprostenol) caused an immediate increase in luteal oxytocin secretion. Similarly, injection of $PGF_{2\alpha}$ analogue into cows during the midluteal phase of the estrous cycle caused an immediate increase in luteal oxytocin secretion detectable in systemic blood within 5–15 min post-injection [42]. Almost concurrently, McCracken and Schramm [43] advanced the hypothesis that episodic secretions of luteal oxytocin and uterine $PGF_{2\alpha}$ are interrelated through a double positive feedback loop and thereby together promote regression of the corpus luteum. This proposed functional interrelationship between the ovary and uterus was supported by the observation of Flint and Sheldrick [44] and Walters et al. [45] who showed that onset of luteal regression in the ewe and cow is characterized by intermittent synchronous pulsatile secretion of oxytocin and $PGF_{2\alpha}$. Whether luteal oxytocin actually is necessary for promoting regression of the bovine corpus luteum is now controversial. In both the cow and ewe luteal concentrations of oxytocin at the end of a normal estrous cycle are less than during the midluteal phase of the cycle. Repeated infusions of norepinephrine into the abdominal aorta of heifers on days 15 and 16 of the estrous cycle caused diminished release of oxytocin in response to each succeeding infusion of the catecholamine [46]. The authors estimated that repeated infusions of norepinephrine on days 15 and 16 depleted luteal oxytocin by 74%. Because this depletion of oxytocin had no effect on duration of the estrous cycle the authors concluded that luteal oxytocin had no direct action in luteolysis in cattle. However, it should be noted that administration of 500 μg of $PGF_{2\alpha}$ analogue to heifers, with a corpus luteum presumably depleted of oxytocin, still caused a release of luteal oxytocin that attained a peak concentration of 50 pg/ml in systemic blood plasma. Some more recent conclusions that luteal oxytocin is nonessential in promoting onset of corpus luteum regression in the cow are based on the results of *in vivo* microdialysis experiments [47,48]. Whether results of this type of invasive experimental approach in assessing luteal function can be considered to actually represent the function of the corpus luteum is questionable. Further, it may be premature to conclude by failure to measure oxytocin in systemic blood or microdialysates that this nanopeptide plays no role in luteal regression in the cow. Negative results obtained by microdialysis experiments may reflect the intraluteal placement of the microdialysis tubing.

Although norepinephrine has been shown to induce the secretion of bovine luteal oxytocin under experimental conditions it is unknown to what extent the endogenous neurotransmitter is involved in regulating the secretion of this nanopeptide. Norepinephrine apparently binds to a

luteal β-adrenergic receptor to provoke release of oxytocin because response to the neurotransmitter is blocked by administration of propranolol but not phentolamine [49]. On the other hand, much more is known about the mechanism of action of prostaglandin $F_{2\alpha}$. Receptors for $PGF_{2\alpha}$ are found predominantly on the large luteal cells [50]. Binding of $PGF_{2\alpha}$ to its receptor activates phospholipase Cβ (PLC) via coupling with a G_q protein [51,52]. Activation of PLC is the initial step of the phosphoinositide cascade that generates the second messengers diacylglycerol (DAG) and Ca^{2+} [53,54]. Both DAG and Ca^{2+} are required for activation of the conventional class of protein kinase C isozymes (α, βI, βII, γ). In contrast, isozymes of the novel class of PKC (δ, ε, η, θ) lack the calcium binding domain found in the cPKCs, however, they are activated by phospholipids and DAG. Both these two classes of PKC isozymes can be activated by phorbol ester. The bovine corpus luteum contains predominantly PKCα and ε although isozymes βI and βII have been reported to be present [52,55]. The increase in intracellular Ca^{2+} that occurs in response to $PGF_{2\alpha}$ is believed to promote translocation of at least some PKC isozymes to the plasma membrane from a cytoplasmic site. Orwig et al. [56] demonstrated that $PGF_{2\alpha}$-induced luteal secretion of oxytocin was correlated with an increase in plasma membrane PKC activity. In order for exocytosis of oxytocin to occur, the vesicle bearing the oxytocin granule must pass through a cytoskeletal cortex consisting of cross-linked monomeric actin filaments that lie in close apposition to the plasma membrane [57]. The actin filaments of the cortex are maintained in a particular geometrical configuration by actin binding proteins. A number of actin binding proteins have been identified but one in particular, a myristoylated alanine-rich C kinase substrate (MARCKS) protein, has been shown to be associated with $PGF_{2\alpha}$-induced secretion of oxytocin in the bovine corpus luteum [58]. As the name implies this protein is phosphorylated by PKC and luteal concentrations of MARCKS mRNA and protein appear to correspond with those of oxytocin throughout the estrous cycle [59]. In its unphosphorylated state, the protein is attached to the plasma membrane via the myristate moiety and its stability is maintained by electrostatic interaction with the membrane due to positively charged Lys/Arg residues found in its phosphorylation site domain [60]. *In vivo* exposure of the bovine corpus luteum to $PGF_{2\alpha}$ during the midluteal phase of the estrous cycle causes an immediate phosphorylation of MARCKS by activated PKC, with a resultant translocation of the phosphorylated MARCKS to the cytoplasm that is highly correlated with exocytosis of oxytocin [58].

Exposure of the mature corpus luteum to $PGF_{2\alpha}$ has been shown to cause activation of MAPKinase-kinase-kinase (Raf-1) and downstream components of the MAPKinase pathway including the transcription factor C-Jun [61,62]. The extent, if any, to which activation of this pathway is involved in the exocytosis of oxytocin is unknown.

Autocrine/Paracrine Action of Ovarian Oxytocin

Chandrasekher and Fortune [63] reported that oxytocin, in a dose-dependent manner, significantly increased progesterone production by cultured granulosa cells of bovine preovulatory follicles. Oxytocin was without effect on granulosa cell progesterone synthesis when cultures contained an oxytocin antagonist. Similarly, addition of graded doses of oxytocin to cultures of theca cells failed to affect steroidogenesis.

Although luteal oxytocin may indirectly promote luteolysis to establish the normal estrous cycle, at least in the ewe, the question remains as to the biological significance of the massive synthesis of the neuropeptide in the developing corpus luteum. Administration of various dosages of oxytocin to ewes for 3 to 4 day intervals throughout the estrous cycle failed to alter the duration of the cycle or affect luteal concentration of progesterone [64,65]. These data might be interpreted to suggest that oxytocin has no direct effect on the corpus luteum. However, research by Tan et al. [66,67] demonstrated that exposure of dispersed bovine and human luteal cells to low concentrations of oxytocin stimulated progesterone synthesis whereas higher concentrations of the nanopeptide were inhibitory. This research could not be confirmed by Richardson and Masson [68] using human luteal cells. On the other hand, using an *in vitro* microdialysis system Miyamoto and Schams [69] reported that oxytocin stimulated progesterone production by cells of the developing bovine corpus luteum (days 5 to 7 of the cycle) but was less effective on luteal cells collected later in the estrous cycle. Bovine luteal cells collected at various stages of the estrous cycle have been shown to possess oxytocin receptors [70]. Surprisingly, addition of $PGF_{2\alpha}$ but not PGE_2 or estradiol to cultures of bovine luteal cells has been reported to cause upregulation of oxytocin receptors without changing the binding affinity [71].

In contrast to the stimulatory effect of oxytocin on bovine luteal cells, oxytocin in doses of 4 to 800 mU (1 mU = 2 ng) added to short term cultures of baboon luteal cells recovered during the early luteal phase of the cycle significantly suppressed progesterone production [7]. When added to cultures of baboon luteal cells recovered during the midluteal phase of the cycle, oxytocin had no effect on progesterone production. *In vitro* progesterone production by cells of the baboon corpus luteum recovered during the late luteal phase of the cycle was suppressed by high doses of oxytocin only. In conclusion it appears that available data on the intraluteal effect of oxytocin on progesterone synthesis are quite equivocal. Further research is

required to resolve the issue of whether or not oxytocin is essential for regulating steroidogenesis by the developing and mature corpus luteum.

Concluding Remarks

While much knowledge exists about the biochemical and endocrine factors involved in regulating luteal oxytocin synthesis it is obvious that much has yet to be learned. Of particular interest is elucidation of the key factor(s) that are absent or present to limit transcription of the oxytocin gene during the cycle. Research is also needed to more precisely define the intraovarian or intraluteal action of oxytocin. It is presumed that future research will contribute vital new information about luteal oxytocin synthesis/secretion and action in the various mammalian species.

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